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Short Communication: The genetic diversity and agronomical characters of local cultivars of tidal rice in East Kalimantan, Indonesia

RUSDIANSYAH[1,♥], TJATJUK SUBIONO[1], WIDI SUNARYO[1], ANDI SURYADI[1], SULASTRI[2], SAMID ANJASMARA[1]

[1]Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman. Jl. Pasir Belengkong Samarinda 75119, East Kalimantan, Indonesia. Tel.: + 62-541-749343, ♥email: rrida_rusdi@yahoo.co.id

[2]Department of Research and Development, PT. Pupuk Kalimantan Timur. Jl. James Simandjuntak No. 1, Bontang 75313, East Kalimantan, Indonesia

Manuscript received: 8 November 2016. Revision accepted: 1 August 2017.

Abstract. *Rusdiansyah, Subiono T, Sunaryo W, Suryadi A, Sulastri, Anjasmara S. 2017. Short Communication: The genetic diversity and agronomical characters of local cultivars of tidal rice in East Kalimantan, Indonesia. Biodiversitas 18: 1289-1293.* Availability of local rice germplasm is very important for plant breeding, especially in the improvement of the genetic diversity. The objectives of the research were to study the genetic diversity and agronomical characters of local characters of tidal rice in East Kalimantan. The research was conducted from May to October 2014 in the Village of Sidomulyo, Anggana Sub-District, Kutai Kertanegara District, East Kalimantan. The experiment was arranged in a randomized complete block design (RCBD) with five replications. Eight selected local tidal rice cultivars from East Kalimantan (Pandan Ungu, Kambang, Amas, Roti, Pudak, Sikin Merah, Sikin Putih and Popot) were used in this experiment as plant materials. The results showed that there was wide genetic diversity among local tidal-rice cultivars selected from East Kalimantan. Number of productive tillers, the percentage of empty grain per panicle, 1000 grain weight and yield per hectare can be used as selection criteria for local lowland rice cultivars from East Kalimantan because they have a high value of heritability (h^2_{bs}), phenotype and genotype and coefficient of variation (PCV and GCV). Pandan Ungu, Kambang, Amas, Roti and Sikin Merah cultivars potential to be used as parents in the breeding program.

Keywords: Genetic diversity, agronomical characters, local cultivars, lowland rice

INTRODUCTION

Indonesia, known as one of megadiverse countries in the world, has abundant natural germplasm resources. One of them is local rice cultivars i.e paddy rice, upland rice and other wild rice species. Up to 2010, more than 2,797 species of rice germplasm consisting of 1,635 local rice cultivars, 978 introduced varieties and 184 new improved varieties have been successfully collected by Rice Research Institute of Sukamandi, Indonesia (Sitaresmi et al. 2013). However, many of these local rice germplasm have depleted due to genetic erosion leading to the extinction of local rice germplasm. This happened due to the lack of conservation efforts (Hendra et al. 2009). Local rice germplasm is a very important source of genetic diversity in plant breeding programs for supporting national food self-sufficiency especially rice.

East Kalimantan with its tropical rain forest climate has genetic diversity resources of local rice which is abundant with exotic characteristics and high economical value. Many of these local rice germplasm have tasty rice flavors and fragrant aromas. In addition, they resistant to abiotic environmental stresses such as drought, aluminum, iron, sour sulfate and salinity; and also biotic stresses such as blast disease, dwarf grass, and others. Of these local rice germplasm, Rusdiansyah (2012) has successfully explored 13 local cultivars of tidal rice paddy which were cultivated by farmers from generation to generation. Nurhasanah et al. (2016) have also successfully collected 73 local rice cultivars consisting of 53 ordinary rice cultivars and 18 glutinous rice cultivars. The initial selection of 13 local cultivars of upland rice tidal showed that eight cultivars have a high potential candidate to be further characterized their agronomical performances and be used as parents in plant breeding program.

In plant breeding programs, genetic variability is a major factor in the development of improved varieties. Extensive genetic variability allows superior varieties to be fast developed. In order to expand the genetic diversity of rice, some extensive methods have been conducted by plant breeders, i.e., the exploration of local cultivars, artificial mutations, and the utilization of germplasm from other countries (Kuswantoro 2017a). The effectiveness of the selection can be achieved when the selected characters used as target has a high value of genetic diversity (Herawati et al. 2009; Ishak 2012). Extensive or high genetic diversity will also provide flexibility in the selection of superior genetic or the genetic improvement of rice (Saleem et al. 2008; Idris and Mohamed 2013). Therefore, the objective of the study was to study the genetic diversity and the agronomical characters of local cultivars of tidal rice in East Kalimantan.

MATERIALS AND METHODS

The study was conducted from May to October 2014 in the tidal rice field of Sidomulyo Village, Anggana Sub-District, Kutai Kartanegara District, East Kalimantan, Indonesia. The used material consisted of eight local cultivars of East Kalimantan tidal rice fields such as Pandan Ungu, Kambang, Amas, Roti, Sikin Merah, Sikin Putih, Pudak and Popot (Figure 1) (Rusdiansyah 2012).

The study was conducted in a randomized block design (RAK) with five replications. Each cultivar was grown on 5 × 10 m^2 plot, with the plant spacing of 30 × 30 cm^2 and each planting hole was planted with 1 seed of rice plant. The first fertilization was performed seven days after planting with a dose of 45 kg N. ha^{-1}, 60 kg $P_2O_5.ha^{-1}$ and 60 kg $K_2O.ha^{-1}$. The second fertilization was given 30 days after planting with a dose of 30 kg N. ha^{-1}. Weed control was done at 2, 4, 6 and 8 weeks after planting. Pest and disease control was performed when the plants showed symptoms of an attack. The observed variables included plant height, number of productive tillers, number of grains of content/panicle, percentage of unhulled grain/panai, grain weight/weed, 1000 grains of grain and grain/hectare production.

The data obtained were analyzed using variance (F test) at the real level of 5% and if there was a real effect continued with the smallest real difference test at 5% real level. Analysis of various components such as genotypic variety (σ2g), phenotypic variety (σ2f), environmental variety (σ2e), phenotypic diversity coefficient (PCV) and genotypic diversity coefficient (GCV) was performed on all observed variables. The genotypic diversity coefficient criteria were in broad performances if GCV ≥ 22 $\sigma_{\sigma}{}^2{}_g$, and in narrow performances, if GCV <2 $\sigma_{\sigma}{}^2{}_g$. All analyses of various components were carried out following Seyoum et al. (2012); Sutjahjo et al. (2013); Bagati et al. (2016); Kuswantoro (2017b).

RESULTS AND DISCUSSIONS

Genetic diversity

The estimation of genetic diversity is necessary and of concern in the selection of parent candidates or hybridization outcomes. The selection will be effective if the influence of environmental factors is small and the genetic diversity within the population is wide. The analysis results of h^2_{bs} of all observed characters ranged from 0.44 to 0.96. The highest values of h^2_{bs} were obtained in the plant height parameters with value of 0.96 followed by successive number of productive tillers, production/ha, percentage of unhulled grain/panai, weight of 1000 grains, weight of grain/clump and number of grain content/panicle 0.83; 0.82; 0.80; 0.78, 0.76 and 0.44 (Table 1). The broad perspective of high heritability means that the characters observed in the selected population had high adaptability to the growing environment.

The results obtained in this study was in accordance with Herawati et al. (2013); Idris and Mohamed (2015) who achieved high heritability values (h2bs) in some parameters including plant height, grain/panai, number of unhulled rice, percentage of void and weight of grain/clump. These results indicated that most genetic factors influence and contribute greater to the diversity of the existed characters than environmental factors. According to Ogunbayo et al. (2014) and Bagati et al. (2016), heritability is an overview of the main contribution of genetic factors toward certain characters. This heritability value could be further used as an easy measurement of those characters to be inherited. Kuswantoro (2017a,b) also reported that heritability determines the progress of selection and also the method of selection to be used in generating diversity. Characters that have high heritability value can be used as selection criteria in plant breeding. According to Singh et al. (2011), heritability is the transmission index of a character from an elder to a descendant. Heritability estimation gives a favor for plant breeders in the selection of plant genotype with superior traits in a population. Yakub et al. (2012), stated that the selection of characters with high h2bs values could be possibly done in the early generations because those characters might be easily inherited by their offspring. In this study, some parameters i.e plant height, the number of productive tillers, the percentage of empty grain/panicle, the weight of grain/clump, the weight of 1000 grains of grain and the production/ha had a high value of heritability value, while the number of grain content/panai had a moderate value of broad sense heritability (Table 1).

Table 1. The variety and broad sense heritability amongst agronomic characters of local cultivars of tidal rice in East Kalimantan, Indonesia

Parameters	σ^2_g	σ^2_p	σ^2_e	h^2_{bs}
Plant height	436.63	451.56	14.93	0.96 T
Number of productive tillers	29.89	36.09	6.20	0.83 T
The number of grain content/panai	398.89	900.59	501.70	0.44 M
Percentage of unhulled grain/panai	32.92	40.96	8.04	0.80 T
Weight of grain/clump	140.92	184.35	43.43	0.76 T
Weight of 1000 grains	1.92	2.46	0.54	0.78 T
Production/ha	0.93	1.13	0.20	0.82 T

Note: σ^2_g = Genotypic variety; σ^2_p = phenotipic variety; σ^2_e = environtmental variety; h^2_{bs}= broad sense heritability; T = high; M = medium/moderate

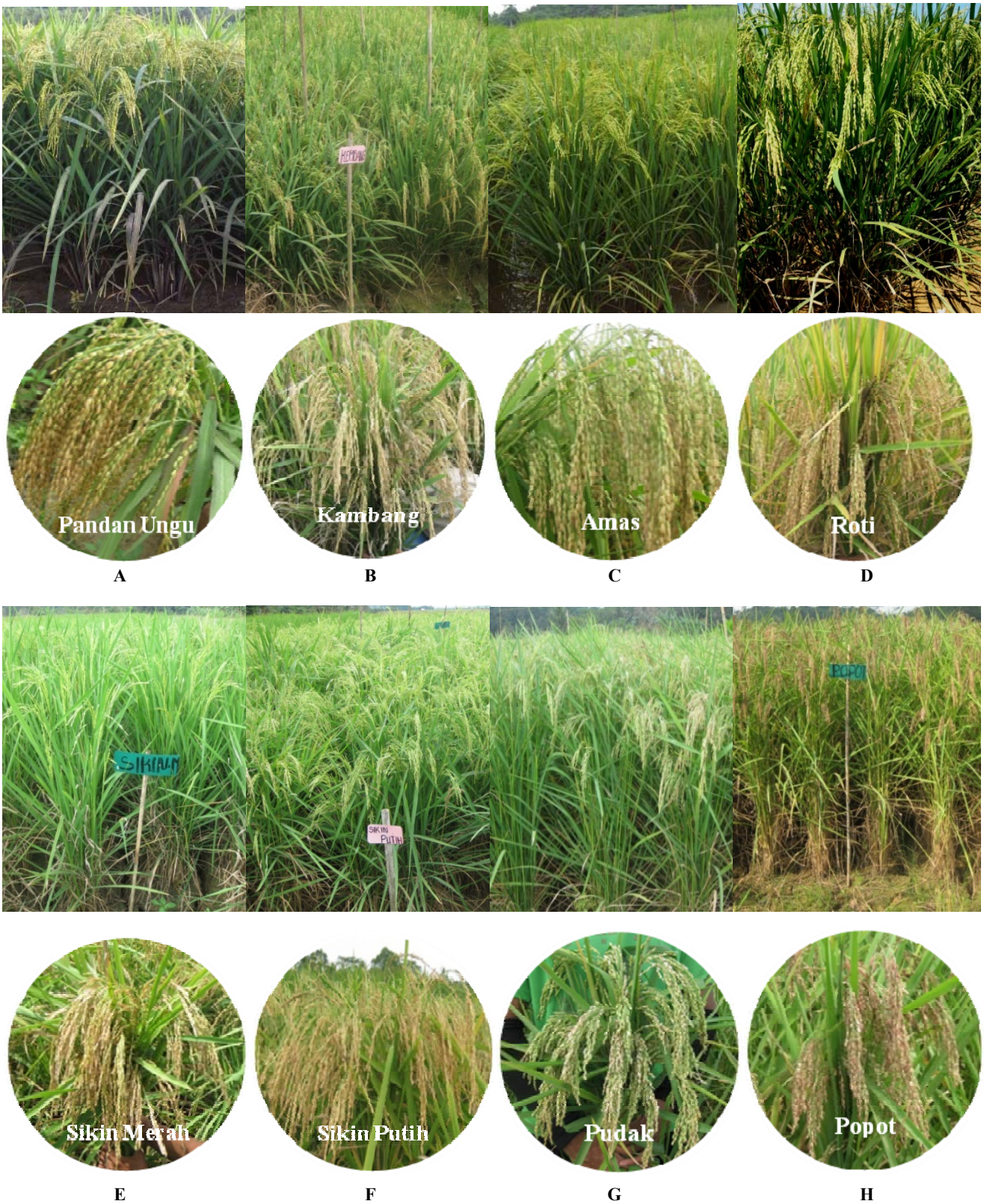


Figure 1. Appearance of plants and panicles from the cultivars of A. Pandan Ungu, B. Kambang, C. Amas, D. Roti, E. Sikin Merah, F. Sikin Putih, G. Pudak, H. Popo

The results showed that the value of phenotypic diversity coefficient (PCV) of all observed characters was higher than the value of genotypic diversity coefficient (GCV) (Table 2). These results were in accordance with several other studies conducted by Seyoum et al. 2012; Idris and Mohamed 2013; Ogunbayo et al. 2014; Bagati et al. 2016; Hefena, et al. 2016. Despite the different value of PCV and GCV, it seemed that the gap value is small. This

showed that the environmental factors had a less influence on the phenotypic and genotypic characters (PCV and GCV) of tidal rice species. Table 2 shows that four agronomical characters including number of productive tillers, percentage of empty grain/panicle, weight of 1000 grains and production per hectare had broad criteria of PCV and GCV. While the other three characters such as plant height, the number of grain content/panicle and the weight of grain/clump had narrow criteria so that they could not be used as selection criteria. Selection of characters with wide genetic diversity will be effective and able to increase the genetic potential of the character in the next generation (Herawati et al. 2013). Based on the results of h2bs values, the agronomical characters including PCV and GCV, the number of productive tillers, the percentage of empty grain/panai, the weight of 1000 grains and the production/ha can be used as the selection criteria for the local cultivars of tidal rice in East Kalimantan.

Agronomical characters

The results of the analysis showed a significant difference in all observed agronomical characters. The mean of plant height was in range between 95,93 and 157,49 cm followed by number of productive tiller/clump, number of grain of content/panicle and percentage of empty grain/panai which in range of 15,07 -31,08 stems, 164,52-237,69 Grains and 15.25-28.99% (Table 3). There are four cultivars, namely Kambang, Amas, Roti and Sikin Merah which had plant height <125 cm, number of productive tillers/clumps> 25 stems and percentage of empty grain/panai <20%. In addition, Kambang, Amas, and Sikin Merah cultivars had a number of grains/panicle around 180 grains. Although the cultivar of Pandan Ungu has the less number of productive tillers/clumps around 15.07 stems, but, it has the highest plant height, the highest number of grains of content/panai, the grain and the percentage of the lowest empty grain of 104.92 cm, 237.69 grains and 15.25%. Plant height, number of tillers, number of grains/panicle and percentage of grain content/panicle is an important character in rice plant breeding especially for the increased production and development of new type of rice varieties. Short stems and tillers characters can represent how much they produce panicle/clump and number of grains/panicles (Abdullah et al. 2008; Abdullah 2009). Based on the selection criteria, Kambang, Amas, Sikin Merah and Pandan Ungu cultivars could be used as parent in the formation of new rice varieties with the range of plant height between 115 and 120 cm, the number of grains between 180 and 240, and the number of grains/panicle around 80%. Abdullah et al. (2008), stated that the generation of new rice cultivars in Indonesia need a genetic diversity source with the number of tillers in moderate level with high productivity (12-18 crops), the number of grains from range of 150 to 250 grains and the number of full grain contents between 85 and 95%.

Table 2. The coefficient of phenotypic and genotypic diversity amongst agronomic characters of local cultivars of tidal rice in East Kalimantan, Indonesia

Parameters	PCV	GCV	$\sigma_{\sigma^2 g}$	Criteria
Plant height	17.48	17.19	208.18	Narrow
Number of productive tillers	26.06	23.72	14.68	Wide
The number of grain content/panai	15.86	10.56	236.77	Narrow
Percentage of unhulled grain/panai	29.67	26.60	16.28	Wide
Weight of grain/clump	29.39	25.69	70.56	Narrow
Weight of 1000 grains	6.18	5.46	0.96	Wide
Production/ha	23.36	21.19	0.46	Wide

Note: PCV = The coefficient of phenotypic diversity; GCV = The coefficient of genotypic diversity

Table 3. The agronomical characters of rice cultivars observed after harvesting times

Cultivars	Plant height (cm)	Number of productive tiller/clump	Number of full grain contents/panicle	Percentage of unhulled grain/panai (%)	Weight of grain/clump (g)	The weight of 1000 grains (g)	Production/ha ($Mg.ha^{-1}$)	The harvest age (days)$^{+}$
Pandan Ungu	104.92 b	15.07 e	237.69 a	15.25 a	51.75 b	28.14 a	5.05 b	115
Kambang	95.93 a	31.08 a	185.52 bc	16.57 a	47.07 bc	26.22 b	4.87 b	106
Amas	104.15 b	25.28 bc	194.72 b	18.55 a	50.02 b	24.68 de	4.87 b	122
Roti	114.53 c	26.10 b	171.82 bc	18.13 a	60.54 a	25.50 bcd	5.42 ab	121
Pudak	133.63 e	21.95 d	164.52 c	28.49 b	30.20 d	24.50 e	3.16 c	149
Sikin Merah	120.95 d	26.92 b	196.92 b	18.41 a	61.11 a	25.70 bc	5.87 a	125
Sikin Putih	140.75 f	22.63 cd	181.88 bc	28.13 b	39.05 c	24.78 cde	3.71 c	134
Popot	157.49 g	15.37 e	180.55 bc	28.99 b	29.89 d	23.34 f	3.44 c	145
BNT 5%	5.01	3.23	29.02	3.67	8.54	0.96	0.58	-

Note: The data with plus sign $^{+)}$ was calculated based on the harvest age of plants in field

Furthermore, of the average analysis of weight grain/clump weight showed that the weight of 1000 grains ranged from 29.89 to 61,11 g and the production of grain/ha ranged between 23,34 and 28,14 g, respectively. The cultivars which had weight of grain/clump> 45 g, production/ha> 4.5 Mg. ha^{-1} and harvest age ≤ 125 days were obtained in Red Sikin, Roti, Pandan Ungu, Amas and Kambang. While from other parameters specify the weight of 1000 grains, none of observed cultivars had the weight of 1000 grains ≥ 30 g. However, four observed cultivars i.e. Pandan Ungu, Kambang, Roti and Sikin Merah produced the weight of 1000 grains in range from 25 g to 28 gr (Table 3). Besides, agronomical parameters such as the number of tillers, the number of grain content and the percentage of grain/panicle are used in the development of new parents in the breeding programs, other parameters such as the character of the number of grains/panicles and the size of a large panicle and weight of 1000 grains between 25 and 26 g are of importance to the successful result of breeding program (Abdullah et al. 2008).

Based on the genetic diversity and agronomic characters observed in this study, five cultivars including Pandan Ungu, Kambang, Amas, Roti and Sikin red are potentially used as parents in the rice plant breeding program. The agronomical characters in terms of character of productive tillers, percentage of unhulled grain/panai, weight of 1000 grains and production/ha can be used as selection criteria for local cultivars of East Kalimantan tidal rice due to its high h2bs value and the wider type of PCV and GCV.

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Physiological responses of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress

RITHA LUSIAN KARUWAL[1,2,♥], SUHARSONO[3], ARIS TJAHJOLEKSONO[3], NOVRIYANDI HANIF[4]

[1]Program of Plant Biology, School of Graduates, Institut Pertanian Bogor. Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia
[2]Programmeof Biology Education, Faculty of Teacher Training and Education, Universitas Pattimura. Jl. Ir M. Putuhena Ambon 97233, Maluku, Indonesia, Telp./Fax. +62-911-3825203/+62-911-3825204, ♥email: rithakaruwal@yahoo.com.
[3]Departement of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia
[4]Departement of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia

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Abstract. *Karuwal Rl, Suharsono, Tjahjoleksono A, Hanif N. 2017. Physiological responses of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress. Biodiversitas 18: 1294-1299.* The aim of this study was to analyze the physiological responses of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress. The physiological responses were analyzed by measuring plant height, number of leaves, relative water content (RWC), and chlorophyll content using Anova and were continued with Duncan test at 5% significance level. Results of this research showed that the varieties and drought stress in the form of watering periods to affect to all variables except plant height. Physiological responses showed that at every ten days watering periods, the crimson varieties gives the highest plant height (33.85 cm), the brown varieties gives the highest number of leaves (30), the dark brown varieties have the highest RWC (88.675%), and the highest content of chlorophyll a (0.5088 mg/L), chlorophyll b (1.595 mg/L), and total chlorophyll (1.5095 mg/L) are found in white varieties. The supply of water at every ten days is the optimum time of drought stress in cowpea. Further research will be needed on the responses of the other variables for screening, selection, then multi-location trials to obtain tolerant local cowpea varieties to drought.

Keywords: Cowpea, drought stress, physiological responses

INTRODUCTION

Cowpea is one of local crop legume that was cultivated in Indonesia, but only by a few of farmers. Morphologically, it has diversity in growing habit, leaf, pod and seeds. Physiologically, it has important traits such as more tolerant to drought, more pest and disease resistant, easier to cultivate, and more ability to produce pods on nutrient poor soil than other legumes (Rukmana and Yuniarsih 2000). Cowpea seeds are used as food materials, livestock, and raw industrial materials. Seeds of cowpea have high protein and low fat just like soybean i.e. between 23,4-25,9% (Purwani and Santosa 1996) and then can be developed as an alternative material to make tempeh (Ratnaningsih et al. 2009).

In Indonesia, a total of 139 accessions of cowpea were listed in germ plasma collection from Java, Sulawesi, and Nusa Tenggara islands (ICABIOGRD 2010). One of areas also having potential of cowpea is Maluku islands namely Southwest Maluku District but it is not listed. In general, the community in this area cultivates cowpea as food materials as carbohydrate complement. Based on exploration results by Polnaya (2008) in Lakor Island, there are ten of local varieties with a local name according to seeds color. According to Esquinas-Alcasar (1993), landrace is a variety growing in a location which can adapt to the adverse condition, with stability of production. Southwest Maluku has harvest area of legumes as much as 149 hectares with the low productivity and human consumption by 50.83 calories (Central of Statistics Southwest Maluku District 2015). From environment factors, small islands in southwest Maluku are an arid area with minimum rainfall and this is limiting factors in the availability of water (Susanto dan Sirappa 2005). According to Pesireron et al. (2013), rainfall distribution in this area consists of eight months of dry season and four months of the rainy season so that water availability is limited and it becomes a limiting factor in the productivity of agricultural crops. Thus, it can also affect the growth and productivity of cowpea due to drought stress as described by Peijic et al. (2013) that drought stress affects cowpea production and it also depends on genotype, intensity and stress periods, stage of plant development. Ndiso et al. (2016) also reported that water stress imposed vegetative growth stage and the impact of water stress on growth is dependent on the cowpea variety.

Drought stress is a condition of reduced water in plants affecting the growth and production (Purwanto and Agustono 2010) depending on period and intensity, and stage of plants development (Bastos et al. 2011). The responses to drought stress at plants growth are the decrease in plant height, stem diameter, number of leaves and also the changing of anatomical structure. Another response is the reduction of water content and chlorophyll

content in leaves, and the accumulation of chemical compounds such as the increase of proline content in osmotic adjustment that will inhibit metabolism process (Nio et al. 2011). In the study about breeding for drought tolerance in progenies of cowpea, Abed (2014) reported that the highest amount of chlorophyll content and relative water content is found in the S3 progeny under period of 10 days irrigation in both seasons. While Okon (2013) reported that seedling of brown variety had more adverse effect than white variety on growth performance which was observed based on water regimes (12, 24, and 48 hours) for 30-days in the green house. Also, Kutama et al. (2014) mentioned that in all the varieties after water stress induction, it generally showed a different rate of drought tolerance and it was observed based on some physiological mechanisms.

Various of studies about the response to drought stress on cowpea were conducted by Bastos et al. (2011); Punggulani et al. (2012); Okon (2013); Uzunova dan Zlatev (2013); Abed (2014); Kutama et al. (2014); Ndiso et al. (2016). However, the study about some local cowpea from southwest Maluku varieties was focused on exploration and agronomic characterization while the observation in response to drought stress and to do screening on tolerant varieties had not been done yet. Therefore, character of plants with physiological responses to drought tolerance must be analyzed to obtain tolerant varieties in the local site and to build a data base. The aim of this study was to analyze the physiological responses of some local cowpea from southwest Maluku varieties to drought stress.

MATERIALS AND METHODS

Study area

A field experiment was conducted at a greenhouse in the Department of Biology, Universitas Pattimura, Ambon, Maluku (Moluccas), Indonesia in September-December 2016.

Design

Randomized complete block design (RCBD) was used in this research with two factors and two replicates. The first factor was cowpea varieties consisting of batik, crimson, pink, white, dark brown, russet, black, yellow, and brown which were collected from Sermatang island. The second factor was drought stress which was in the form of watering period of two days (P0), ten days (P1), and 15 days (P2).

Procedures

The soil was obtained from Taeno Rumahtiga village, then it was screened for dirt and litter, and was dried and filtered. A 3 kg of the soil was poured into polyethylene bags with size of 30 x 40 cm and then was watered before the cowpea was planted in it. Two healthy seeds of cowpea were planted in each polyethylene bags with a depth of 3 cm. Supply of water was done in accordance with combination treatments on each variety from the first day of planting and it was 300 mL of water.

Physiological responses

Physiological responses which were measured were plant height in cm, number of leaves/plant, relative water content (RWC) in percent, and chlorophyll content in mg/L. Plant height was measured from the surface of soil to the first branching point using measure tape on tenth week after planting. While, on number of leaves only fully formed leaves were calculated. RWC was measured following the method of Abed (2014) with modification. The second fresh leaf from the shoot of the plant in each pot was detached and was put in a labeled aluminum foil. 0.5 g of fresh leaf was weighed and it was considered as fresh weight (FW), then it was soaked in water for 4 hours, and after being picked up from water, it was weighed again and this time, it was considered as turgid weight (TW). Next, the leaves were dried in an oven at 80° C for 24 hours, then it was weighed again and its weight was considered as dry weight (DW). RWC was calculated using formula:

$$RWC\ (\%) = (FW-DW)/\ (TW-DW) \times 100$$

Chlorophyll content was measured at tenth week after planting following the method from Setiari and Nurchayati (2009) with modification. The second fresh leaf from apex was picked and 0.1 g of it was spread out. This leaf was extracted with 10 mL of acetone 80%. The extract was sifted with Whatman filter paper number 2. Absorbance of filtrate was calibrated on UV-Vis spectrophotometer at a wavelength of 644 nm and 663 nm. Calculation of chlorophyll content was estimated using formula:

$$\text{Chlorophyll a} = 1.07\ (OD\ 663) - 0.094\ (OD\ 644)$$
$$\text{Chlorophyll b} = 1.77\ (OD\ 644) - 0.28\ (OD\ 663)$$
$$\text{Total of chlorophyll} = 0.79\ (OD\ 663) + 1.076\ (OD\ 644)$$

Statistical analysis

The data obtained were analyzed with analysis of variation (ANOVA) and Duncan test at 5% significance level using SPSS 16.0 software.

RESULTS AND DISCUSSION

The effects of drought stress on plant physiology activity are determined by periods and intensity of stress, stage of plant development (Bastos et al. 2011), and also the characteristics of plant (Fathi and Tari 2016). This opinion is also in line with that explained by Peijic et al. (2013) that the production of cowpea exposed by drought is affected by genotype, period and intensity of stress, stage of plant development. Each genotype of plant will give different physiology responses based on its sustainability or controlled by genetic factors. The physiological responses such as plant height, number of leaves, relative water content, and chlorophyll content were measured in this study. The differences in the physiological responses to

drought stress among the nine cowpea varieties show that each variety has different ability in drought stress treatment because cowpea is tolerant plant to drought (Bastos et al. 2011). This is the same for the local cowpea varieties from Southwest Maluku, although these varieties are landrace that naturally grows in the same area, their resistance to drought has not been studied yet and therefore, they are still unknown. In general, the highest result of physiological responses was obtained in the period of watering of treatment P1 (every ten days).

Results show that period of watering affects the fluctuation of plant height in each cowpea varieties (Figure 1). The highest plant height is found on crimson variety (33.85 cm) and the lowest is found on brown variety (12.35 cm) on treatment P1. The russet variety gives the highest (33.40 cm) and the lowest is crimson variety (23.75 cm) on treatment P2. Compared to control, on treatment P1 varieties of crimson, pink, white, dark brown are more tolerant than varieties of batik, russet, black, yellow, and brown based on plant height. Results of ANOVA show that varieties, drought stress, and the interaction with them give no effect on plant height. The fluctuation in plant height of cowpea makes them drought tolerant plant varieties (Bastos et al. 2011). Treatment P1 lead to the reduction of water on growing media but the growth of cowpea is optimal on certain varieties, even it has the highest growth number in treatment P2. Besides that, process of growth requires different amounts of water depending on the type of plant (Hendriyani dan Setiari 2009), so there are varieties with optimal plant height in the water with small amount. Treatment P0 on cowpea made the growing media in humid conditions all the time, so it disturbed aeration of soil and it resulted in stunted growth of cowpea. Results of research show that varieties and periods of watering give no affect on plant height of cowpea, because cowpea has character of tolerant to drought. Moreover, the character of cowpea which is resistant to drought will make the plant to reach its maximal height in a longer time at the vegetative stage.

In contrast, the number of cowpea leaves on the treatment showed that each variety had a declining number of leaves in line with the watering period (Figure 2). The highest leaf number was found in the russet variety (30) and the lowest was on brown variety (17) in the P1 treatment. The highest number of leaves was found in yellow variety (22.50) and the lowest was in pink variety (15 strands) in P2 treatment. Compared with controls, all variety in P1 treatment were more tolerant than P2 based on leaf number. ANOVA results show that variety, water intervals, and interactions between them have an effect on the number of leaves. The decrease in the number of leaves, which is in line with the increasing period of water administration in each variety, is significantly due to cell division at the time of leaf formation. According to Yunusa et al. (2014) reduction of leaf number is caused by decrease of leaf initiation and leaf area so that the activity of photosynthesis is also decreased and this is also explained by Kariuki et al. (2016). Gardner et al. (1991) state that in line with the increasing age of the plant there is a reduction in the number of leaves. Once the leaves become mature and aging, the leaves may not be able to meet their own energy needs. On the other hand, the reduction of the number of leaves can be due to the use of more energy in increasing the plant height in which the energy is obtained from its own leaves as a source of energy. At the vegetative growth stage, water is used by plants for cell division and enlargement in plant height increase and leaf propagation.

Similar to the number of leaves, RWC also shows the same trend (Figure 3). In the P1 treatment, dark brown variety gave the highest RWC (88.675%) while the lowest was on batik variety (69.645%). For P2 treatment, dark brown variety gave the highest RWC (89.615%) while the lowest was on batik variety (63.115%). Compared with control, all variety of P1 treatment were more tolerant than P2 treatment. ANOVA results show that variety, watering intervals, and their interactions have effect on RWC.

Water content is relatively associated with water availability in leaf tissues (Pungulani et al., 2012). The height of relative water content in water stress condition indicates the ability of accession to maintain the metabolism process so that it can survive. The results show that the value of relative water content i.e. 77% in cowpeas after being treated with stress for two weeks is high, while in well-watered condition the cowpea has a relative water content value of 88-91%. After four weeks of treatment, the accession of cowpea has water content of above 50% which is significant to the ability of metabolic processes during extreme conditions. Maintenance of high relative water content in some genotypes after four weeks can be used to screen for drought tolerance in cowpea. When compared to this study, the relative water content measured at plant age of six weeks after the drought stress treatment has a range of above 60%. This suggests that cowpea varieties have the ability to survive even under stressful conditions.

The measurement of chlorophyll content consists of chlorophyll a (Fig. 4), chlorophyll b (Fig. 5), and total chlorophyll (Fig. 6). White variety gives the highest levels of chlorophyll a (0.5088 mg/L), chlorophyll b (1.595 mg/L) and total chlorophyll (1.5095 mg/L) while the lowest is on brown variety in P1treatment. Batik variety gives the highest level and the lowest level is black variety in P2 treatment. Compared to control, in P1 treatment, based on chlorophyll content, all variety is more tolerant except for yellow and brown variety. ANOVA results show that the interaction has an effect on chlorophyll a content while the period of watering affects chlorophyll b content and total chlorophyll content.

Chlorophyll as one indicator of drought-stricken plants is synthesized with the aid of light, water, temperature, nutrients, as well as genetic factors and the numbers are different for each species (Hendriyani and Setiari, 2009). The measurement of chlorophyll content can evaluate the process of photosynthesis in tolerant plants. Water as one of the factors influencing chlorophyll synthesis can determine chlorophyll content. This means that plants that absorb water from growing media containing a lot of water lead to high level of chlorophyll. In this study, cowpea variety has decreasing chlorophyll level which is in line

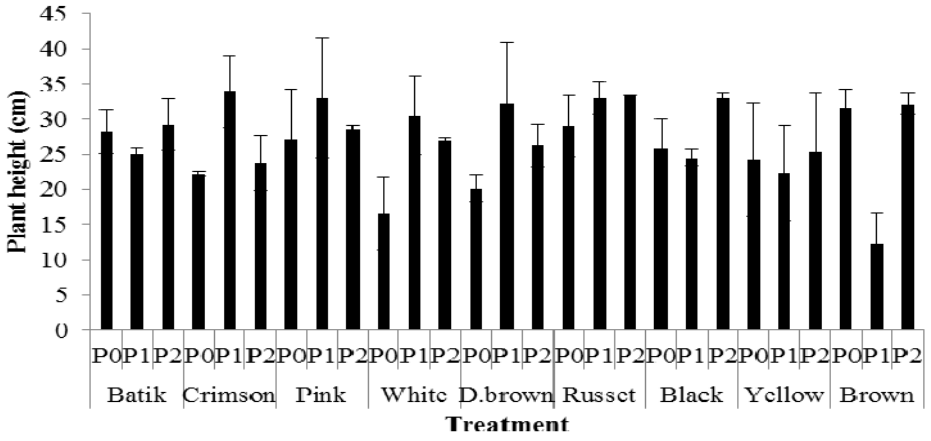


Figure 1. Effect of three periods of watering and nine varieties of cowpea on plant height

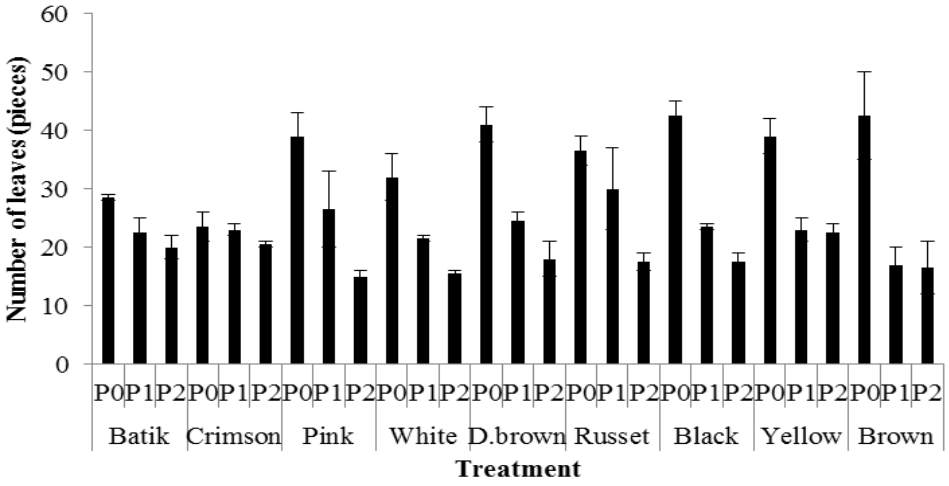


Figure 2. Effect of three periods of watering and nine varieties of cowpea on the number of leaves

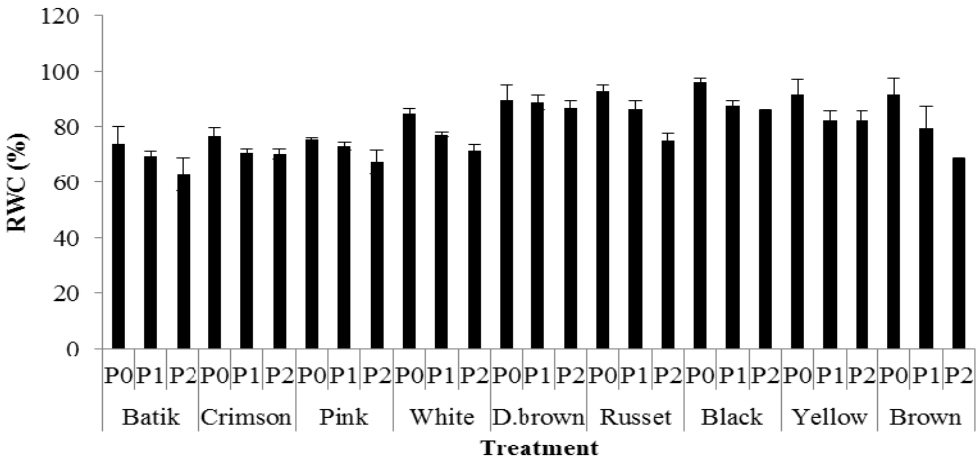


Figure 3. Effect of three periods of watering and nine varieties of caudal on RWC

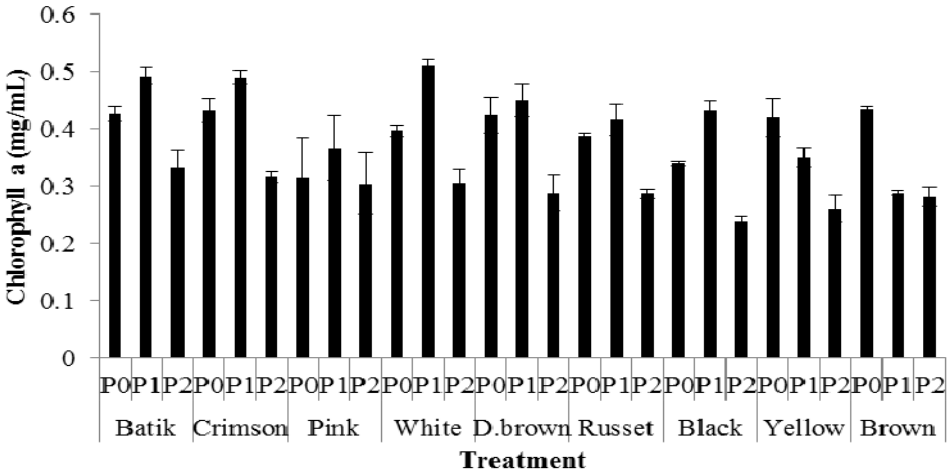


Figure 4. Effect of three periods of watering and nine varieties of cowpea on chlorophyll a

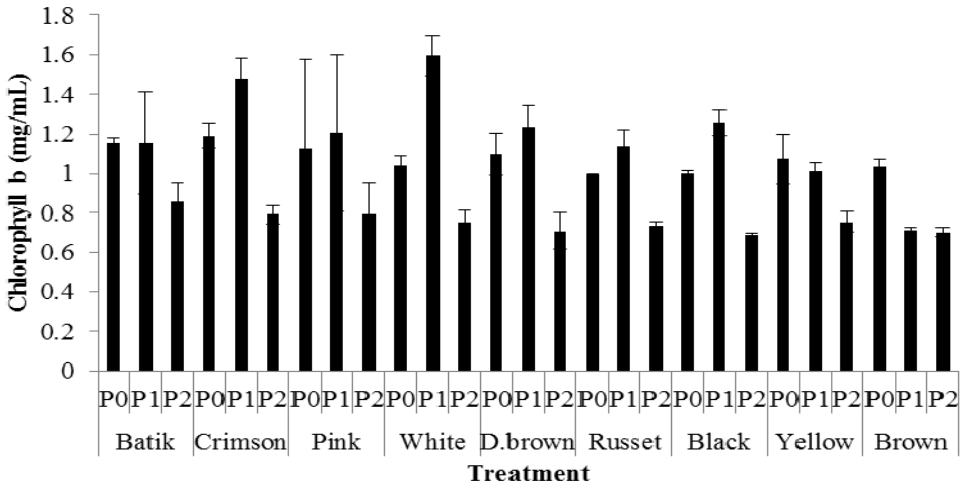


Figure 5. Effect of three periods of watering and nine varieties of cowpea on chlorophyll b

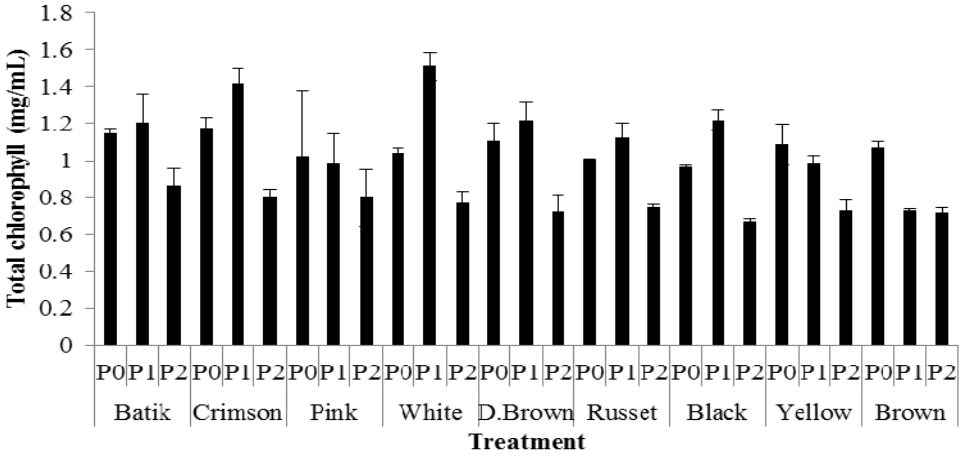


Figure 6. Effect of three periods of watering and nine varieties of cowpea in total chlorophyll

with the period of watering due to the lack of water up taking in planting medium. On the other hand, the synthesis of chlorophyll is determined by the element of nitrogen that can be fixed directly by the cowpea plant having symbiotic relation with the fixing bacteria. In moist or wet conditions of planting media, the fixing bacteria cannot enter the pores of the soil because they are aerobic, on the other hand, they can bind the nitrogen and form the optimal chlorophyll in the lack of water condition.

Based on the result of the research, it can be concluded that the physiological response of nine local varieties of cowpea of Southwest Maluku on drought stress significantly influence leaf number, relative water content, and chlorophyll content and physiological response differences among varieties can be used as indicator of sustainable selection for cowpea variety with drought tolerance. Providing water once every 10 days is the optimum time of drought stress on cowpeas. Through screening and other variable selection and multi-site testing, local cowpeas from Southwest Maluku can be developed to obtain local varieties tolerant to drought.

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Diversity of mung bean insect pests and their natural enemies in farmers' fields in East Java, Indonesia

SRI WAHYUNI INDIATI♥, BEJO, M. RAHAYU
Indonesian Legumes and Tuber Crops Research Institute. Jl. Raya Kendalpayak Km 8, PO Box 66, Malang 65101, East Java, Indonesia.
Tel.: +62-341-801468, Fax.: +62-341-801468, ♥email: swindiati@yahoo.com

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Abstract. *Indiati SW, Bejo, Rahayu M. 2017. Diversity of mung bean insect pests and their natural enemies in farmers' fields in East Java, Indonesia. Biodiversitas 18: 1300-1307.* A survey was conducted from June to September in 2016 in mung bean fields in East Java. The objectives of this survey were to identify the mung bean pests that were present, and to identify potential natural enemies of these pests at each location. Insect pests recorded in low numbers in all fields surveyed were bean fly (*Ophiomyia phaseoli*), thrips and grasshoppers. Pod sucking bugs (*Nezara viridula*, *Piezodorus hybneri*, and *Riptortus linearis*) were found in all fields in all months. The population densities of pod sucking bugs were relatively high, and represent a major threat to mung beans in Indonesia.White flies (*Bemicia tabaci*) and leafhoppers (*Empoasca* spp.) were found in low numbers in all fields surveyed during the August-September period. Several new pests of mung bean were recorded in this survey: the leaf beetle (*Megacopta cribraria*) was recorded in fields in the villages of Pungkurlawas, Brondong, Lamongan; and pod boring weevils (*Apion clavipes*, Curculionoidae) were found in high numbers in Madiun and Nganjuk, resulting in significant damage to mung bean pods. Natural enemies of insect pests recorded during the survey were spiders, dragonflies, Hymenoptera (Braconidae and Trichogrammatidae), Diptera (Tachinidae and Syrphidae), and Coleoptera (Coccinellidae and *Paederus fuscipes*). Natural enemies - spider and Coccinella predators - were present in low numbers in all fields surveyed, although Coccinella predators were not present in Lamongan.

Keywords: East Java, insect pests, mung bean, natural enemies

INTRODUCTION

In Indonesia, mung bean (*Vigna radiata* L.) is the third largest Leguminosae crop after soybean and peanuts. This plant is cultivated by farmers because the price is relatively stable and more competitive than soybean (Basuki et al. 2011). In 2011, the production of mung bean in Indonesia reached 341,342 tons of dry grains with an average of 1.148 t/ha (Directorate General of Food Crops 2012). Mung bean production still does not meet the increasing demand of the community. An average of 322,098 tons of mung bean are needed, while an average of only 313,229 tons of mung bean are produced each year, so the government has had to import about 29,443 t/year (BPS 2012).

Mung bean can be grown in a wide range of soil types, and can be grown throughout the year in Indonesia. Planting of mung bean in paddy fields is done in the dry season after the rice harvest, with the cropping pattern rice-rice-mung bean. In dryland fields, mung beans are planted at the end of the rainy season after upland rice or maize (Radjit and Prasetyawati 2012). Mung beans can be planted in dryland areas because it has a short life (matures quickly), is resistant to drought, and is less susceptible to pests than soybean (Radjit and Prasetyawati 2012). Various methods have been used to increase the production of mung beans, including system intensification, extensification, and land rehabilitation, as well as by reducing attack by pests and diseases (Radjit and Prasetyawati 2012).

Insect pests on mung bean can be classified into: (i) stem feeders, (ii) foliage feeders, and (iii) pod feeders (Tengkano 1986). At the seedling stage, agromyzid beanflies *Ophiomyia phaseoli* (Tryon), are the most common pest. *Ophiomyia phaseoli* larvae feed on the cortex within the stem and pupate in the cortex. Sometimes pupae can be seen under the membranous epidermis. The foliage feeders, especially defoliators that belong to Lepidoptera and Coleoptera, include: the leaf-folder, *Lamprosema indicata* (F.); caterpillars of *Spodoptera litura* (F.); hornworms, *Agrius convolvuli* (L.); ladybirds, *Henosepilachna* spp.; leaf beetles of the Chrysomelidae; and the grasshoppers, *Attractomorpha crenulata crenulata* (F.). The sap-feeding insects include *Aphis craccivora* Koch; the jassid, *Empoasca kerri* Pruthi; white flies, *Bemisia tabaci* Gennadius; thrips from the genus *Megalurothrips* Bagnall; the pod sucking bugs, *Riptortus* spp., *Nezara viridula* (L.), and *Plautia fimbriata* (F.); and pod bugs, *Clavigralla* spp. These pests cause significant damage to mung bean foliage and pods. Beetle of the genus *Mylabris* (F.) cause serious damage to the flowers, especially to the second and third flush of flowers during August-September, in most mung bean cultivation areas in Muneng, Prolinggo, East Java. The lepidopteran pod borer, *Maruca vitrata* (F.), has also been reported as a major pest of mung bean.

In Indonesia, some of the major pests that are considered harmful to mung bean crop are thrips, armyworm (*Spodoptera litura*), pod borer (*M. virata*), and pod sucking bugs (*Riptortus linearis* (F.), *Nezara viridula* (L.) and *Piezodorus hybneri* (Gmelin) (Baliadi 2007; Indiati 2015; Marwoto and Harsono 2008).

According to Kogan and Turnipseed (1987), pest status may be altered by one or a combination of several factors: changes in cultivation, changes in the efficiency of natural enemies (pathogens, parasitoids, and predators), changes in application frequency of insecticides, changes in the value of commodities that will change the value of the level of economic damage, and changes in planted varieties. Recording of insects associated with mung bean plants in some agricultural ecosystems in Indonesia is indispensable. Information on the composition of pest species and their natural enemies at various mung bean sites is vital as a basis for determining the policy of integrated pest management. In addition, such information is used to determine the priorities for research into pest control technology for mung beans. The objectives of the survey reported in this paper were to determine the mung bean pest status and to identify their natural enemies in sampled locations in East Java, Indonesia.

MATERIALS AND METHODS

Study area

The locations in East Java sampled for the survey of mung bean pests and their potential natural enemies, conducted in the dry season of 2016, are shown in Figure 1.

Procedures

The survey began at the end of May and continued until early September 2016. Sampling in the mung bean fields was accomplished using a sweep net (Ø = 40 cm). Three locations in each field (village) were swept.The first survey was conducted in Lamongan, Ngawi, Madiun, Nganjuk, and Mojokerto from the end of May until early June 2016. The second survey took place in Madiun at the end of August 2016. The latest survey was conducted in early September 2016 in four districts; Tuban, Ngawi, Madiun and Nganjuk. The districts, sub-districts and villages used as sampling sites are detailed in Table 1. Mung bean pest species and their natural enemies were counted *in situ*, and crop damage was assessed visually using a simple scoring method. Besides identification by direct observation, mung bean pest species and potential natural enemies of these pests were captured using five single swings of a sweep net in each location (15 single swings for three locations per sample site). Insects captured in the sweep net samples were put into pockets, killed with insecticide, dried in the sun, counted and identified under a stereo microscope (magnification 40 x), in the laboratory. Identification of insects and natural enemies was achieved by comparison with insect identification key books, preserved specimen samples or illustrated instructions (Boror 1976). If parasitoids emerged from insects, they were collected for

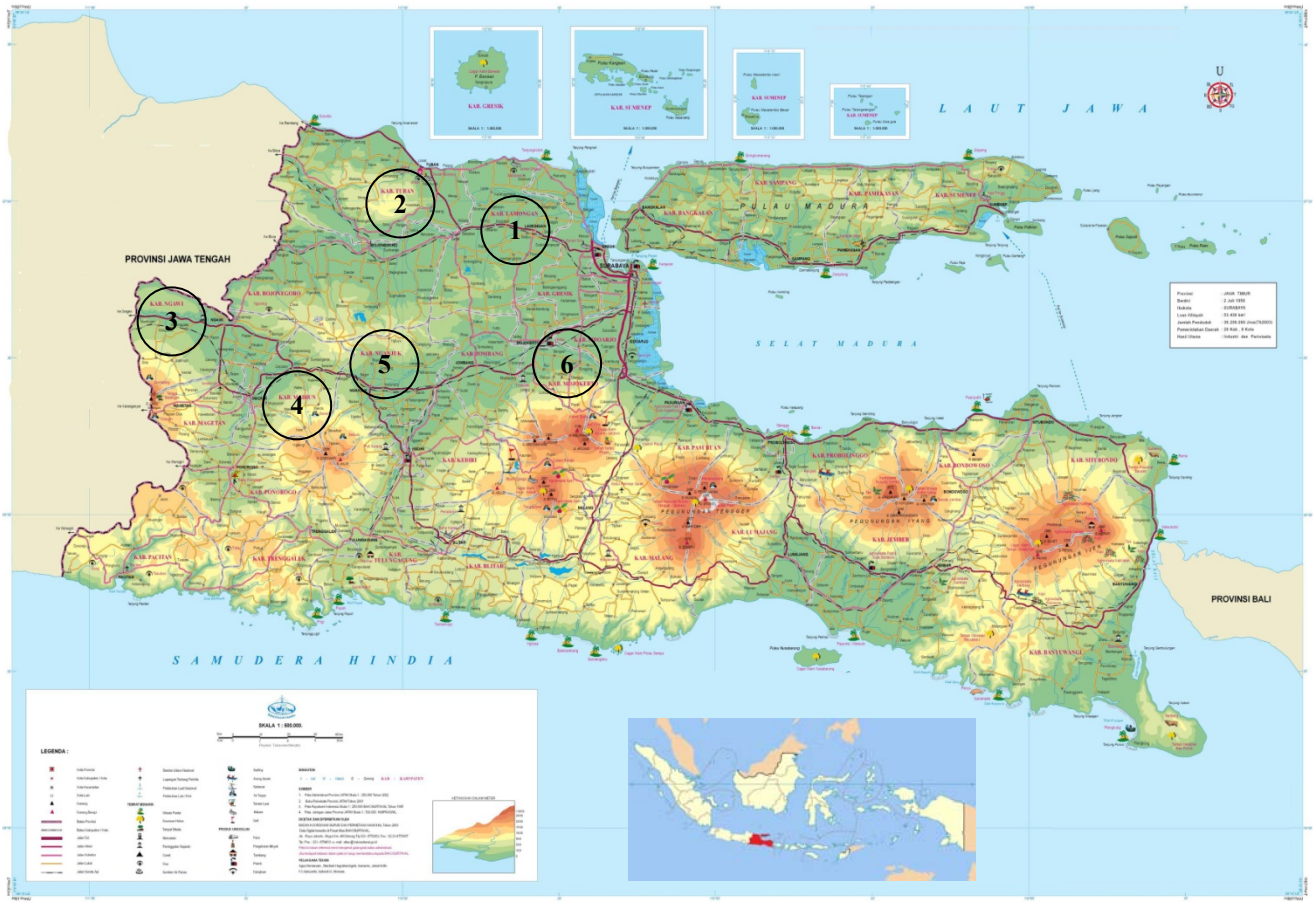


Figure 1. Sampling for the survey of mung bean insect pests and their natural enemies in East Java, Indonesia, was conducted in six areas, i.e. 1. Lamongan, 2. Tuban, 3. Ngawi, 4. Madiun, 5. Nganjuk, 6. Mojokerto

Table 1. The location of sampling sites in the mung bean pest survey of East Java in 2016

District	Sub-district	Village	Time of samping
Lamongan	Brondong	Pungkurlawas	Late May to early June
Tuban	Ranggel	Plumpung	Early September
Ngawi	Paron	Ngale	Early September
	Karangjati	Regundi	Late May to early June
Madiun	Balerejo	Simo	Late May to early June
	Saradan	Sugihwaras	End August
	Mejayan, Caruban	Mejayan	End August
	Madiun	Nglames	End August
	Pilangkenceng	Muneng	Early September
Nganjuk	Gondong	Balunggebang	Early September
	Wilangan	Wilangan	Early September
	Bagor	Selorejo	Late May to early June
	Rejoso	Ngangkatan	Early September
Mojokerto	Mojosari	Pekukuhan	Late May to early June

identification in the laboratory. The following data were recorded in the field for each survey location: name of county, district, village, host plant species, age of host plants, pest and disease control used by farmers (both insecticides and fungicides were used).

RESULTS AND DISCUSSION

Lamongan

In Lamongan, most farmers planted corn and rice. Mung bean was only found in Pungkurlawas village, Brondong. The mung bean was about 50 days old, planted as an intercrop with corn cv. Bisi-2. Both of them were planted at the same time. Farmers in this area generally did not take action to control pests and diseases, so many mung bean plants were found to be infected with leaf spot disease. In this area, we found the bean fly, *Ophiomyia phaseoli*, thrips, grasshoppers (*Atractomorpha crenulata* and *Xenocatantops* sp.), as well as the leaf eating bug *Megacopta cribraria* (F.) (Table 2). Spiders were the only potential natural pest enemies we observed in this area (Table 3).

Table 2. Species abundance data for insect pests found in mung bean crops in fields sampled in East Java. The survey was conducted in the dry season, 2016.

Sampling location	Abundance of pests captured in sweep net*																
	Rl	Ac	Nv	Pd	Acl	Th	Ud	Em	Kk	Lk	Bll	Co	Cl	Lt	Mr	A.crs	M.cr
Kab. Nganjuk																	
Kec. Bagor																	
Ds. Selorejo	4	11	15	7	21	2	-	-	-	-	-	-	-	-	-	-	-
Kec. Rejoso																	
Ds. Ngangkatan	-	-	-	4	-	27	4	34	124	1	-	3	-	-	-	-	-
Kec. Gondong																	
Ds. BalongGebang	-	-	-	-	-	2	3	29	-	1	6	17	-	-	-	-	-
Kec. Wilangan																	
Ds. Wilangan	-	-	2	-	-	4	3	107	-	-	6	13	-	-	-	-	-
Kab. Madiun																	
Kec. Balerejo																	
Ds. Sinan I	2	1	-	-	2	-	2	-	-	7	3	-	6	-	-	-	-
Ds. Sinan II	1	-	1	-	-	1	4	-	-	7	1	-	4	-	-	-	-
Kec. Pilangkuncung																	
Ds. Muneng	1	-	-	-	-	3	-	40	11	6	6	9	-	-	-	-	-
Kec. Saradan																	
Ds. Sugihwaras	-	-	-	-	-	-	-	28	-	14	6	-	-	-	-	-	-
Kec. Caruban																	
Ds. Mejayan	-	-	-	-	-	-	-	4	-	22	3	-	-	-	-	-	-
Kec. Madiun																	
Ds. Nglames	-	-	-	-	-	-	-	4	-	1	5	-	-	1	1	-	-
Kab. Mojokerto																	
Kec. Mojosari																	
Ds. Perkukuhan	-	-	16	-	-	3	1	-	-	16	-	-	-	-	-	1	-
Kab. Lamongan																	
Kec. Brondong																	
Ds. PungkurLawas	-	-	-	-	-	2-	-	-	-	17	3	2	-	-	-	-	2
Kab. Tuban																	
Kec. Rangel																	
Ds. Plumpung	-	-	-	1	-	5	-	28	8	2	6	3	-	-	-	-	-
Kab. Ngawi																	
Kec. Paron																	
Ds. Ngale	1	-	-	1	-	5	-	27	-	1	6	20	-	-	-	-	-

Notes: Rl=*Riptortus linearis*, Ac=*Anoplocnemis curvipes*, Nv = *Nezara viridula*, Pd=*Piezodorus*, Acl=*Apion clavipes*, Th=thrips, Ud=*Spodoptera litura*, Em=*Empoasca,* KK = whitefly, Lk= bean fly, Bll = Grasshopper, Cl =*Cletus sp*, Lt =*Longitarsus,* Mr=*Maruca virata*, A.crs =*Apoderus crenatus*, M.cr =*Megacopta cribraria,* Co =*Coleoptera*. * Number of each species per 15 single swings of the insect net; - = no insect found. Kab. = district, Kec. = subdistrict, Ds. = village

Table 3. Abundance of natural enemies of insect pests on mung bean crops in East Java. Dry season 2016

Sampling location	Species and population numbers of natural enemies*														
	Rv	H Pd	Lb	Tg	Br	Sy	Hm	Ich	Co	Tc	H Pr	Lb K	LHP	Cp	Pa
Kab. Nganjuk															
Kec. Bagor															
Ds. Selorejo	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Kec. Rejoso															
Ds. Ngangkatan	-	-	7	8	2	-	-	-	-	-	-	-	-	-	-
Kec. Gondong															
Ds. BalongGebang	-	-	9	7	14	1	-	-	-	-	-	-	-	-	-
Kec. Wilangan															
Ds. Wilangan	-	-	14	-	4	-	-	1	2	-	-	-	-	-	-
Kab. Madiun															
Kec. Balerejo															
Ds. Sinan I	-	-	4	-	-	-	1	-	1	-	-	-	-	-	-
Ds. Sinan II	-	-	2	-	1	-	1	-	-	-	-	-	-	-	-
Kec. Pilangkuncung															
Ds. Muneng	-	-	15	-	4	-	-	-	-	-	-	-	-	-	-
Kec. Saradan															
Ds. Sugihwaras	-	-	18	-	-	-	59	-	2	4	-	-	-	-	-
Kec. Caruban															
Ds. Mejayan	-	-	6	-	-	-	52	-	1	-	-	-	-	-	-
Kec. Madiun															
Ds. Nglames	-	-	5	-	-	-	17	-	3	1	-	-	-	-	-
Kab. Mojokerto															
Kec. Mojosari															
Ds. Perkukuhan	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-
Kab. Lamongan															
Kec. Brondong															
Ds. PungkurLawas	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
Kab. Tuban															
Kec. Rangel															
Ds. Plumpung	-	-	7	-	2	-	-	-	3	1	-	-	-	-	-
Kab. Ngawi															
Kec. Paron															
Ds. Ngale	-	-	6	-	3	-	-	-	3	-	-	-	-	1	-

Notes: Rv = *Reduviidae*, H Pd =*Hymenoptera* Predator, Lb = spiders, Tg = Trichogrammatidae, Br = Braconidae, LHP =spider black white, Sy =*Syrphidae*, Hm = *Heminoptera*, Ich =*Ichneumonidae*, Co = *Coccinellidae*, Tc =*Tachinidae*, H Pr=*Heminoptera* Parasite, Lb K = spider yellow, Cp =dragon flies, Pa =*Paederus*; * Number of each species type per15 single swings of the insect net; - = no species type found. Kab. = district, Kec. = subdistrict, Ds. = village

Tuban

In Tuban, the survey was conducted in early September. Mung bean crops were only found in the village of Plumpung, in sub-district Ranggel. At that time, the mung bean plants were in the vegetative phase and were planted as a monoculture. Mung beans grew well, so that farmers did not need to conduct pest control measures. In the area around the sampling location, most farmers planted corn.The pests that we found were Homoptera (*Empoasca* spp.), thrips, Hemiptera (*Piezodorus* spp.), grasshoppers (*A. crenulata),* and whitefly (*Bemisia tabaci*) (Table 2). Natural enemies that we found in this area were spiders, coccinellid beetles and parasitoids of the families Tachinidae and Braconidae (Table 3).

Ngawi

In Ngawi, sampling in the mung bean fields was carried out in early September. At this time, most farmers had planted corn and legumes. In general, pest control was rarely carried out by farmers in their mung bean fields. Observation of mung bean pests was conducted in the Experimental Garden of Ngale, Paron. Pest control is regularly carried out at this institution. The pests we found were grasshoppers (*A. crenulata*), Hemiptera (*Piezodorus hybneri; Riptortus linearis)*, Homoptera (*Empoasca spp.),* and thrips (order Thysanoptera) (Table 2). The natural enemies we found at this location were spiders, a dragonfly, coccinellid beetles, and Braconidae (Table 3).

Madiun

Observations of mung bean pests were conducted three times in Madiun. In early June, farmers were still planting rice. In the village of Simo, Balerejo sub-district, the farmers planted mung beans in monoculture. At that time, the plants were 40 days old. Farmers planted mung bean for seed for the next planting season (July). In this area we found grasshoppers, pod sucking bugs (*Nezara viridula, P. hybneri, R. linearis, Cletus sp.*), leaf-eating caterpillars

(*Spodoptera litura)*, bean fly, and thrips, all in low numbers (Table 2). We also discovered a new pest of mung beans: *Apion clavipes* Gerst. (Curculionidae). The second survey in Maduin district was conducted in late August 2016; samples were taken in three sub-districts Saradan, Caruban, and Madiun. In each sub-district, the mung bean crops sampled were between 30-days old and harvest. In this area we found grasshoppers, bean fly, and Homoptera (*Empoasca* spp), all in low numbers (Table 2). The third survey was conducted at the beginning of September 2016, with sampling conducted in Village Muneng, Sub-district Pilangkenceng. Most farmers in this area were planting rice, soybeans and mung beans at the time of the survey. Mung bean plants in the survey areas were 45 days old. Insect pests found during this third survey were grasshoppers (*A. crenulata),* whitefly *(B. tabaci)*, Coleoptera (*Longitarsus* sp.), Hemiptera (*M. testulalis*, *R. linearis, Empoasca* spp.), and thrips (Table 2). Natural enemies found based on all three survey visits in Madiun were spiders, Tachinidae, Braconidae, Hymenoptera, and coccinellid beetles (Table 3). In general, pest control was rarely carried out by farmers in these mung bean fields.

Nganjuk

Survey visits were carried out twice in four sub-districts Gondang, Bagor, Wilangan and Rejoso. In early June, most farmers were still planting rice in their fields. In June, sampling was carried out at Selorejo, Sub-district Bagor. In this village, we found one farmer's field had mung bean as a monoculture. Plants had reached the pod production stage, but the crop was not in good condition, having many pests and diseases (Figure 2). The population of pod sucking bugs and pod borers was relatively high, with many flowers falling, which was an indicator of thrip attack in the flowering phase. As a result, the extent of pod production was poor, and most pods were holey. Insects caught in the sweep net were *N. viridula, P. hybneri, R. linearis*, *Cletus* sp., *Anoplocnemes curvipes* (F.), thrips and *A. clavipes* (Curculionidae), in high number (Table 2). The pod borer weevil, *A. clavipes* (Curculionidae) is a new mung bean insect pest in this area and was found in high number, so that many mung bean pods were severely damaged. In the second survey, in early September 2016, samples were taken at three villages, Balunggebang, Ngangkatan and Wilangan. Around these sample locations, farmers' fields were mostly planted with rice in a cropping pattern of rice, soybeans and mung beans. Pests species found were leaf-eating caterpillars *S. litura*, whiteflies *B. tabaci*, *Empoasca* sp., *P. hybneri*, bean fly, thrips, leaf-eating beetles, grasshoppers *A. crenulata*, and pod-sucking bugs *N. viridula* (Table 2). Natural enemies found in Nganjuk were spiders, Tachinidae, Braconidae, Trichogrammatidae and Syrphidae (Table 3). Pest control of mung bean is rarely carried out in these mung bean fields.

Mojokerto

Observation of mung bean pests was conducted in the Mojosari Research Station (Pekukuhan village), in early June. Mung bean variety Vima-3 was planted in monoculture, and the plants were 35 days old. Pest control was done periodically at this institution. At this site, we found pod sucking bugs (*N. viridula, R. linearis*), leaf-eating caterpillars (*S. litura),* bean fly, thrips and leaf beetles *Apoderus crenatus* (Voss) (Table 2). Natural enemies of pests found were a spider, *Paederus fuscipes* (Curtis), and *Coccinella* (L.) beetles (Table 3)

Discussion

In general, a major pest of mung bean was found in each district surveyed (Table 2). The species composition of the main pest populations differed in each district. Differences in species composition can be attributed to differences in the distribution of host plants, the growth stage of host plants, pest control and cropping patterns in the area (Evans et al. 2013). Matsubayashi et al. (2011) consider that the specificity of a host as a feed source is one of the most important factors explaining insect diversity. Most pests have several species of host plants in one Family as a potential food source, but less than 10% of pest species have more than three plant Families as their potential hosts (Schoonhoven et al. 2005).

The pests species present in the mung bean fields in East Java were found to be: pod sucking bugs (*N. viridula, P. hybneri, R. linearis*), thrips, armyworms, whitefly, leafhoppers (*Empoasca* sp.), beans fly, bean pod borer (*M. virata)*, leaf-beetles and grasshoppers (*A. crenulata)*. Among these pests, pod sucking bugs were the most important pests in all mung bean field areas. Pod sucking bugs are an important pest and are polyphagous insects (Figure 3). These pests attack several species in the family Leguminosae, such as soybeans, mung beans, peanuts, pigeon pea, cowpea, common beans, alfalfa, *Crotalaria* sp., and *Sesbania rostrata* (Funayama 2006; Nakamura and Numata 2006). Pod sucking bugs are widespread in tropical and sub-tropical areas, including Indonesia, South Japan, America, Australia, and Europe (Musser et al. 2010; Temple et al. 2011; Wada et al. 2006). Pod sucking bugs cause economic damage as a result of a decreased ability for seeds to grow; a decrease in grain quality and yield; a decline in seed vigor because of tissue damage due to puncture of the seed; as well as a delay in the maturing of the plant (Leonard *et al.* 2011). According to Arifin and Tengkano (2010), nymph and adult stages of *P. hybneri* attack the plants at 42 days after planting (DAP), but the symptoms of the damage are observed at 63 DAP. Based on our observation of species composition, status, and regional spread of pests, mung bean cultivation in East Java appears seriously threatened, especially by pod sucking bugs, *N. viridula, P. hybneri* and *R. linearis,* such that insect population reduction or other preventative action needs to be carried out.

Another important outcome of this survey was the discovery, in the village of Pungkurlawas, Brondong, Lamongan, of a new mung bean pest species, *Megacopta cribraria* (F). Kudzu bug, *M. cribraria* (Hemiptera: Heteroptera: Plataspidae), is an insect native to Asia and Australia, previously known as the bean plataspid, lablab bug or globular stink bug (Eger et al. 2010). In October 2009, a large population of kudzu bug was found in the

walls of houses near the leguminous species kudzu, *Pueraria montana* Lour. (Merr.), in nine counties in northeast Georgia, USA. In August 2010, the insect was found in 48 counties in northeast Georgia and 13 counties in northwest South Carolina (Eger et al. 2010). Two years latter, *M. cribraria* populations had spread to most of Georgia, South Carolina and North Carolina (Zhang et al. 2012), with additional sightings in Virginia and Alabama (Gardner et al. 2013; Reisig and Bacheler 2013). Currently, kudzu bug populations have spread to all districts of South Carolina; most counties in Georgia and North Carolina; to Alabama, and most districts that grow soybeans in Virginia, Florida, Tennessee and Mississippi and in parts of Arkansas and Louisiana; and continue to spread every year (Gardner 2015). In Malang (East Java, Indonesia), this insect was found for the first time on soybean plants in 2013. Now, in 2016, this bug is reorded here as a new pest of mung beans, in Lamongan.



Figure 2. Insect samples were taken using a sweep net in a mung bean farmer's field at Nganjuk (*left*); mung bean crop damage by a complex of pests - including thrips, pod-sucking bugs, and pod-borers - as well as by plant diseases (*right*).



Figure 3. Pod sucking bugs (*Nezara viridula* and *Riptortus linearis*) are dominant pests on mung bean, and predator *Paederus fuscipes* were found in East Java

Kudzu bugs have a piercing-sucking mouth type. They are suspected of eating the phloem, reducing soybean yields by sucking stem, leaf petioles and leaves (Suiter et al. 2010; Thippeswamy and Rajagopal 2005), and by diverting nutrients and moisture away from plants' vegetative and reproductive growth (Waldvogel and Alder 2011). Kudzu bugs are commonly found forming aggregates on the main stem of soybean (Del Pozo-Valdivia 2016; Suiter et al. 2014). On soybean crops, kudzu bugs cause a significant reduction in crop yields (Blount et al. 2016; Del Pozo-Valdivia et al. 2016; Seiter et al. 2013). Studies in Southern and Central China show that severe pest attacks cause defoliation and disorders of photosynthesis as a result of sooty mold which grows on the excretion of *M. cribraria* (Zhang et al. 2012). Soybean yield losses due to damage from kudzu bug have been reported in the range of 1-50% (Eger et al. 2010). In addition, this pest has the potential to cause direct damage to the skin and eyes of humans from their defensive chemicals (Ruberson et al. 2013).

Another new finding from this survey was the recording of the pod borer weevil, *Apion clavipes* Gerst. (Curculionidea), in Madiun and Nganjuk. The population of this pest was so high that it resulted in damage to the mung bean pods. Occurrence of new pests should be anticipated and monitored at all times, in order to forestall economic damage to crops. Besides its occurrnce as a new pest on mungbean, this weevil has also been recorded on other host plant species i.e. blackgram, redgram, soybean and cashew (Umesha 2006). In India, *A. amplum* (Faust) (Apionidae: Coleoptera) is an important pest in mung beans and blackgram, and it may cause damage pods up to 49% (Deshmukh et al. 2007). In India, this insect is a major pod-borer pest of pigeon pea, and has caused high yield losses (Azad Thakur and Firake 2013; Dialoke et al. 2014; Sandip Patra et al. 2016)

Survey of pests and natural enemies in specific agroecosystems should be done periodically so that the fluctuations in pest populations, changes in pest status and pest dominance, and emergence of new primary pests can be identified quickly and accurately, and appropriate action taken.

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Use of endophytic bacteria from roots of *Cyperus rotundus* for biocontrol of *Meloidogyne incognita*

MARDHIANA, ANKARDIANSYAH PANDU PRADANA♥, MUH ADIWENA, DWI SANTOSO, RIZZA WIJAYA, ADITYA MURTILAKSONO

Program of Agrotechnology, Faculty of Agriculture, Universitas Borneo Tarakan. Jl Amal Lama No 1, Tarakan 77123, North Kalimantan, Indonesia, ♥email: pandupradana.id@gmail.com

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Abstract. *Mardhiana, Pradana AP, Adiwena M, Santoso D, Wijaya R, Murtilaksono A. 2017. Use of endophytic bacteria from roots of* Cyperus rotundus *for biocontrol of Meloidogyne incognita. Biodiversitas 18: 1308-1315.* Yield loss due to *M. incognita* infection in tomato plants cultivation can reach 60%. The problem is able to be solved through the application of endophytic bacteria. In this study, endophytic bacteria from root *Cyperus rotundus* were isolated using Tryptic Soy Agar media. The bacteria isolates were then tested their safety against plants and mammals. The phenotypic and physiological properties of selected isolates were characterized and tested to know their resistance to antibiotics, and their ability in suppressing the infection rate of *M. incognita* on tomato. Eighteen bacterial isolates were obtained and 8 of them are categorized as safe bacteria for plants and mammals, which could be used in further tests. A result of the physiological test showed that bacterial isolates were able to produce protease enzyme (87.5%), chitinase enzyme (62.5%), and HCN (37.5%), having urease activity (75%) and could dissolve phosphate (87.5%). Based on the test results, all endophytic bacteria effectively increased tomato growth and suppressed the severity of *M. incognita* infection with the most stable isolate as a biocontrol agent of *M. incognita* was CRS16.

Keywords: Antibiotic resistance, biosafety, lytic enzymes, plant growth

INTRODUCTION

Root-knot nematodes (RKN, *Meloidogyne* spp.) are one of plant pest, which has significant negative impact on the tropical area. They can have a wide host range and interact with other pathogens causing the yield loss in some agricultural commodities. Infestation of *Meloidogyne* spp. can reduce the production up to 40-60% on tomato, 35% on potato, and 20% on bean. Symptoms caused by infestation with *M. incognita* are indicated by gall at the root. The existence of gall can disrupt water and minerals distribution systems from the soil through the roots to all parts of the plant leading to the disruption of new root growth, plant wilting, slow plant growth, stunted growth, and chlorosis (Moens et al. 2009). *Meloidogyne* spp. Infestations can also increase the severity of the wilt disease caused by *Fusarium oxysporum* and *Ralstonia solanacearum* in tomato (Muthamia and Ravichandra 2012).

Researches on chemical, physical and mechanical control of nematodes had been conducted, but the nematodes infestation is still a major problem, which could not be fully resolved. One of efforts to control nematodes is using endophytic bacteria as biocontrol agents. According to Hardoim et al. (2015), endophytic bacteria are bacteria living in plant tissues without causing disease symptoms within the plant and can be isolated from sterilized surface of plant tissue. Action mechanisms of endophytic bacteria as biocontrol agents include the production of antimicrobial compounds, spatial and nutrients competition, micronutrient competition, the production of siderophores, and the induction of plant resistance against pathogen infestation.

Biological control using endophytic bacteria is one of the alternative controls, which can be expected to tackle the issue. The endophytic bacteria were reported to effectively control nematodes in several crops. Endophytic bacteria isolated from potato such as *Pseudomonas* and *Streptomyces* species were reported to be able to reduce the population of *Meloidogyne incognita* (Krechel et al. 2002). Application of endophytic bacteria through seed treatment could reduce 30-50% galls of *M. incognita* on tomato (Munif et al. 2013). Treatment of *P. chlororaphis* strain Sm3 in strawberries could reduce population of root-lesion nematode (*Pratylenchus penetrans*) by 41-61% and was able to enhance plant growth (Hackenberg et al. 2000).

The advantages of endophytic bacteria as biocontrol agents are their ability to increase the growth of plants, known as Plant Growth Promoting Bacteria (PGPB), by increasing the availability of nutrients, and also their ability to produce the growth hormone for inducing plant resistance, known as induced systemic resistance (ISR). ISR is an alternative mechanism to control the broad-spectrum plant diseases (Pieterse et al. 2014, Chebotar et al. 2015).

The endophytic bacteria were reported to have a broad host specificity. Mekete et al. (2009) have successfully isolated endophytic bacteria from coffee plant to control the root-knot nematodes on tomato. In addition, Mohanty et al. (2017) have also isolated endophytic bacteria from *Jatropha curcas* to promote the growth of maize.

Therefore, there are great opportunities to explore the endophytic bacteria from various plants. The species of plant that their endophytic bacterial effectiveness as a biocontrol agent has not been widely reported is *Cyperus rotundus*. Therefore, this study was aimed to isolate and characterize the most effective endophytic bacteria originated from *C. rotundus* roots as a biocontrol agent of *Meloidogyne incognita* on tomato.

MATERIALS AND METHODS

Study area

This research has been done in the laboratory and greenhouse experiment at Laboratory of Plant Nematology (Department of Plant Protection), and green house Faculty of Agriculture, Bogor Agricultural University, Bogor, Indonesia.

Isolation of endophytic bacteria

The endophytic bacteria were isolated from the roots of *C. rotundus*. Samples were taken around Bogor Agricultural University campus, Indonesia. Roots of plant were taken as much as 1 g, which was then washed using tap running water to remove soil particles. Roots surface were further sterilized using 1% sodium hypochlorite (NaOCl) solution for 1 minute, 70% alcohol for 1 minute, and rinsed with sterile distilled water 3 times. Subsequently, the roots were dried using a sterile tissue paper. Samples were further placed on the Tryptic Soy Agar (TSA) media to determine the success of surface sterilization.

Sterile root samples were then macerated using a sterile mortar until smooth with the addition of 1: 10 (w/v) distilled water. The suspension was diluted 10-fold using sterile distilled water. A total of 0.1 mL 10^{-3} dilution suspension was further spread on 20% TSA media using sterile microbiology glass beads then incubated at 28°C for 72 hours. Single colonies with different colony shape, size, texture, and color were further sub-cultured in respective media (100% TSA), followed by the incubation for 48 hours.

Hypersensitivity reactions test

The endophytic bacteria were grown on TSA media using 9 cm diameter petri dish and incubated for 24 hours. The growing bacteria were then harvested using 2 mL of sterile distilled water. The suspension was further infiltrated on the leaf blade of tobacco (Kemloko 3 variety) at the bottom and incubated for 48 hours. The occurrence of necrosis on leaves of tobacco plants was observed. Bacteria that did not show necrosis on leaves of tobacco were used for further testing (Klement and Goodman 1967).

Hemolysis activity test

Endophytic bacteria with no indication of hypersensitivity reaction were used in this test. The bacteria were grown on blood agar media, incubated for 24 hours. The formation of hemolysis zones was observed. α-hemolysis toxin produced by endophytic bacteria would form a dark zone, while β-hemolytic toxin would form a light zone, and αβ-hemolysis toxin will form a zone of light followed by a bit dark around the colony. Bacteria with hemolytic activity were not used in the further test (Tille 2015).

Characterization of phenotypic and physiological properties of endophytic bacteria

Phenotypic characters of endophytic bacteria colonies

The phenotypic performance of Endophytic bacteria colony was observed based on the shape of the colony, size, texture, color, and elevation.

Gram staining

Endophytic bacteria Gram staining was conducted using Himedia Gram-stain kit. 24-hour-old bacterial colonies were taken using a needle loop and placed on a slide surface contained a drop of sterile water. The suspension of bacteria on the slide was flattened and passed through the bunsen burner.

Crystal violet was dripped over the preparation of bacteria that had been fixed, left to stand for 1 minute and then washed with distilled water and left for a while. Iodine solution was dripped on the dried preparations, left to stand for 1 minute and washed with 96% alcohol until the solution flow was not colored. Preparations were washed with distilled water and left for a moment. The next step, dried preparations were dripped with safranin, left to stand for 1 minute and washed again using distilled water. Observations were conducted after preparations were dried, using a light microscope with a magnification of 1000x. Gram-positive bacteria bound the crystal violet after washing with alcohol so that preparations would be dark violet after staining and Gram-negative bacteria did not bind the crystal violet so they would be red after staining (Beveridge 2001).

Proteolytic activity

Proteolytic activity was tested using skim milk media at pH 6.5. Media composition consisted of 10 g of skim milk, 15 g of TSB, 7.5 g of agar and 500 mL of distilled water. Agar, TSB and distilled water were sterilized by autoclaving at 121°C for 15 minutes. After sterilization was finished, skim milk was further added when the media was still hot. Bacterial cultures were streaked on the media added to skim milk. Proteolytic activity was indicated by a clear zone around the colonies of bacteria, 48 hours after treatment (Sokol et al. 1979).

Chitinolytic activity

Test medium used was 1% chitin, consisted of 15 g of agar, 5 g of glucose, 2 g of peptone, 10 g of colloidal chitin, 0.5 g of K_2HPO_4, 0.5 g of MgSO4, 0.5 g of NaCl in 1 L of distilled water with a pH of 6.2. Bacterial cultures were streaked onto the surface of the medium and incubated at room temperature for 4 days. Chitinolytic activity was observed with the clear zone around the bacterial streak (Kuddus and Ahmad 2013).

HCN production

The medium used to grow the bacteria consisted of 4.4 g of glycine, 30 g of TSB, 15 g of agar in 1 L of distilled water. The medium was poured into a 9 cm diameter petri dish. Preparation of cyanide detection solution (CDS) paper was performed using 2 g of picric acid and 8 g of sodium carbonate, then dissolved in 200 mL of sterile distilled water. The solution was used to soak the 1x1 cm sterilized filter paper. The filter paper was soaked up to bright yellow; then the filter paper was dried in a laminar air flow.

The endophytic bacteria were streaked at test medium. Filter paper which had been dipped in a solution of CDS was attached to the inside of the petri dish lid. The bacteria were incubated for 7 days at room temperature, and then on the 7th day of treatment, the color filter paper was observed. Bacteria that can produce cyanide was detected by a color change on filter paper from yellow to orange-brown (Lorck 1948).

Urease activity

The test was performed on NFB semi-solid medium with pH of 6.8. The compositions of this medium were 0.5 g of malic acid, 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.1 g of NaCl, 0.02 g of $CaCl_2.2H_2O$, 2 mL of the micronutrient, 2 mL of Bromthymol blue, 4 mL of Fe (III) EDTA (1.64%), 1 mL of vitamins solution, and 0.5 g of agar. The micro-nutrient solution used in this medium were conducted with the composition of 0.4 g of $CuSO_4.5H_2O$, 0.12 g of $ZnSO_4.7H_2O$, 1.4 g of H_3BO_3, 1 g of $Na_2MoO_4.2H_2O$, 1.5 g of $MnSO_4.H_2O$, in 1 L of distilled water. Vitamins solution was conducted with the composition of 10 mg of biotin, 20 mg of Pyridoxol HCL in 1 L of distilled water.

The test was performed by growing 1 mL of the bacterial suspension with the density of 10^8 cfu mL^{-1} in 9 mL of NFB semi-solid media and incubated for 48 hours. Bacterial ability in fixing nitrogen was indicated by a color change, turned blue or dark blue, and mucus or pellicle layers on the surface of the media appeared (Baldani et al. 1986).

Phosphate dissolving activity

The test was performed using Pikovskayas Agar medium (Himedia, India). Bacterial cultures were streaked onto the surface of the medium and incubated at room temperature for 48 hours. Bacterial ability in dissolving phosphate was indicated by the clear zone around the bacterial streak (Sharma et al. 2011).

Antibiotic resistance test

24-hours-old endophytic bacteria isolates were grown in TSB medium containing (25, 50, 75, and 100) µg mL^{-1} of chloramphenicol, amoxicillin and rifampicin antibiotics. If the media color turned murky then the isolates were resistant to antibiotics with the tested doses. The test was repeated 3 times (Vibhaw et al. 2017).

Propagation of *Meloidogyne incognita* inoculum

M. incognita used as materials was taken from the collection of Plant Nematology Laboratory, Department of Plant Protection, Bogor Agricultural University. The nematode was reproduced on Tantyna F1 variety of tomato plants.

Effect of filtrate culture of endophytic bacteria on nematode egg hatching

The endophytic bacteria were grown in 100 mL of TSB medium and shaken for 24 hours at room temperature. After 24 hours, the suspension of the endophytic bacterial was centrifugated at 12000 rpm for 15 minutes. Furthermore, the supernatant was filtered using 0.22 µm and diameter of 25 mm sterile syringe filter. The filtered supernatant was used to test the ability of endophytic bacteria on egg hatching of *M. incognita*.

M. incognita eggs isolated from extract of the roots were sterilized by soaking in a solution containing 600 ppm of Streptomycin sulfate for 20 minutes then washed and soaked in sterile water for 30 seconds. About 0.5 mL (containing 50 eggs) of suspension of *M. incognita* eggs was added into 5 mL of endophytic bacterial filtrate and incubated at room temperature for 48 hours. As a control, the eggs were added into 5 mL of sterile distilled water. After incubation, the eggs were washed with sterile water and incubated at room temperature for 14 days.

This experiment used a completely randomized design (CRD) with bacterial culture filtrate as fixed factor and each isolate was repeated 3 times. The percentage of eggs hatching indicating nematodes was out of the cuticle (the egg protectors) was recorded. Data were then analyzed using DSAASTAT program version 1.021 (Siddiqui and Shaukat 2003).

Effect of filtrate culture of endophytic bacteria on mortality of *M. incognita* J2

The test of the effect of bacterial filtrate on *M. incognita* was conducted by taking a 5 mL of bacterial culture filtrate and put into a small petri dish (diameter of 6 cm) then 0.5 mL of the suspension of *M. incognita* juvenile (containing 100 juveniles) was added and stored at room temperature for 24 hours. This test used a completely randomized design (CRD) with bacterial culture filtrate as fixed factor and repeated 3 times. As a control, sterile water and TSB were used to prove that *M. incognita* juveniles died because of bacterial filtrate. Observations were conducted on the dead and living *M. incognita*. The dead *M. incognita* were characterized by straight shape and did not move after 2 hours in sterile water. The data were then analyzed using the DSAASTAT program version 1.021 (Siddiqui and Shaukat 2003).

Selection of endophytic bacterial as RKN controlling agent and plant growth promoter

The endophytic bacteria were grown in TSB medium and incubated for 48 hours. F1 Tantyna tomato variety seeds sterilized using NaOCl 1% for 1 minute and sterile distilled water were soaked in the suspension of endophytic bacteria for 12 hours. Then, tomato seeds were sown in sterile soil and after four main leaves appeared, the seeds were moved and planted in pots with a diameter of 15 cm. Planting was conducted in a completely randomized design

(CRD) with three replications, each test consisted of two test plants. Afterward, tomato plants were watered with 100 mL of the bacterial suspension with a density of 10^8 cfu mL^{-1} at the first, 2nd, and 5th week. Watering was conducted around the base of the stem. Watering was applied in the afternoon at 16.30.

M. incognita infestation was performed 1 month after tomato plants were moved. Total *M. incognita* infested to the plants was 500 nematodes in the infective stage (J2). Measurement of observation variables was conducted at 40 days after inoculation. Agronomic variables included height, root length, both fresh and dry weight of plants. Pathological variables included the number of nematodes per 5 g of roots and the number of gall at the root. Data analysis was performed at the end of the observation using analysis of variance at the 5% level confidence. If they are significantly different then Duncan Multiple Range Test (DMRT) at 95% level confidence was applied. The analysis was performed using DSAASTAT program version 1.021 (Munif et al. 2013).

RESULTS AND DISCUSSION

Biosafety of endophytic bacteria

Eighteen isolates of endophytic bacteria were isolated from the roots of *C. rotundus*. Among those bacteria isolates, 33.33% (6 isolates) of them caused necrosis based on hypersensitivity reaction test. Therefore, those isolates were not used in the next test. The remained isolates (12 isolates) were tested for their safety using hemolysis test. The hemolysis test showed that 16.66% of endophytic bacteria were able to produce β-hemolysis toxins, and other 5.55% were able to produce α-hemolysis toxins. Bacteria with the indication of both negative hypersensitivity test and negative toxic hemolysis test were used in the further test. The biosafety test showed that eight isolates from plant roots of *C. rotundus* could be used in the advance test (Table 1).

Phenotypic characters and endophytic bacterial Gram

Among 8 isolates of endophytic bacteria, 100% of colonies were circular of shape. Colony size of endophytic bacteria is varied; the observations showed that 12.5% of punctiform, 37.5% of small, 37.5% of moderate and 12.5% of large size. The texture of endophytic bacterial colonies consisted of 2 types, smooth and rough. 25% were rough, and 75% were smooth. There were five colors of endophytic bacterial colonies, white (50%), yellow (12.5%), brown (12.5%), red (12.5%), and green (12.5%). Furthermore, elevation of endophytic bacteria colonies consisted of 4 types; those were flat (25%), raised (50%), convex (12.5%), umbonate (12.5%). Based on the observations, 37.5% of endophytic bacteria were Gram-positive, and 62.5% were Gram-negative (Table 2).

Physiological characteristics of endophytic bacteria

Five physiological activities were tested included proteolytic, chitinolytic, production of HCN, urease activity and phosphate dissolving activity. The tests showed that 87.5% of bacteria had proteolytic activity, 62.5% of bacteria had activity chitinolytic, 37.5% of bacteria were able to produce HCN, 75% of bacteria had urease activity, and 87.5% of bacteria were able to dissolve phosphate (Table 3).

Table 1. Hypersensitive reaction and hemolysis activity of endophytic bacterial isolates originated from roots of *C. rotundus*

Isolate code	Hypersensitive reaction	Hemolysis activity
CRS01	-	β
CRS02	-	-
CRS03	-	-
CRS04	+	x
CRS05	-	β
CRS06	+	x
CRS07	+	x
CRS08	+	x
CRS09	-	-
CRS10	+	x
CRS11	-	-
CRS12	-	-
CRS13	-	α
CRS14	-	-
CRS15	-	β
CRS16	-	-
CRS17	-	-
CRS18	+	x

Note: (+) endophytic bacterial isolates tested caused necrosis on tobacco leaf in hypersensitivity test, (−) endophytic bacterial isolates tested did not cause necrosis on tobacco leaf in hypersensitivity test dan had no hemolysis activity, (α) endophytic bacterial isolates tested could produce α-hemolysis toxins in blood agar media, (β) endophytic bacterial isolates tested could produce β-hemolysis toxins in blood agar media, (x) endophytic bacterial isolates were not tested in hemolysis test because they were detected to cause necrosis in hypersensitivity test on tobacco plant.

Table 2. Phenotypic and Gram characteristics of endophytic bacterial isolates originated from roots of *C. rotundus*

Isolate code	Shape	Size	Texture	Color	Elevation	Gram
CRS02	Circular	Small	Smooth	White	Flat	Negative
CRS03	Circular	Moderate	Smooth	White	Raised	Positive
CRS09	Circular	Small	Rough	Brown	Flat	Negative
CRS11	Circular	Small	Smooth	White	Raised	Negative
CRS12	Circular	Moderate	Smooth	Red	Umbonate	Positive
CRS14	Circular	Moderate	Rough	Yellow	Raised	Negative
CRS16	Circular	Large	Smooth	White	Convex	Positive
CRS17	Circular	Punctiform	Smooth	Green	Raised	Negative

Endophytic bacterial resistance to antibiotics

In general, endophytic bacteria tested for resistance varied to three types of antibiotics. Most of the endophytic bacteria had antibiotic resistance in the concentration of 25 µg mL^{-1} up to 50 µg mL^{-1}. Results of test on the antibiotic chloramphenicol with concentration of 25 µg mL^{-1}, 50 µg mL^{-1}, 75 µg mL^{-1}, and 100 µg mL^{-1} were 100%, 100%, 37.5% and 0%, respectively. Furthermore, the results of the test on amoxicillin antibiotic with the concentration of 25 µg mL^{-1}, 50 µg mL^{-1}, 75 µg mL^{-1}, and 100 µg mL^{-1}, respectively were 100%, 62.5%, 25% and 0%. Another antibiotic tested was rifampicin, with the results in the concentration of 25 µg mL^{-1}, 50 µg mL^{-1}, 75 µg mL^{-1}, and 100 µg mL^{-1} were 100%, 100%, 50%, and 0%, respectively (Table 4).

Effectiveness of endophytic bacteria as biocontrol agents against *M. incognita* on tomato plants

Filtrate cultures of endophytic bacterial were able to inhibit eggs hatching of *M. incognita* up to 87.33% (CRS09), 85.33% (CRS12), 78.66% (CRS11), 69.33% (CRS03), 67.33% (CRS17), 61.33% (CRS14), 52% (CRS16), 49.00% (CRS02). Furthermore, the same filtrate cultures were also able to kill *M. incognita* J2 by 85% (CRS09), 81.67% (CRS12), 76.66% (CRS11), 74.33% (CRS14), 74% (CRS17), 72% (CRS03), 65% (CRS02), and 60% (CRS16) (Table 5).

Table 3. Physiological characteristics of endophytic bacterial isolates originated from roots of *C. rotundus*

Isolate code	Proteolytic activity	Chitinolytic activity	Production of HCN	Urease activity	Phosphate dissolving activity
CRS02	+	-	+	+	+
CRS03	+	-	+	+	+
CRS09	+	+	-	+	+
CRS11	+	+	-	+	-
CRS12	+	+	-	+	+
CRS14	-	+	-	-	+
CRS16	+	-	-	-	+
CRS17	+	+	+	+	+

Note: (+) endophytic bacterial isolates had physiological activities and were able to produce compounds tested, (−) endophytic bacterial isolates had no physiological activities and were not able to produce compounds tested

Table 4. Resistance of endophytic bacteria originated from roots of *C. rotundus* on chloramphenicol, amoxicillin, and rifampicin antibiotics on various concentrations.

Isolate code	Chloramphenicol (µg mL^{-1})				Amoxicillin (µg mL^{-1})				Rifampicin (µg mL^{-1})			
	25	50	75	100	25	50	75	100	25	50	75	100
CRS02	+	+	+	-	+	+	+	-	+	+	+	-
CRS03	+	+	-	-	+	+	-	-	+	+	-	-
CRS09	+	+	+	-	+	-	-	-	+	+	+	-
CRS11	+	+	+	-	+	-	-	-	+	+	-	-
CRS12	+	+	-	-	+	+	-	-	+	+	-	-
CRS14	+	+	-	-	+	-	-	-	+	+	+	-
CRS16	+	+	-	-	+	+	+	-	+	+	+	-
CRS17	+	+	-	-	+	+	-	-	+	+	-	-

Table 5. Effectiveness of filtrate culture of endophytic bacterial originated from roots of *C. rotundus* in inhibiting eggs hatching and killing *M. incognita* J2 in vitro.

Isolate code	Inhibition of eggs hatching of *M. incognita* (%)	Mortality of *M. incognita* J2 (%)
CRS02	$49.00^{e} \pm 6.24$	$65.00^{de} \pm 2.65$
CRS03	$69.33^{c} \pm 1.15$	$72.00^{cd} \pm 7.94$
CRS09	$87.33^{a} \pm 4.93$	$85.00^{a} \pm 4.36$
CRS11	$78.66^{b} \pm 1.53$	$76.66^{bc} \pm 2.08$
CRS12	$85.33^{a} \pm 2.08$	$81.67^{ab} \pm 2.08$
CRS14	$61.33^{d} \pm 1.53$	$74.33^{bc} \pm 3.79$
CRS16	$52.00^{e} \pm 3.00$	$60.00^{e} \pm 6.24$
CRS17	$67.33^{c} \pm 3.79$	$74.00^{bc} \pm 2.65$
Control	0^{f}	0^{f}

Note: Values followed by different superscript letters are significant at $P \leq 0.05$ over control

In general, applications of endophytic bacteria in tomato plants infested with *M. incognita* had positive effects. Plants treated with endophytic bacteria had higher plant height compared with plants untreated with the endophytic bacteria. Differences in height on the plant treated and control were 32.38% (CRS03), 32.41% (CRS02), 39.75% (CRS17), 44.78% (CRS09), 46.30% (CRS11), 46.54% (CRS14), 48.70% (CRS12), and 59.78% (CRS16). In addition to plant height, all of endophytic bacteria also had a positive effect on the growth of roots. The roots of plants treated with endophytic bacteria were longer by 39.07% (CRS09), 41.16% (CRS17), 43.26% (CRS16), 43.54% (CRS02), 46.90% (CRS14), 47.03% (CRS11), 49.20% (CRS03), and 51.31% (CRS12). On the other hand, all isolates were able to increase the fresh weight of the plant. The bacteria that were able to increase the fresh weight of the plants were CRS03 (10.47%), CRS09 (12.19%), CRS16 (20.25%), CRS12 (22.41%), CRS11 (23.17%), CRS02 (25.19%), CRS14 (29.28%), and CRS17 (30.11%). Furthermore, endophytic bacteria were also able to increase plant dry weight. The isolates, which effectively increased plant dry weight were CRS02 (24.83%), CRS12 (24.95%), CRS16 (25.92%), CRS17 (29.70%), and CRS14 (31.45%).

All endophytic bacteria tested were also able to effectively reduce the number of *M. incognita* in the roots of tomato plants and reduce the number of gall caused by the infestation of *M. incognita* (Figure 1). Reduction of *M. incognita* in the roots occurred at 25.30% (CRS12), 26.51% (CRS11), 35.54% (CRS17), 39.16% (CRS03), 42.77% (CRS02), 45.18% (CRS09), 49.40% (CRS14), and 58.43% (CRS16). Endophytic bacteria isolates were also able to reduce the number of gall formed by 24.38% (CRS12), 29.80% (CRS11), 34.44% (CRS17), 37.58% (CRS03), 38.76% (CRS09), 38.76% (CRS09), 39.67% (CRS02), 41.70% (CRS14), and 56.67% (CRS16).

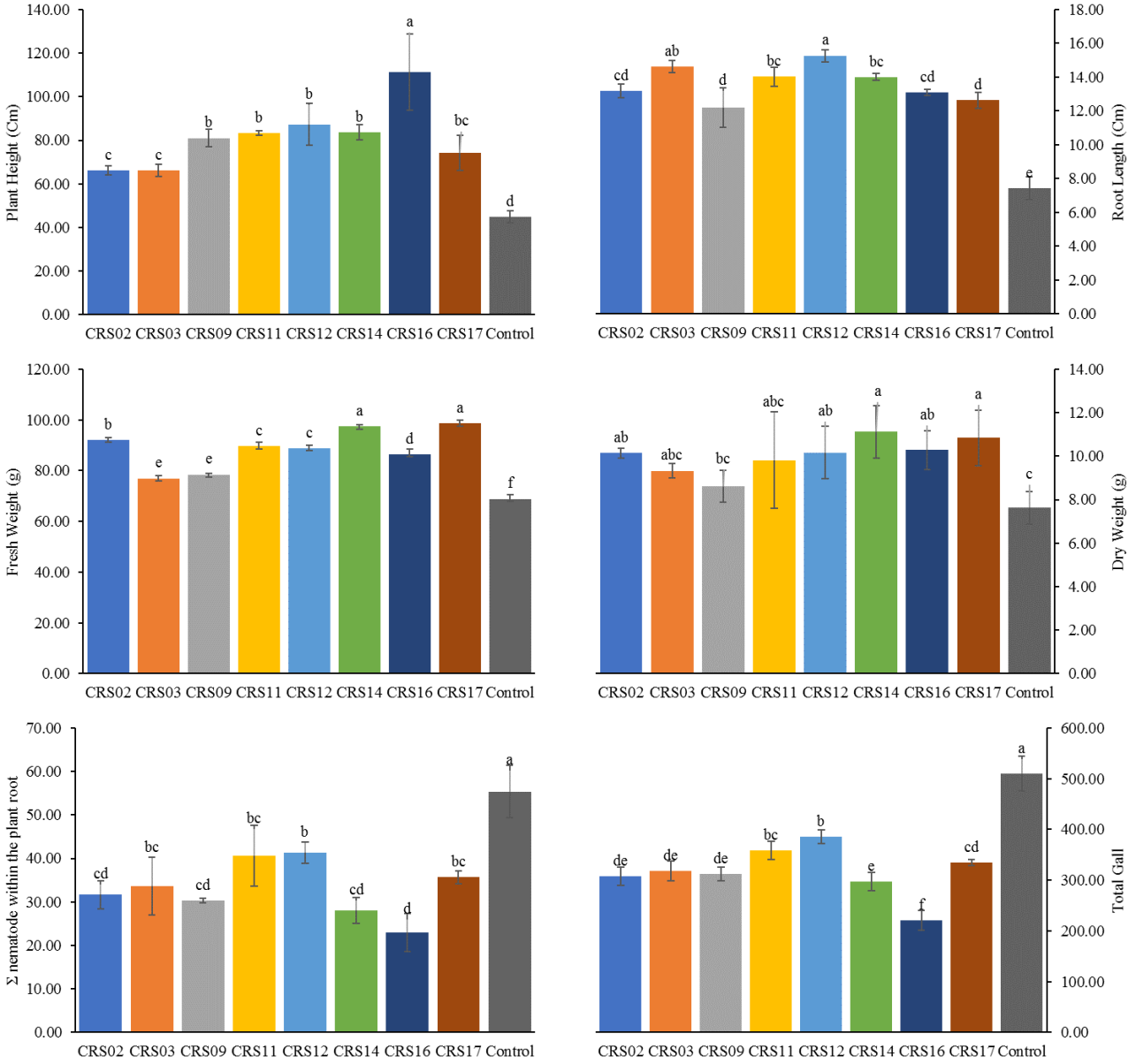


Figure 1. Growth of tomato plant infested with *M. incognita* and treated using endophytic bacteria from roots of *C. rotundus* and number of *M. incognita* and gall on each test plant. Note: values followed by different letters are significantly different from control

Discussion

The endophytic bacteria are known to be associated with almost all part of the plants, including the roots, stems, leaves, and seeds. In terms of the life cycle of endophytic bacteria, all or part of the life cycle was in the plant tissue. Not all the bacteria living within the plant tissues are beneficial to the host plant. Some endophytic bacteria are neutral, and some are pathogenic (Hardoim et al. 2015). The bacteria caused necrosis of hypersensitivity test show that these bacteria are pathogenic to plants. Hypersensitivity reactions are response of plants to pathogens infection in plant tissue that is an attempt to inhibit the growth of pathogens. Hypersensitivity reactions are influenced by *hrp* gene commonly found in plant pathogenic Gram-negative bacteria, such as *Xanthomonas* sp. groups (Zhu et al. 2000). Hypersensitivity reactions are part of the cell death program that occurs very fast and localized. The cellular membrane on the leaves of tobacco plants that have contact with pathogenic bacteria will be destroyed, undergoing drying and necrosis (Klement and Goodman 1967). Safety of bacteria as biocontrol agents is also determined from their effects on mammals. The bacteria producing hemolysis toxin cannot be used as a biocontrol agent. Hemolysis toxin is highly cytotoxic to humans granulocytes, monocytes, and lymphocytes (Herlax and Bakas 2002).

Each bacteria can have the same or different characters from other bacteria. Phenotypic character is expression of the interaction between genes and the environment. The same bacteria grown in different environments can show different phenotypic characters. Pathogenic bacteria *Ralstonia solanacearum* , for example, is white turbid in Nutrient Agar media, but the colony will turn red when it is grown on media containing tetrazolium chloride antibiotics (Williamson et al. 2002). The diversity of phenotypic characters of endophytic bacteria colonies observed in this study is the result of the interaction between genes and the environment.

As biocontrol agents, endophytic bacteria can act directly or indirectly. Endophytic bacteria can directly control pathogens by producing antagonistic enzymes against pathogens. The protease and chitinase enzymes are reported to have a nematicidal effect on root-knot nematodes. HCN produced by endophytic bacteria is also reported to be able to kill nematodes. Endophytic bacteria can indirectly increase plant resistance through induced systemic resistance (ISR) mechanism. Some studies have also stated that the endophytic bacteria were capable of fixing nitrogen from the environment, and dissolving phosphorus. The abilities are closely related to the mechanisms of plant growth promoting on endophytic bacteria (Brader et al. 2014, Chebotar et al. 2015, Ma et al. 2016).

As biocontrol agents, endophytic bacteria can be applied in a vast environment. Such capabilities are indicated from how bacteria survive on the stress of antibiotics. Agricultural environment, especially in developing countries has been contaminated by a wide variety of active compounds of pesticides (De Bon et al. 2010). The bacteria which are able to survive in media containing antibiotics is expected to survive well in the environment (Myresiotis et al. 2012).

Extracellular protease enzyme produced by the endophytic bacteria has an important role in controlling some species of plant pathogens. Tian et al. (2007) reported that the production of extracellular protease by bacteria is one of the mechanisms of bacteria as control agents of root-knot nematodes *Meloidogyne* spp. In addition to the protease, other enzymes such as chitinase, lipase, catalase, also have a role in the antagonistic activity of endophytic bacteria against nematodes. Chitinase has an important role in nematode control because this enzyme is able to degrade the middle layer of *M. Incognita* eggs (Van Nguyen et al. 2007). Cronin et al. (1997) described that chitinase can inhibit eggs hatching of *Globodera rostochiensis* up to 90%. This explains why the hatching of nematode eggs in this study may be delayed, and the cause of death of *M. incognita* J2 treated with filtrate cultures of endophytic bacteria. This also explains why the number of *M. incognita* in the roots and the number of gall on tomato plant roots are less than the control plants.

Several growth variables of tomato plants treated with endophytic bacterial showed better results than the control due to the activities of endophytic bacteria that are able to fix nitrogen and dissolve phosphorus. Some species of endophytic bacteria in symbiosis with plants are known to be able to fix nitrogen from the environment. Elbeltagy et al. (2001) successfully isolated the endophytic bacteria of wild rice, which the bacteria showed the ability to fix nitrogen. The bacteria are then inoculated on rice seedlings. Rice seedlings treated with endophytic nitrogen-fixing bacteria showed better growth than the control. Phosphate solvent bacteria are able to produce organic acids, such as citric, glutamate, succinate, lactate, oxalic, glyoxylic, malate, fumarate, tartrate, and α-ketobutyric. Such organic acids have an important role in the process of dissolving phosphate that is difficult to dissolve in the medium and in the soil (Mohammadi 2012). Manzoor et al. (2017) reported that some phosphate solvent bacteria are able to promote the growth of corn plants.

This study provides new information that endophytic bacteria originated from roots of *C. rotundus* have potential as a biocontrol agent. Endophytic bacteria are able to reduce the number of *M. incognita* in the roots of plants and are able to reduce the number of gall on the roots of tomato plants infested with *M. incognita*. Each isolate had activities of proteolytic, chitinolytic, and was able to produce HCN, fix nitrogen and dissolve phosphate. In general, CRS16 isolate has the best performance and stable as a biocontrol agent of *M. incognita* on tomato plants.

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Carapace length-weight and width-weight relationships of *Scylla serrata* in Bulungan District, North Kalimantan, Indonesia

BAMBANG WIDIGDO[1,♥], RUKISAH[2], ASBAR LAGA[2], AGUS A. HAKIM[1], YUSLI WARDIATNO[1]

[1]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jl. Raya Darmaga, Kampus IPB, Bogor 16680, West Java, Indonesia. Tel./Fax. +62-251-8622932, ♥email: bbg_widigdo@yahoo.co.id

[2]Faculty of Fisheries and Marine Sciences, Universitas Borneo Tarakan. Jl Amal Lama No 1, Tarakan 77123, North Kalimantan, Indonesia

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Abstract. *Widigdo B, Rukisah, Laga A, Hakim AA, Wardiatno Y. 2017. Carapace length-weight and width-weight relationships of* Scylla serrata *in Bulungan District, North Kalimantan, Indonesia. Biodiversitas 18: 1316-1323.* The mud crab, *Scylla serrata* lives associated with mangrove ecosystems and is widely distributed in the Indo West-Pacific. The present work was aimed to reveal the carapace length/width-weight relationships of *Scylla serrata* in two mangrove ecosystem area of Bulungan District, North Kalimantan-Indonesia, i.e. Kahayan Delta and Tibi Island. The crabs were collected by using *ambau pancang* (trap) for two months from November to December 2016. A total of 522 individuals were collected and their carapace length, carapace width, and weight were measured. The sex ratio of total collected samples (male to female) was 1:0.79. Carapace length and width of *S. serrata* caught in these two study area ranged from 40 to 89 mm for male; 32 to 91 mm for female and 59 to 128 mm for male; 53 to 122 mm for female, respectively. The carapace length-weight relationships of total samples showed logarithmic equations as follow: W = -3.2194 + 2.9725 Log CL for male and Log W = -2.2010 + 2.3750 Log CL for female. While the carapace width-weight relationships are as follow: W = -3.9162 + 3.1012 Log CW for male and Log W = -2.6001 + 2.3968 Log CW for female. The b values of carapace width-weight relationship indicated that the growth pattern of male was positive allometric and female was negative allometric. Size frequency of crab population is also presented.

Keywords: Allometric relationship, crustacea, growth pattern, mangrove, mud crab

INTRODUCTION

The mud crab, *Scylla serrata* is known to live associated with mangrove ecosystems and is widely distributed in the Indo West-Pacific. The mud crab is a nocturnal organism and is mostly burrowing during low tide (Hill 1976; Barnes et al. 2002), but they will be very active to forage when the habitat is covered by seawater at high tide (Nirmale et al. 2012). The crab is abundant in estuaries and swamps water with dense of mangrove vegetation (Keenan et al. 1998; Barnes et al. 2002). Genus *Scylla* is a commercially important species of the Indonesian crustaceans and has become a high economic value commodity (Cholik 2005). Increasing of the mangrove crabs demand can threaten existence of the population. So, the biological information is needed as a basis for utilization and management of mud crab resources.

One of potential location to catch mud crabs is mangrove ecosystem in Bulungan District, North Kalimantan-Indonesia. The ecosystem is located between 2°26.5'-3°45' North latitude and 117°18'-117°58' East longitude. The local fishermen catch the crab by using a local trap, so-called *ambau pancang*. Up to now, there were only a few researches on mud crab of North Kalimantan, e.g. resource potential (Natanael and Wiharyanto 2016), meroplankton of *Scylla* sp. (Triana and Wiharyanto 2016), and reproductive biology (Farizah 2011; Iromo et al. 2013; Iromo and Farizah 2014).

The study of carapace width-weight relationships assumes an important prerequisite in biology, physiology and ecology (Froese 2006; Mohapatra et al. 2010; Thirunavukkarasu and Shanmugam 2011). The estimation is useful to determine the variations in expected weight from the known length groups which are one after another the indication of size, reproduction and nourishing states (Gayathre et al. 2016). Application of this study for aquatic animals has done in delineating the growth patterns during their developmental pathways (Bagenal 1978). Information about individual carapace width-weight relationship is important to estimate the population size or stock structure (Josileen 2011) and to use the stock assessment model (Moutopolos and Stergiou 2002). Some crustacean research used the carapace width-weight relationship as more suitable for evaluating populations (Atar and Sector 2003; Gorce et al. 2006, Sangun et al. 2009). The present work was aimed to reveal the carapace width-body weight relationship of mangrove mud crab (*Scylla serrata*) in two area of mangrove ecosystem in Bulungan District, North Kalimantan, Indonesia. The result is compared with the different population of the same species in other locations.

MATERIALS AND METHODS

Study site and sampling methods

The samples were collected on November until December 2016 from Kahayan Delta and Tibi Island, Bulungan District, North Kalimantan Province, Indonesia (Figure 1). Totally 522 samples were caught using *ambau pancang* (local name) by fishermen. The specimens were transported to the laboratory for identification and measurement of carapace width and weight. Identification of species was based on FAO book identification (Ng 1998) and Keenan et al. (1998). Carapace Length (CL) was taken as the distance between the tips of the posterior most lateral carapace spines. Carapace Width (CW) was measured dorsally along the midline, between the frontal notch and the posterior margin of the carapace (Josileen 2011). Individual crab weight (W) was measured by digital scales. All samples were analyzed in fresh condition (living crabs).

Data analysis

The sex ratio was calculated using the formula: total number of males (∑M)/total number of females (∑F). The ratio was tested to see if it differs from a 50/50 ratio by Chi-Square test (Steel and Torrie I980). The carapace length/width-weight relationship was estimated using the exponential equation $W = aL^b$ (Ricker 1975). The exponential equation was transformed to logarithmic equation log W = log a + b log L, where W = weight, L = carapace width, a = 'y'-intercept and b = the slope or growth coefficient. The R-squared was determined to identify the association degree of two variables. Growth pattern was determined by value of growth coefficient (b value). The pattern was defined as follows: isometric growth (b = 3), or positive allometric (b > 3) or negative allometric (b < 3). The differences of the carapace width-weight relationship between sexes were tested by one-way ANOVA at p = 0.05.

RESULTS AND DISCUSSION

Results

A total of 522 mud crabs were sampled in two locations, 291 samples from Kahayan Delta and 231 samples from Tibi Island. The sex of mud crabs was determined in accordance to Keenan et al. (1998) and showed in Figure 2. The shape of the abdomen or tail flat is a thin triangular apron for males, while the female's is larger.

Sex ratios of male to female of total collected sample and each location (Kahayan Delta and Tibi Island) were 1:0.79, 1:0.82 and 1:0.76, respectively. Chi-Square test of sex indicated a significantly different (p < 0.05), meaning that the population was not in balance between of the number on male and female crab the two locations. Detail information of the sex ration at the two location is presented in Table 1.

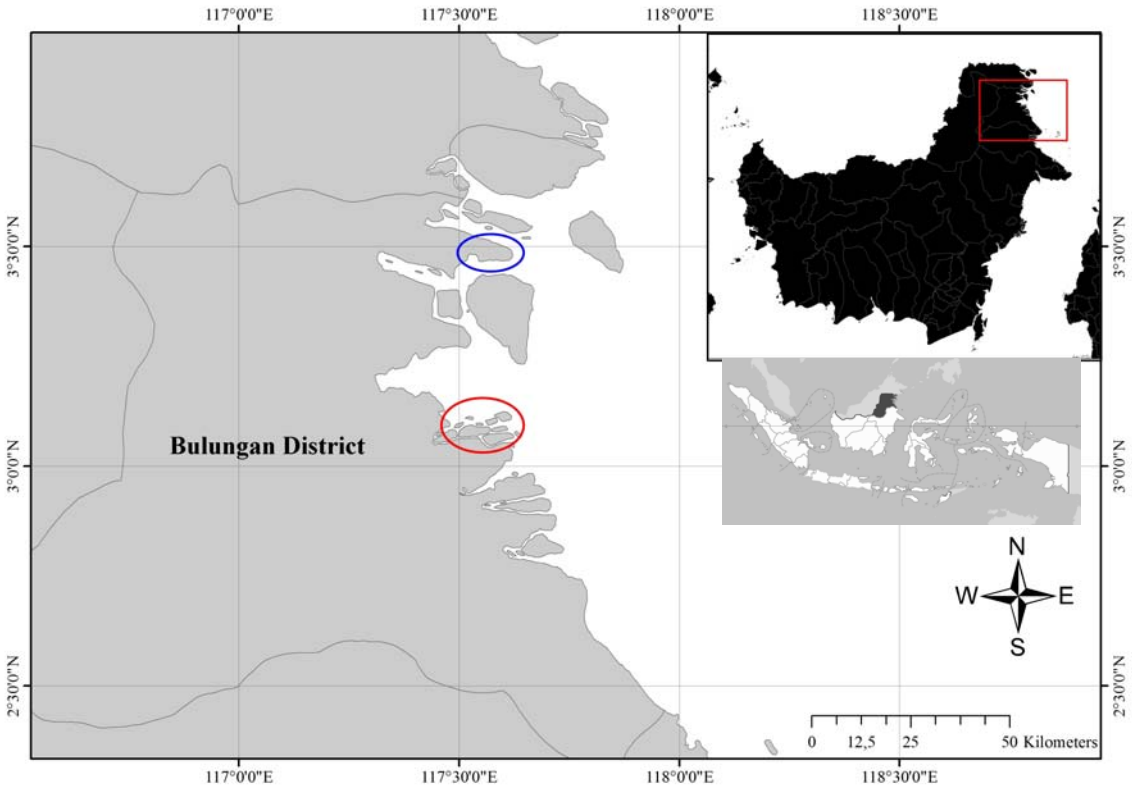


Figure 1. Locations where the mud crabs were collected. The red ellipse indicates Kahayan Delta and the blue ellipse indicates Tibi Island, Bulungan District, North Kalimantan, Indonesia



A **B**

Figure 2. Sex of mud crabs (A) male, (B) female. The shape of the abdomen or tail flat is a thin triangular apron for males, while the females is larger

Tabel 1. Sex ratio of *Scylla serrata* from Bulungan District, North Kalimantan, Indonesia

Location	Sex	Number of samples	Carapace Length (mm)		Carapace Width (mm)		Sex ratio
			Min	Max	Min	Max	
Total	Male	291	40	89	59	128	1: 0.79
	Female	231	32	91	53	122	
Kahayan Delta	Male	159	40	89	59	128	1: 0.82
	Female	131	32	89	53	122	
Tibi Island	Male	132	40	87	62	127	1: 0.76
	Female	100	43	91	61	122	

In total carapace length of *S. serrata* caught in the two study area ranged from 40 to 89 mm for male and 32 to 91 mm for female. In terms of location, the carapace length of the crab ranged from 32 to 89 mm in Kahayan Delta and 40 to 91 mm in Tibi Island (Table 1). The minimum size of carapace length was found in Kahayan Delta and the maximum size was found in Tibi Island. Size frequency distribution of carapace length is shown in Figure 3. Based on the figures, male and female crabs exhibited some size variation. The maximum size of *S. serrata* was found in 84-89 mm CL for male and 90-95 mm CL for female. No male was found with carapace length more than 89 mm. The situation is the same with Tibi Island, but different with Kahayan Delta. In Kahayan Delta, no male and female were found more than 89 mm. The dominant size of *S. serrata* in total, Kahayan Delta, and Tibi Island were found 60-65 mm for male and 54-59 mm for female.

Carapace width of *S. serrata* caught in the two study area ranged from 59 to 128 mm in male and 53 to 122 mm in female. While, the carapace width ranged from 53 to 128 mm in Kahayan Delta and 61 to 127 mm in Tibi Island (Table 1). The minimum and maximum size of carapace width were found in Kahayan Delta. Size frequency distribution of carapace width is shown in Figure 4. Base on the figure, male and female crabs exhibited some size variation. The maximum size group of *S. serrata* was found in 127-133 mm CW for male and 120-126 mm CW for female. No female was found with carapace width more than 133 mm. The condition occurred in both locations. The dominant size of *S. serrata* in total, Kahayan Delta, and Tibi Island were 92-98 mm for male, but it was not the case for female. The total and Kahayan Delta for female were dominated by 78-84 mm sizes, but it was 71-77 mm in Tibi Island.

A Scatter diagram was composed by plotting logarithm of carapace length/width against logarithm of weight of individual crabs (Figure 5 and Figure 6). The logarithmic equation log W = log a + b log L was used to estimated carapace length/width-weight relationship of mud crabs. The carapace length-weight relationship results of total showed logarithmic equations are as follows: W = -3.2194 + 2.9725 Log CL for male and Log W = -2.2010 + 2.3750 Log CL for female. While, the result of carapace Length-weight relationship in Kahayan Delta and Tibi Island are-3.0117 + 2.8570 Log CL for male and Log W = -1.9701 + 2.2455 Log CL for female, and Log W = -3.4634 + 3.1081 Log CL for male and Log W = -2.6013 + 2.6017 Log CL for female. The R-squared obtained for the carapace length and weight of males and females were nearly equal to 1 (0.83-0.91). The value indicated a high degree of positive correlation of length and weight for male and female crabs.

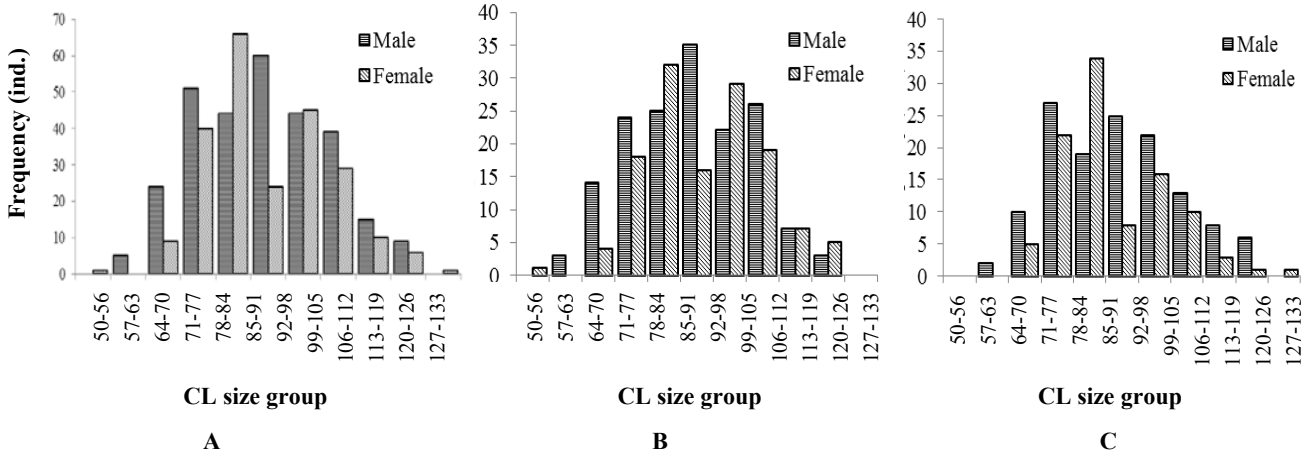


Figure 3. Size frequency distribution of carapace length of *Scylla serrata* in different location (A) total, (B) Kahayan delta, (C) Tibi island, Bulungan District, North Kalimantan, Indonesia

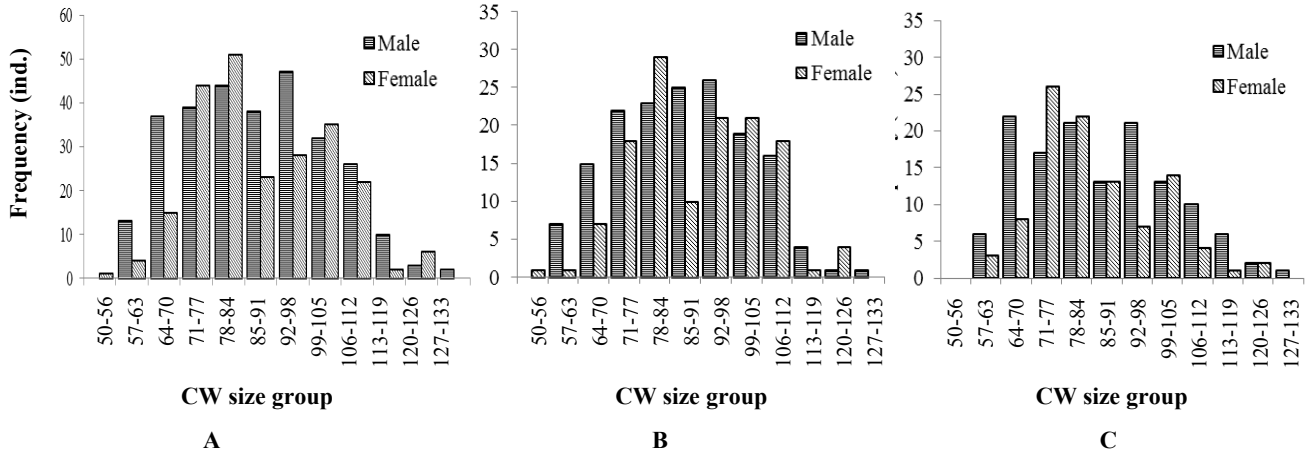


Figure 4. Size frequency distribution of carapace width of *Scylla serrata* in different location (A) total, (B) Kahayan Delta, (C) Tibi Island, Bulungan District, North Kalimantan, Indonesia

The carapace width-weight relationship results of total showed logarithmic equations are as follows: W = -3.9162 + 3.1012 Log CW for male and Log W = -2.6001 + 2.3968 Log CW for female. While, the result of carapace width-weight relationship in Kahayan Delta and Tibi Island are-3.6917 + 2.9809 Log CW for male and Log W = -2.6134 + 2.3982 Log CW for female, and Log W = -4.1882 + 3.2474 Log CW for male and Log W = -2.7538 + 2.4840 Log CW for female. The R-squared obtained for the carapace width, and weight of males and females were nearly equal to 1 (0.86-0.92). The value indicated a high degree of positive correlation of width-weight for both sexes.

The growth pattern was estimated by b value from carapace width-weight relationship. The growth coefficient values (b) of male and female width-weight were 3.1012 and 2.3968. Based on the values, we found b value more than 3 for male indicating a positive allometric. It means the increase of weight is faster than the increase of carapace width. But, it was not the case in female with b value less than 3. The value indicates negative allometric of growth pattern. The b values were different between male and female crab (ANOVA, $P < 0.05$).

Discussion

The information on sex ratio is important in the interpretation of the population structure and reproductive performance in crustaceans (Cheewasedtham 1990; Wardiatno 2004; Wardiatno and Mashar 2010). In our study, sex ratio showed that male is excessive than female in Bulungan District, North Kalimantan. The result of the present study agreed with other studies by Akter (2003), Ali et al (2004), Ikhwanuddin et al. (2010) and Musa et al. (2017). The dominance of males over females was proved to occur frequently in crab populations (Warburg et al. 2012).

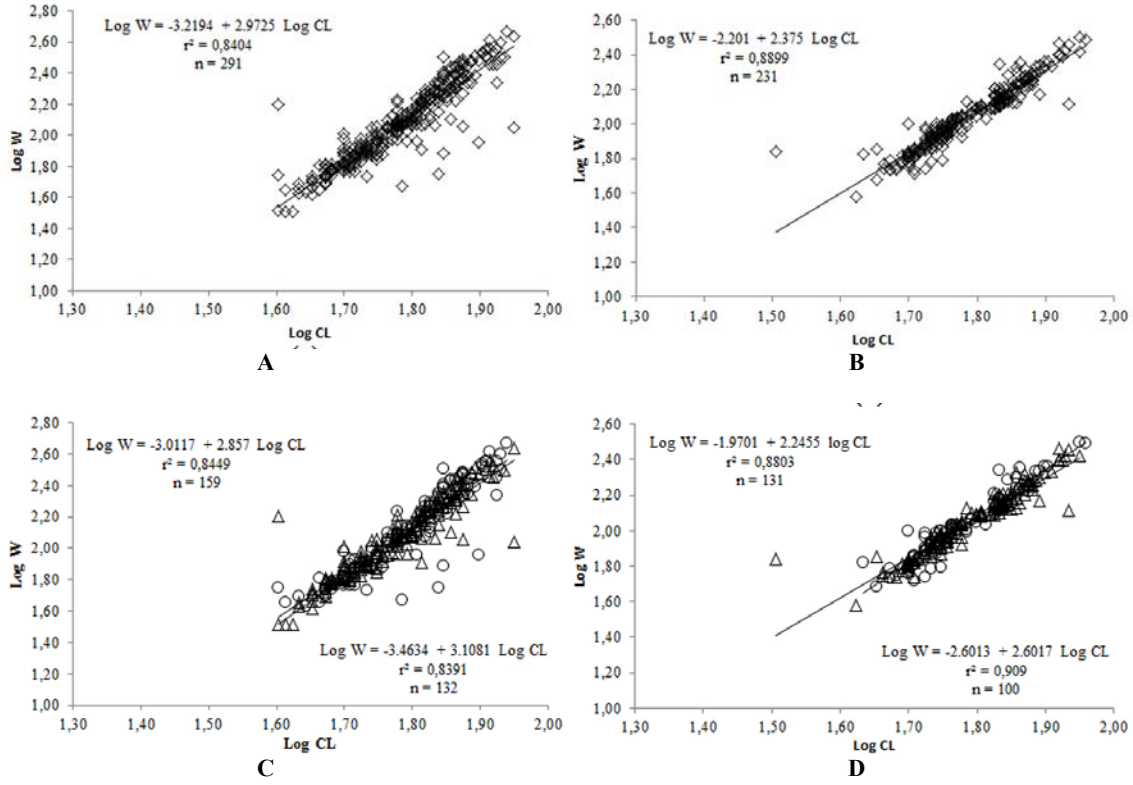


Figure 5. Carapace length (CL)-weight (W) relationship of *Scylla serrata.* (A) total male n = 291, (B) total female n = 231, (C) male, ○: Kahayan Delta n = 159; Δ: Tibi Island n = 132 (d) female, ○: Kahayan Delta n = 131; Δ: Tibi Island n = 100

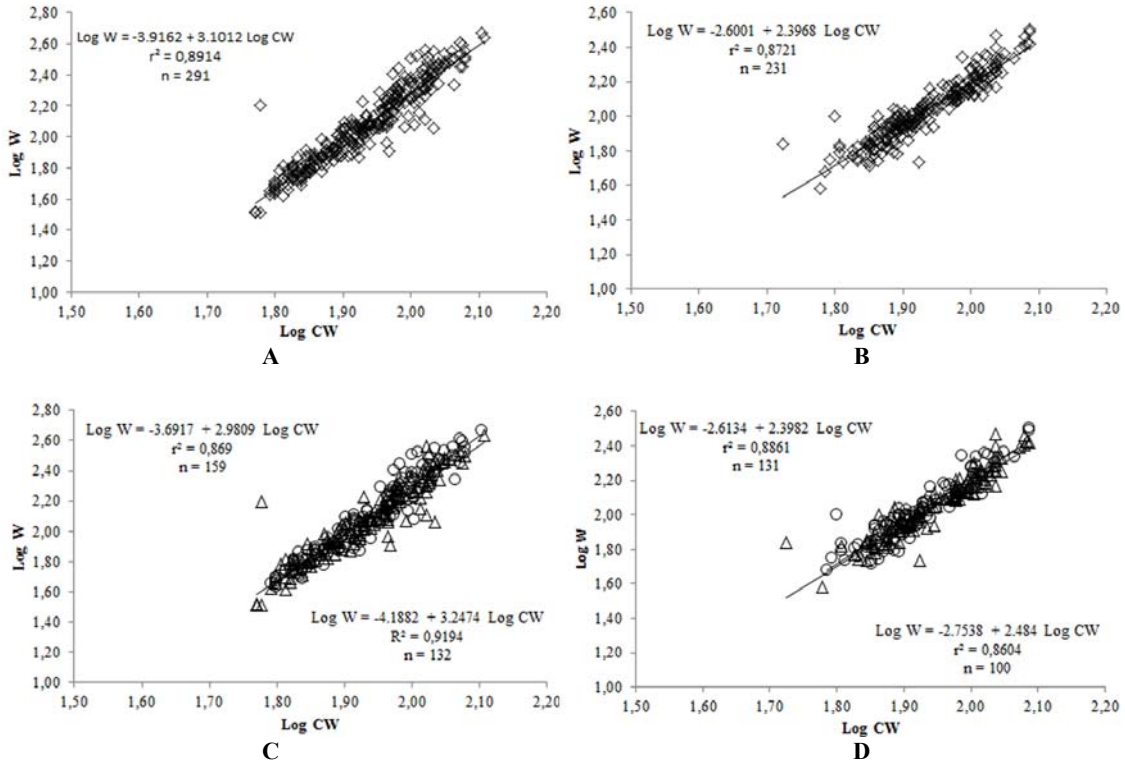


Figure 6. Carapace width (CW)-weight (W) relationship of *Scylla serrata.* (A) total male n = 291 (B), total female n = 231, (C) male, ○: Kahayan Delta n = 159; Δ: Tibi Island n = 132 (d) female, ○: Kahayan Delta n = 131; Δ: Tibi Island n = 100

Table 2. The growth coefficient values of *Scylla serrata* and *S. tranquebarica* in some locations.

Species	Sex	Location	b value	Reference
S. serrata	Male	Chilika Lagoon, India	3.2150	Mohapatra et al. (2010)
	Female		2.7510	
	Male	Inhace Island, Mozambique	3.0244	Toivio (2015)
	Female		2.4345	
	Male	Khulna region, Bangladesh	3.0600	Ali et al. (2004)
	Female		1.8928	
	Male	Bulungan district, Indonesia	3.1012	Present study
	Female		2.3968	
S. tranquebarica	Male	Chilika Lagoon, India	3.0060	Mohapatra et al. (2010)
	Female		2.7820	
	Male	Parangipettai coastal, India	3.2718	Thirunavukkarasu and Shanmugam (2011)
	Female		3.0202	

In terms of size the crabs collected from Kahayan Delta was smaller than that from Tibi Island. This phenomenon might be caused by the discrepancies of habitat conditions in both locations (Tahmid et al. 2015a, 2015b). Similar result was also found in east Kutai (Wijaya et al. 2010), that have crabs size smaller than in mangrove habitat or the sea. In fishery point of view the smaller size may also be due to higher effort in capture fisheries (Sharpe and Hendry 2009).

The b value of carapace width-weight relationship ranged from 2.3968 for female and 3.1012 for male. According Allen (1938), an ideal organism which maintains its shape throughout, the value of 'b' will be '3'. But in few of organisms, the value of 'b' lies between 2.5 and 4.0 (Martin 1949). The b value of male crab was higher than female crab. Higher b value of male crabs was observed in other research of *S. serrata* in India (Mohapatra et al. 2010), Andaman Sea (Poovachiranon 1992), and Bengkulu (Herliany and Zamdial 2015). Other brachyuran species had estimated to have higher b value in males than female (Josileen 2011; de Araújo and de Lira 2012). The male has the b value more than 3, but the female has the b value less than 3. This condition indicated the growth pattern is different for male and female. The growth pattern male is positive allometric and female is negative allometric. The growth is influenced by internal and external factors. Sex, age, nutrition and disease resistance include of internal factors, while water quality, predator, and habitat include of external factors (Effendie 1997; Levay and Walton 2007).

By comparing with some studies of *S. serrata,* the carapace width-weight relationship also showed similar results in the b value. The b value of male (more than 3) was higher than that of female (less than 3) (see Table 2). The similar results were found in some locations such as: India (Mohapatra et al. 2010), Mozambique (Toivio 2015), and Bangladesh (Ali et al. 2004). This biological character was also discovered in *S. tranquebarica*. The b value of male in *S. tranquebarica* was higher than that in female in India (Mohapatra et al. 2010; Thirunavukkarasu and Shanmugam 2011). The relationship between carapace width and weight showed that the growth pattern indicated faster and larger in male. It is common phenomenon since the claws of male grows significantly larger than female in mud crabs. Pinheiro and Fiscarelli (2009) mentioned that bigger claw in male is enabled them to protect the females successfully during and after copulation. Pinheiro and Hattori (2006) revealed that females tend to reduce their chelae growth as they grow bigger, while males tend to do the opposite. Mature females utilize much of their energy into egg production and cause reducing their somatic growth (Pinheiro and Fransozo 2002; Ferkau and Fischer 2006).

Study of carapace width-weight relationship is important in fishery biology. If data of size frequency is available, it can be used to convert length to weight in order to estimate biomass (Effendie 1997; Froese 2006). In addition, it can be purposed for proper conservation and management of mud crabs (Ali et al. 2004).

In conclusion, the sex ratio of total collected samples (male to female) was 1:0.79. Crab size of Kahayan Delta was smaller than that of Tibi Island. The carapace length-weight relationship of total samples showed logarithmic equations as follow: $W = -3.2194 + 2.9725$ Log CL for male and Log $W = -2.2010 + 2.3750$ Log CL for female. While the carapace width-weight relationships are as follow: $W = -3.9162 + 3.1012$ Log CW for male and Log $W = -2.6001 + 2.3968$ Log CW for female. Based on the b values of carapace width-weight relationship, the growth pattern of male was positive allometric and female was negative allometric.

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Short Communication: New record of wild banana (*Musa balbisiana* Colla) in West Kalimantan, Indonesia

ARI SUNANDAR
Program of Biology Education, Faculty of Teaching Training and Education, Universitas Muhammadiyah Pontianak. Jl. A. Yani No. 111, Pontianak 78124, West Kalimantan, Indonesia. Tel./Fax. +62-561-764571, email: arisunandar@unmuhpnk.ac.id, arisunandar031@gmail.com

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Abstract. *Sunandar A. 2017. Short Communication: New record of wild banana (*Musa balbisiana *Colla) in West Kalimantan, Indonesia. Biodiversitas 18: 1324-1330.* Kalimantan Island (Indonesia) is part of the center of origin and diversity of wild bananas in Indo-Malesia region. West Kalimantan is one of province in Kalimantan Island. However, only a little information is available about diversity of wild bananas in West Kalimantan. The aims of this study were to study a possible new record of wild banana species in Nipah Panjang and Teluk Nibung Villages, Kubu Raya District and to describe its phenetic relationships among wild banana species based on morphological characters. By examination based on the morphological character, one species of wild banana was identified, i.e. *Musa balbisiana* found in Teluk Nibung Village (Local name: Pisang Klotok). However, we didn't found any wild bananas in Nipah Panjang Village. *M. balbisiana* was a new record in West Kalimantan. Previously, *M. balbisiana* is widely distributed only in Java and Sulawesi. *M. balbisiana* was found in roadsides or backyards of residents in Teluk Nibung Village. Dendrogram of phenetic relationship showed that *M. balbisiana* from West Kalimantan had similarity with *M. balbisiana* from Sulawesi with coefficient of similarity 80%.

Keywords: Musa balbisiana, pisang klotok, Teluk Nibung, West Kalimantan, wild banana

INTRODUCTION

Musaceae family consists of three genera, namely *Musa* L, *Musella* C.Y. Wu and *Ensete* Horan (Nasution and Yamada 2001). The genus of *Musa* is divided into three groups, namely giant bananas such as *M. ensete*, edible bananas viz. *M. paradisiaca*, *M. sapientum* and *M. troglodytarum*, and ornamental bananas which have certain morphological characteristics such as upright inflorescences and brightly coloured bracts i.e. *M. ornata*, *M. sanguinea* and *M. coccinea* (Sagot 1887). In 1893, Baker divided the genus of *Musa* into three subgenera: *Physocaulis* with bottle-shaped stem and inedible fruits, *Eumusa* having cylindrical stem and edible fruits, and *Rhodoclamys* having cylindrical stem, non edible fruits, and brightly colored bracts (Baker 1893). In 1947, based on number of chromosomes, Cheesman divided the *Musa* into four sections: *Australimusa* (x=10), *Callimusa* (x=10), *Eumusa* (x=11), and *Rhodochlamys* (x=11) (Cheesman 1947). In general, *Musa* is grouped into seeded bananas (wild), which consists of approximately 70 species (Häkkinen 2008) and seedless bananas (edible bananas), which consists of approximately 500 cultivars (Valmayor et al. 2002).

Indonesia is the center of origin of bananas (Simmonds 1966) and also the center of diversity (Daniells et al. 2001). Approximately, more than 325 cultivars of bananas were found in Indonesia (Valmayor et al. 2002) and 12 wild banana species have been documented (Nasution and Yamada 2001). The wild banana species can be found in all islands in Indonesia, such as Sumatra, Java, the Lesser Sunda Island, Kalimantan, Sulawesi, Maluku, and Papua (Sulistyaningsih et al. 2014). Kalimantan Island is located on the equator, so it has a rainy humid equatorial climate. It has high mountains which provide many different habitats supporting Kalimantan Island as part of the center of origin and diversity of wild bananas (Häkkinen 2004a).

Kalimantan Island has 20 species of endemic wild bananas, but only 15 species that have been previously described (Häkkinen 2004a). Endemic wild bananas were found in Sarawak, Malaysia, such as *M. bauensis* (Häkkinen and Meekiong 2004), *Musa voonii* (Häkkinen 2004a), *M. campestris* Becc (Häkkinen 2004b), *M. azizii* (Häkkinen 2005), *M. beccarii* (Häkkinen et al. 2005), *M. suratii* and *M. monticola* (Argent 2000). There was one study about distribution of wild banana in West Kalimantan-by Sulistyaningsih and Irawanto (2011) reporting that *M. campestris* Becc. var. *sarawakensis* Häkkinen or Pisang Kera was found in Nek Rokon hill, Raya Pasi Natural Resource area, Singkawang -West Kalimantan.

On Recent years, the human activities, such as illegal logging, illegal mining, and forest fire have damaged habitat of wild bananas and influenced the population of wild bananas. Deforestation and forests fragmentations are major cause on the loose of genetic resources of *Musa* (Hapsari 2014). Between 1973 and 2015, an estimated 18.7 Mha of Borneo's old-growth forest were cleared (14.4 Mha and 4.2 Mha in Indonesian and Malaysian Borneo) and it is contested in tropical biodiversity hotspots where objective

data are limited (Gaveau et al. 2016). Therefore a study on wild bananas in Kalimantan Island is necessary to conduct. The aims of this study were to study wild banana species in Nipah Panjang and Teluk Nibung Villages, Kubu Raya District-West Kalimantan, to propose a possible new record, and to describe phenetic relationships among wild banana species based on morphological characters.

MATERIALS AND METHODS

Study area

The study on wild bananas species was conducted in two villages of Batu Ampar Sub-district, Kubu Raya District, West Kalimantan, Indonesia, i.e. Nipah Panjang and Teluk Nibung Villages (Figure 1)._Batu Ampar is located about 108 km from the capital city of Kubu Raya District. Geographically, it is located between 109º26.936' East longitude and 00º47.308' South latitude, with total area of 2.002 km^2. This region has the following boundaries, namely: northern side borders on Kubu Sub-district, while eastern side borders on Sanggau District, Southern border borders on Ketapang District, and western border borders on Karimata Strait. Average rainfall is 229.6 mm with daily temperatures range from 22.8ºC to 33.2ºC (BPS Kubu Raya 2015).

Method

The study was carried out in May 2016. Morphological characters were documented with digital camera. Morphological characterizations were conducted by following the instruction on "Descriptors for Banana (*Musa* spp.)" from International Plant Genetic Resources Institute (IPGRI 1996). Morphological character records include plant's general habit; as well as characteristics of pseudostem; petiole; leaf; penducle; male bud; male flower; fruit; seed (shape, diameter, and color) (Table 1). Morphological characteristics were obtained from field then were crosschecked with references.

Phenetic relationships among *M. balbisiana* were analyzed by comparing the morphological characters of *M. balbisiana* from Teluk Nibung Villages, West Kalimantan to *M. balbisiana* (Klutuk Wulung) and *M. balbisiana* (Klutuk ijo) from Java (Hapsari 2014), and *M. balbisiana* from Sulawesi (Sulistyaningsih et al. 2014). NTSYSpc version 2.11a was used in this study. Coefficient of similarity was analyzed by SIMQUAL (Coefficient of similarity for Qualitative Data) using the simple matching (SM) method. Clustering analysis was analyzed using SAHN (Sequential Agglomerative Hierarchical and Nested clustering) by the method of unweighted pair-group method with arithmetic average (UPGMA). Dendrogram was produced using tree display (TREE).

RESULTS AND DISCUSSION

By observing the morphological characters, one species of wild banana was identified in Kubu Raya District, West Kalimantan, i.e. *M. balbisiana* Colla (Figure 2-3). From this study, it was found that there were not any wild bananas in Nipah Panjang Village. Information from local people in Teluk Nibung Village shows that *M. balbisiana* Colla is originally available in the village by nature and is not introduced by the villagers recently.

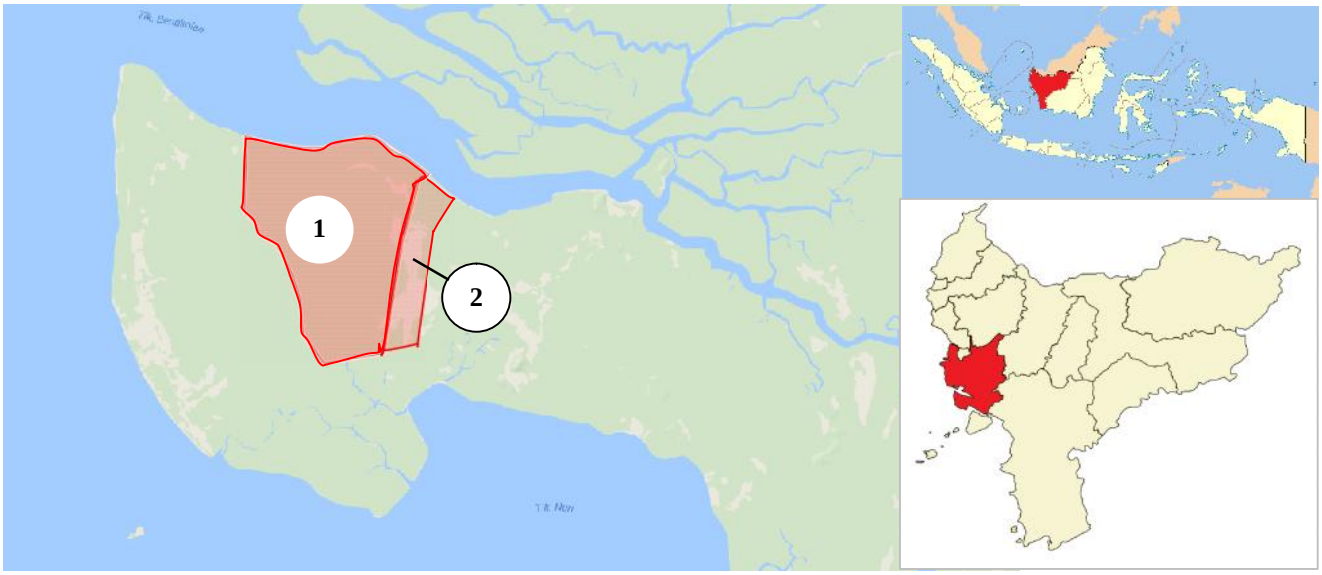


Figure 1. Location of study area in Batu Ampar Subdistrict, Kubu Raya District, West Kalimantan, Indonesia. 1. Nipah Panjang Village, 2. Teluk Nibung Village



Figure 2. *Musa balbisiana* morphology. A. Pseudostem, B. Bunch

***Musa balbisiana* Colla**

Vernacular name: Pisang Klotok

Characteristics: intermediate *Leaf habit*, 4-6 m high of *Pseudostem* of mature plant, green color. Closed to parent *Sucker*, growing at angle. 54-94 cm *Petiole*, inward curved of petiolar canal leaf margins. 160-260 x 60-100 cm *Leaf*, upper surface dark-green, lower surface medium green, midrib dorsally green-yellow, midrib ventrally light green, round on both sides of leaf bases. 30 cm *Penducle*, 7-9 width, green-color. Ovoid *male bud*, normal male bud, bract base shape small shoulder, obtuse apex, external red-purple and internal pink-purple of bract, tinted with yellow, moderate grooving, not revolute bract behavior, lifting two at a time on male bract, and rachis position at an angle. *Male flower* falling with bract, compound tepal cream with yellow lobes, translucent white free tepal, oval, triangular apex; five stamens, yellowish, exserted, white filament; straight style, same level exertion style, cream stigma, arched ovary, cream. *Fruit* is 13 cm long with 9-12 fruits per hand in two rows, straight and apex blunt-tipped with persistent style, green peel on immature fruit but yellow peel on mature fruit. *Seed* is globular and wrinkled, brown, 3.67-5.23 mm in diameter (Table 1-2).

Location found: Teluk Nibung Village

Habitat: *M. balbisiana* can be found in open spaces along roadsides or backyards of residents in Teluk Nibung Village, Kubu Raya District, West Kalimantan (Figure 3). Nasution and Yamada (2001) reported that *M. balbisiana* can never found in wild. *M. balbisiana* were founded in Indonesia banana plantations. Sulistyaningsih et al. (2014) reported that *M. balbisiana* can be found on the roadside in the suburb and grow widely on forest border at Manado and North Sulawesi.

Geographical distribution: *M. balbisiana* from Teluk Nibung Village, West Kalimantan is a new record. Previously, *M. balbisiana* was reported to be in distribution in Myanmar, Sri Lanka, India, Malaya, New Guinea, Philippines, Thailand, and Indonesia. In Indonesia, *M. balbisiana* is widely distributed in Java (Cheesman 1948) and Sulawesi (Sulistyaningsih et al. 2014).

Uses: Local people in Teluk Nibung Village processed the fruit into *sale* (dried banana). Dry leaves are used as cigarette paper, exudates deposited from pseudostem are used to treat diabetes.

Many parts of *M. balbisiana* are used as potential medicines in India. Borborah et al. (2016) reported that exudates deposited from pseudostem of *M. balbisiana* were used to expel intestinal worm infection and to treat infertility in women. Furthermore, *M. balbisiana* had higher accumulation level of potassium and chloride causing high alkalinity in the plant which justified their medical uses (Mudoi et al. 2011). The inflorescence was used to treat jaundice. The fruit bark was used to treat gout.

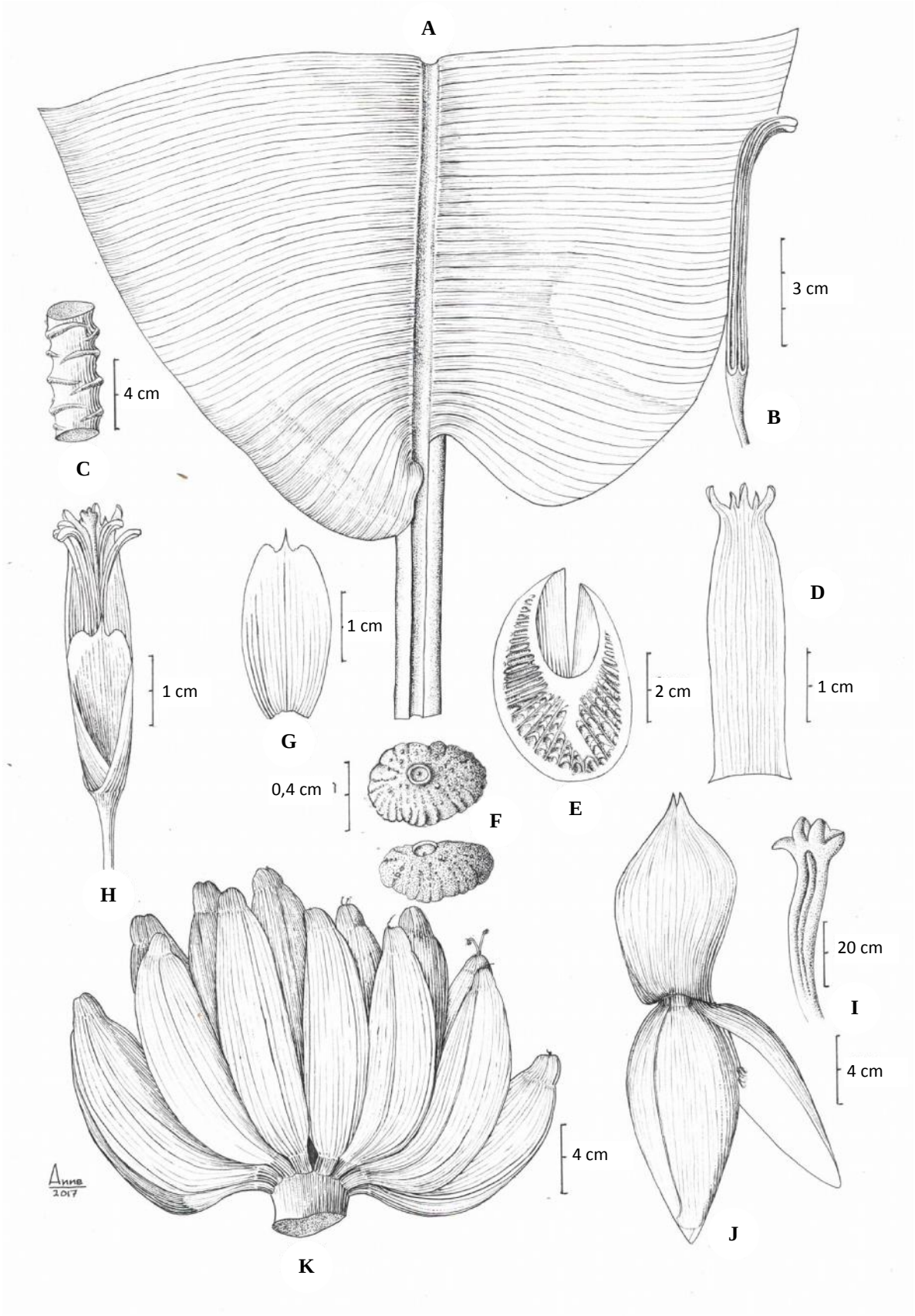


Figure 3. *Musa balbisiana* Colla botanical illustrations. A. Leaf, B. Stamen, C. Peduncle, D. Compound tepal of male flower, E. Petiole canal leaf, F. Seed, G. Free tepal of male flower, H. Male flower, I. Style, J. Male bud, K. Fruit

The dried peels were used to heal gastritis and cough or as health tonic. The fresh peel of ripe banana was used to heal dysentery (Borborah et al. 2016). The paste of fresh or dried seeds of *M. balbisiana* was used as contraceptive in Tripura, India. The tablets were prepared from the paste of fresh or dried seeds weighing 5 g and taken orally twice a day in empty stomach for 7 days (Das et al. 2014).

In Indonesia, *M. balbisiana* has some miscellaneous uses. Hapsari et al. (2017) reported that local people in Java, particularly in East Java used leaves for wrapping and medicinal use, immature fruit and male bud were for consumption, and fruit was used as medicine. Leaves of *M. balbisiana* are preferred as wrapper because they were thick, wide, and were not easily torn. Fruits of *M. balbisiana* were used to cure diarrhea. Unripe fruit of *M. balbisiana* was added onto *rujak cingur*, a popular side dishes in East Java. The male bud of *M. balbisiana* was commonly eaten as a *pecel*, *urap*, and *gulai sayur* in rural communities of East Java, the male bud of *M. balbisiana* had lesser astringent taste than *M. acuminata*.

Key to the species of *Musa balbisiana*, a new record in West Kalimantan

Sucker is close to parent and grows at angle; petiolar canal leaf margins curve inward; bract apex is obtuse and split; bract behavior makes no revolute before falling; seeds are globular, wrinkled, brown …… *Musa balbisiana*

In Indonesia, *M. balbisiana* had some variation in local names i.e., Pisang Klutuk Wulung, Pisang Klutuk Batu, Pisang Klutuk Sukun, Pisang Roti, Pisang Pataga (Ahmad et al. 2014), and Pisang Klutuk Ijo (Hapsari 2014). Pisang Klutuk Ijo was different from Pisang Klutuk Wulung on its pseudostems color, which is dark purple to black for Pisang Klutuk Wulung and green on Pisang Klutuk Ijo (Hapsari 2014). *M. balbisiana* found in West Kalimantan had similarity with Pisang Klutuk Ijo, with pseudostem color are green. *M. balbisiana* from Sulawesi had similarity with *M. balbisiana* from West Kalimantan in type of petiol canal leaf III, color of leaf upper surface, color of leaf lower surface, color of midrib dorsal and ventral surface, male bud shape, free tepal color, free tepal apex shape, fruit apex, remains of flower relicts, and color and shape of seed (Tabel 2).

Phenetic relationship

Phenetic relationship analysis showed that two groups were formed among *M. balbisiana* at the coefficient of similarity of 51% (Figure 4). Group I contained Klutuk Wulung and Klutuk Ijo from Java. Group II contained *M. balbisiana* from Sulawesi and *M. balbisiana* from West Kalimantan. They were separated from the others by type of petiolar canal leaf III, fruit apex, remnants of flower relicts, and seed surface. Dendrogram shows that *M. balbisiana* from West Kalimantan had similarity *M. balbisiana* from Sulawesi with coefficient of similarity of 80%.

Importance of *M. balbisiana*

M. balbisiana is important species in the origin of cultivated bananas (Simmonds 1966); it is believed to be one of progenitors of cultivated banana contributing B-genome (Davey et al. 2013). B-genome of banana is a target for breeding program because B-genome has high tolerance to both biotic and abiotic stresses (Hu et al. 2015). B-genome in *M. balbisiana* corresponds to a more tolerant on drought condition and more disease resistant than A-genome in *M. acuminata* (Daniels et al. 2001) and more tolerant to BBTV (Hapsari and Masrum 2012), Panama wilt, and leaf spot diseases (Singh et al. 2001).

Tabel 1. Wild banana's morphological characters

Characters	Character set (code)
Leaf habit	Erect (0), intermediate (1)
Dwarfism	Dwarfism (0), normal (1)
Pseudostem Height (m)	3 (0), 4(1)
Pseudostem color	Black-dark purple (0), green (1), brown-purple (2)
Position of sucker	Growing at angle (0), vertical growth (1)
Petiole canal leaf III	overlapping Margin (0), curved inward (1)
Leaf blade Length (cm)	171-220 (0), 151-170 (1), 180-300 (2), 160-220 (3)
Leaf blade width (cm)	51-60 (0) 60-100 (1)
Color of leaf upper surface	Dark green with red-purple (0), dark green (1)
color of leaf lower surface	Green (0), medium green (1)
Shape of leaf base	pointed on both sides (0), round sides (1)
Color of midrib dorsal surface	Green (0), light green (1), greenish yellow (2)
Color of midrib ventral surface	Green-purple (0), light green (1)
Peduncle length (cm)	≥31 (0), ≤30 (1)
Male bud type	Like true-horn 'Plantain' (0), normal (1)
male bud shape	Rounded (0), ovoid (1)
Bract apex shape	Obtuse and split (0), intermediate (1), obtuse (2)
Color of bract external face	Purple (0), red-purple (1)
Color on the bract apex	Not tinted with yellow (0), tinted with yellow (1)
Male bract lifting	Lifting one at a time (0), lifting two or more at a time (1)
Bract behavior before falling	Revolute (0), not revolute (1)
Compound tepal basic color	White (0), cream (1)
Lobe color of compound tepal	Cream (0), yellow (1)
Free tepal color	Tinted with pink (0), tinted with yellow (1), translucent white (2)
Free tepal shape	Rectangular (0), oval (1)
Free tepal apex shape	Thread-like (0), triangular (1)
Ovary shape	Arched (0), straight (1)
Number of fruit	≥ 13 (0), ≤ 12 (1)
Fruit length (cm)	15 (0), 14 (1), 13 (2)
Fruit shape	Curved (0), straight (1)
Fruit apex	Pointed (0), blunt (1)
Remains of flower relicts	Without any floral relicts (0), persistent style (1)
Seed surface	Slightly wrinkled (0), wrinkled (1)
Seed shape	Rounded (0), globular (1)
Seed color	Dark brown (0), brown (1)

Table 2. Morphological characteristics differentiation of *M. balbisiana* (Klutuk Wulung) and *M. balbisiana* (Klutuk Ijo) from Java, *M. balbisiana* (Sulawesi) and *M. balbisiana* (West Kalimantan)

Description	*M. balbisiana* (Klutuk Wulung) (Hapsari 2014)	*M. balbisiana* (Klutuk Ijo) (Hapsari 2014)	*M. balbisiana* (Sulawesi) (Sulistyaningsih et al. 2014)	*M. balbisiana* (West Kalimantan) *This research*
Leaf habit	Intermediate	Intermediate	Erect	Intermediate
Dwarfism	Normal	Normal	Normal	Normal
Pseudostem height (m)	3	3	4	4
Pseudostem color	Black-dark purple	Green	Brown-purple	Green
Position of sucker	Vertical growth	Vertical growth	Vertical growth	Growing at angel
Petiole canal leaf III	Margins overlapping	Margins overlapping	Curved inward	Curved inward
Leaf blade length (cm)	171-220	151-170	180-300	160-220
Leaf blade width (cm)	51-60	51-60	60-100	60-100
Color of leaf upper surface	Dark green with red-purple	Dark green	Dark green	Dark green
Color of leaf lower surface	Green	Medium green	Medium green	Medium green
Shape of leaf base	Both side rounded	Both side rounded	Both side rounded	Both side rounded
Color of midrib dorsal surface	Green	Light green	Green yellow	Green yellow
Color of midrib ventral surface	Green purple	Light green	Light green	Light green
Peduncle length (cm)	31-60	31-60	30	30
Male bud type	Normal	Normal	Normal	Normal
Male bud shape	Ovoid	Ovoid	Ovoid	Ovoid
Bract apex shape	Obtuse and split	Obtuse and split	Intermediate	Obtuse and split
Color of bract external face	Red-purple	Purple	Red-purple	Red-purple
Color on the bract apex	Tinted with yellow	Tinted with yellow	Tinted with yellow	Tinted with yellow
Male bract lifting	Lifting two or more at time	Lifting two or more at time	Lifting two or more at time	Lifting two or more at time
Bract behavior before falling	Not revolute	Not revolute	Not revolute	Not revolute
Compound tepal basic color	Cream	Cream	Cream	Cream
Lobe color of compound tepal	Yellow	Yellow	Yellow	Yellow
Free tepal color	Tinted with pink	Tinted with yellow	Translucent white	Translucent white
Free tepal shape	Oval	Oval	Oval	Oval
Free tepal apex shape	Triangular	Thread-like	Triangular	Triangular
Ovary shape	Straight	Straight	Straight	Arched
Number of fruit	12	13-16	12	12
Fruit length (cm)	10-15	10-15	10-14	13
Fruit shape	Straight	Straight	Straight	Straight
Fruit apex	Pointed	Pointed	Blunt	Blunt
Remains of flower relicts	Without any floral relicts	Without any floral relicts	Persistent style	Persistent style
Seed surface	Slightly wrinkled	Slightly wrinkled	Wrinkled	Wrinkled
Seed shape	Globular	Globular	Globular	Globular
Seed color	Brown	Dark brown	Brown	Brown

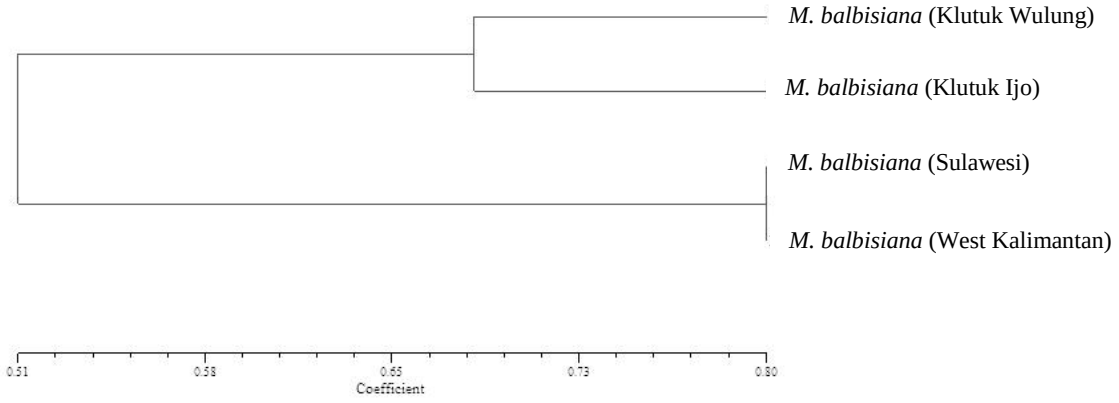


Figure 3. Dendrogram of phenetic relationship among *M. balbisiana*: *M. balbisiana* from West Kalimantan, *M. balbisiana* from Sulawesi (Sulistyaningsih et al. 2014), *M. balbisiana* (Klutuk Wulung) and *M. balbisiana* (Klutuk Ijo) from Java (Hapsari 2014)

bZIP genes in B-genome of banana showed 74% of increased transcription levels under cold, drought, and salt stresses (Hu et al. 2015). Banana cultivars from ABB group i.e Kepok, Siem, and Cepatu tend to be more tolerant to drought and more disease resistant than *M. acuminata* cultivars (AAA and AA) such as Ambon, Berangan, and Mas (Nasution and Yamada 2001).

Bioactive compound is another potential of *M. balbisiana.* Tin et al. (2016) reported three triterpenes were isolated from *M. balbisiana* inflorescence. They are 31-norcyclolaudenone, cycloartenol and (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol. Triterpene from *M. balbisiana* inflorescence is a new source of natural products for food or pharmaceutical applications in future.

It can be concluded from this study that one wild banana species was successfully identified from Teluk Nibung Village, Kubu Raya District, West Kalimantan, i.e. *M. balbisiana* and it is considered as new record. *M. balbisiana* from West Kalimantan was closely related to *M. balbisiana* from Sulawesi with coefficient of similarity of 80%. Conservation of *M. balbisiana* and other wild banana in Indonesia is important to be prioritized both *in-situ* and/or *ex-situ* before it goes to extinction. Conservation of wild bananas will provide long term and sustainable conservation of genetic diversity. Genetic diversity of wild banana is important resources to improve quality of cultivated banana using genetic manipulation in the future.

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Short Communication: Strong genetic differentiation of the endemic rosin-producing tree *Styrax sumatrana* (Styracaceae) in North Sumatra, Indonesia

HENTI HENDALASTUTI RACHMAT[1,♥], ARIDA SUSILOWATI[2,♥♥], DENI ELFIATI[2], KANSIH SRI HARTINI[2], WIZA NONI FARADILLAH

[1] Forest Research and Development Center. Jl. Gunung Batu No. 5. PO Box 165, Bogor 16001, West Java, Indonesia. Tel.: +62-251-8633234; 7520067. Fax. +62-251-8638111, ♥email: hendalastuti@yahoo.co.uk

[2] Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Dharma Ujung No 1, Kampus USU, Medan 20155, North Sumatra, Indonesia. Tel./Fax.: +62-61-8211633, ♥♥ email: arida_iswanto@yahoo.co.id

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Abstract. *Rachmat HH, Susilowati A, Elfiati D, Hartini KS, Faradillah WN. 2017. Short Communication: Strong genetic differentiation of the endemic rosin-producing tree* Styrax sumatrana *(Styracaceae) in North Sumatra, Indonesia. Biodiversitas 18: 1331-1335. Styrax sumatrana* is an economically important rosin-producing tree endemic to North Sumatra, Indonesia. Distribution of this species is very limited, and the high rate of forest degradation in Sumatra is increasing the necessity for conservation. To quantify genetic variation and population structuring, we collected individuals from 3 populations namely Pakpak Bharat, Humbang Hasundutan (Humbahas) and Tapanuli Utara in which each of the population was represented by 10 individuals. However, the successful rate of amplification was varied among populations and for later analysis, we only took an account those sequences showing clear electropherograms and disposed those which showed ambiguity. We sequenced *trnL-trnF* chloroplast DNA (cpDNA) region yielded 941 bp after alignment. The *trnL-trnF* assigned the species into 4 haplotypes in which Pakpak Bharat was differentiated significantly and not shared any similar haplotypes with two others populations. Humbahas and Tapanuli Utara was shared one common haplotype. Mean nucleotide diversity at silent sites ranged 0 - 3.33 x 10^{-3}, while nucleotide diversity at non-synonymous site ranged 0 - 5.9 x 10^{-4}. Strong genetic differentiation was also found among 3 origin populations, with the highest pairwise genetic differentiation found on Pakpak Bharat and Tapanuli Utara (F_{ST}= 0.80952). Clear and apparent genetic structuring was possibly caused by geographical barriers such as highland and mountain ranges (Bukit Barisan mountain ranges) which acted as effective barriers to gene flow among population. The findings suggest that conservation efforts should focus on every population because each of the population maintains distinct genetic identity.

Keywords: *trnL-trnF*, genetic diversity, *Styrax sumatrana*, genetic differentiation

INTRODUCTION

Styrax is a genus of about 130 species in the family Styracaceae with the major habitat in eastern and southeastern Asia. For local communities in North Sumatra, Indonesia, this rosin-producing tree has been an economically important commodity since centuries. The tree, locally known as kemenyan, has been cultivated in many localities including Tapanuli Utara, Dairi, Pakpak Bharat and Humbang Hasundutan (Humbahas). Statistical data (BPS 2008) showed that Tapanuli Utara owned the largest cultivated area covering 22.670 Ha with the resin production of about 321,3 kg/ha/yr with total production of about 4.247 tones/yr. Humbahas District is also known to have huge kemenyan cultivation namely 9.594 Ha.

Styrax sumatrana is an economically important rosin-producing tree, endemic to North Sumatra, Indonesia and is locally known as *kemenyan toba* or *haminjon* (Lubis et al. 1984). In North Sumatra, this species has long been cultivated since 18th century and economically important for local communities. Rosin from this family has long been known for medicinal, ritual, and pharmaceutical use and globally named benzoin resin. In pharmaceutical and medicinal preparation, it is used as an expectorant tincture for bronchitis and laryngitis, as an antiseptic to prevent infection and to cure stomachache and skin diseases (Burkill 1935; Katz et al. 2002). Later research found wider application and use of the resin, e.g to cure mastitis disease in dairy cattle (Mooventhan et al. 2016), to prevent cast slippage in cubfoot (Ibrahim 2016) and event to be patented as a trans-mucosal delivery of pharmacologically active agent (Battaglia and Beim 2016).

Sumatran rain forests were cleared and converted at an annual rate of 500,000 hectares or 2.56 % yr^{-1} and since 2011 it was listed as a site of World Heritage in Danger (Curran 2004; Gaveau 2009). Consequently, an emergency conservation plan is a priority. Through the economic benefit of the species, *S. sumatrana* (kemenyan Toba) has long been cultivated and this made the species gained more concern on conservation aspect. Local communities plant and cultivate the species on their private lands. This kind of cultivation usually develops the local kemenyan variant in each of the localities. Hence, there are several local names standing both for similar and different species of the *Styrax*, for example: *kemenyan toba, kemenyan bulu, kemenyan batak, kemenyan durame, kemenyan minyak,* and *kemenyan dairi*. Each shows slightly different morphological tree and resin characters. Even though for

local communities there is clear separation of the *kemenyan* growing and planting in their gardens, genetic analysis for the separation of the species has not been reported before.

The chloroplast genome is considered a single, non-recombining unit of inheritance and that is structurally stable (primarily maternally in angiosperms) (Olmstead and Palmer 1994; Birky 1995). Molecular marker-based genetic studies are crucial for elucidating appropriate management units for both conservation and production purposes. An understanding of genetic identity of the species and/or population is great practical importance both to conservation biologists and silviculturist. In the present study, we hypothesize that (i) since *S. sumatrana* is an endemic species and cultivated by localities with significant geographical barriers among localities, each locality or sub population will harbor distinct genetic identity; and (ii) as a result of significant geographic barriers, there will be strong genetic structure among population. We used *trnL-trnF* cpDNA region to address the objective of identifying the level of genetic variation and describing population differentiation among the populations of *S. sumatrana* and discussing the causes of shaping of the population genetic structure.

MATERIALS AND METHODS

Plant material

Sampling sites in North Sumatra, Indonesia were selected based on the existence and abundance of *kemenyan toba* cultivation, those including three districts of Pakpak Bharat, Tapanuli Utara and Humbang Hasundutan (Humbahas) with 10 productive trees which were sampled at each site (Figure 1). Here, productive trees refer to trees which, currently being tapped for resin, generally aged 15 years or more with the average diameter at breast height was 21 cm. Sampled leaves were dried with silica gel and used for DNA extraction. Total genomic DNA was extracted from leaf samples using a modified CTAB method (Murray and Thompson 1980).

DNA sequencing of *trnL-trnF* chloroplast DNA (cpDNA)

trnL-trnF region was amplified by PCR using the universal c and f primers described in Taberlet et al. (1991). PCR was performed in 20 uL of a solution containing 10 ng of genomic DNA, five pmol of each forward and backward primer, and 10 uL of Go Taq® Hot Start Colourless Master Mix (Promega, Wisconsin, USA) according to the manufacturer's instructions. Initial denaturation was performed at 95°C for 2 min, followed by 30-35 cycles of denaturation at 95°C for 1 min, annealing at 50°C and polymerization at 72°C for 45 min, and final extension at 72°C for 7 min. Prior to sequencing, the PCR products were purified and automatic sequenced by Genetic Science (Singapore). DNA sequencing was performed for both strands with the primers were used for the PCR amplifications.

Data analysis

DNA sequences were checked visually and forward and backward traces were assembled using the ATGC program (Genetyx Corporation, Japan). Haplotype diversity and nucleotide diversity (Nei 1987) were calculated using DnaSP version 5.10 (Rozas et al. 2005) Tests of neutrality, Tajima's *D* (1989), Fu and Li's *D** and *F** (1993), Fu's *Fs* (1997), and test of pairwise population differentiation were also performed using DnaSP.

RESULTS AND DISCUSSION

Results

Sequences of 941 bp were determined after alignment with 5 segregating sites in all individuals. The *trnL-trnF* region defined individuals into 4 haplotypes (Figure 1-2) (Table 1-2) in which haplotype 2 shared between Humbahas and Tapanuli Utara while the rest are distinct for each of the population. Pakpak Bharat population was assigned only into one distinct haplotype (Haplotype 3), while Tapanuli Utara consisted of two haplotypes (Haplotype 1 and 2) and Humbahas also consisted of two haplotypes (Haplotype 2 and 4). Genetic identity for each of population is shown in Table 3. Since Pakpak Bharat was only assigned into one haplotype, the numbers of nucleotide differences or also known as nucleotide diversity were 0.000 both for silent and non-synonymous sites. In Humbahas population, the mutation occurred in synonymous and non-coding with the nucleotide diversity at silent site (π_s) was 2.67 x 10^{-3} and θ value was 2.4 x 10^{-3}. Contrary to that of Humbahas, mutation in Tapanuli Utara population occurred at non-synonymous site with $\pi_{non\text{-}syn}$ was 1.25 x 10^{-3} and θ value was 8.4 x 10^{-3}. The average number of nucleotide differences per silent sites (π_s) in total populations was 3.33 × 10^{-3} and θ value was 5.7 x 10^{-4}. Tajima's *D* showed no significant results (Table 3) and neither did the other three neutrality tests (data not shown).

Pairwise population structure showed that population differentiation between Pakpak Bharat and two other populations were very high (Table 4), while Humbahas and Tapanuli Utara shared similar haplotypes and those showed weak population structuring.

Table 1. Single nucleotide polymorphism found among *S. sumatrana* tree that divided the species into 4 distinct haplotypes

Haplotype	Single Nucleotide Polymorphism at (bp)				
	146	585	617	680	755
Haplotype I	C	A	A	T	T
Haplotype II	A	A	A	T	T
Haplotype III	A	C	A	T	T
Haplotype IV	A	A	C	G	C

Table 2. Sampling number per population and its successful amplification

Population	Sample (N)	Successful amplification
Pakpak Bharat	10	10
Humbahas	10	6
Tapanli Utara	10	8
Total	30	24

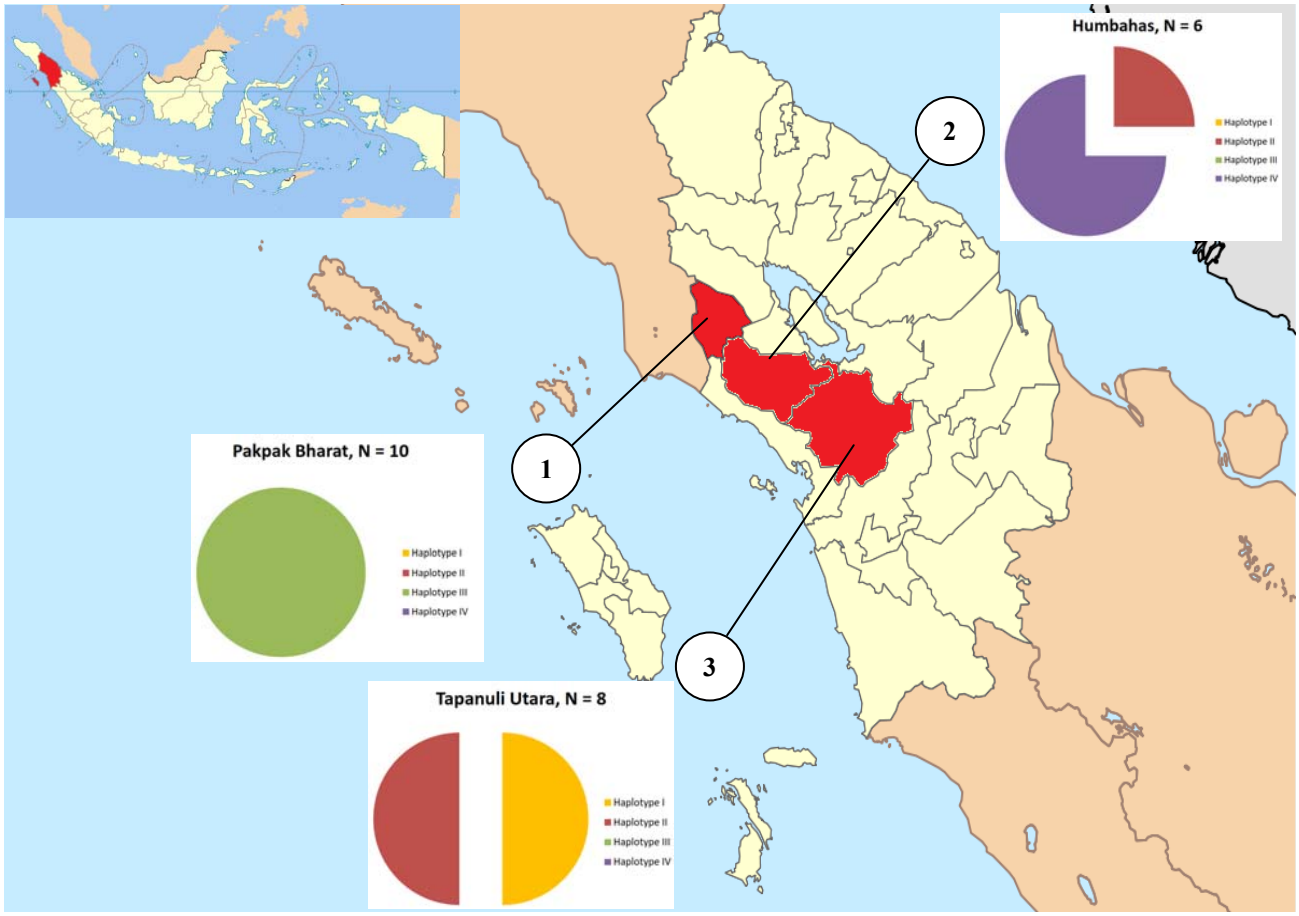


Figure 2. Haplotype assignment based *on trnL-trnF* cpDNA region among three population of *S. sumatrana* in North Sumatra, Indonesia. Note: 1. Pakpak Barat, 2. Humbang Hasundutan, 3. Tapanuli Utara

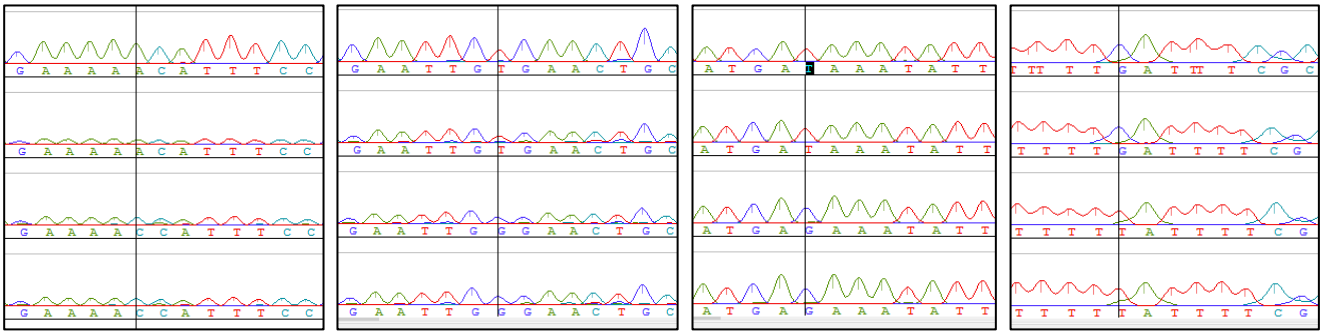


Figure 2. Sequences performance showing several segregating sites that divided the sequences into four haplotypes

Table 3. Nucleotide variation in *S. sumatrana* as inferred from *trnL-trnF* cpDNA regions

Pop.	L	*N*	*S*	H_d	($\times 10^{-3}$) π_s	θ_s	*D*
Pakpak Bharat	941	20	0	0.000	0.00	0.00	na
Humbahas	941	12	3	0.429	2.67	2.40	0.458
Tapanuli Utara	941	16	1	0.571	0.00	0.00	1.444
Total	941	48	4	0.751	3.33	2.18	1.212

Length sequences aligned (L), population (Pop), number of sequences analyzed (*N*), haplotype diversity (H_d); number of segregating sites (*s*); number of nucleotide differences per silent sites (π_s), theta per silent sites (θ_s), Tajima's D (*D*).

Table 4. Pairwise F_{ST} between six populations of *S. sumatrana*

Population	Pakpak Bharat	Humbahas	Tapanuli Utara
Pakpak Bharat	—		
Humbahas	0.8022	—	
Tapanuli Utara	0.8095	0.0029	—

Discussion

Endemic species tend to have a low level of genetic diversity due to their small population sizes (Hamrick and Godt 1989). Therefore, considering the restricted distribution of *S. sumatrana* to only grow in North Sumatra, it was expected that populations would show lower genetic variation than those of wider distribution. However, this study revealed that *S. sumatrana* maintains high genetic variation. This result is congruent with the pattern of other *Styrax* species that previously studied using different molecular markers. Using AFLP markers, Wang et al. (2015) determined that *S. obassia* possessed high genetic diversity at the species level (Na = 1.9960, Ne = 1.2782, Nei's gene diversity H = 0.1764) but no positive correlation between genetic and geographical distance. By using SRAP markers, Nan et al. (2012) showed that *S. tonkinennsis* reflected a high level of genetic diversity and significant genetic differentiation within and among population with also positive correlation between genetic and geographical distance. Iwasaki et al. (2006) studied several deciduous broad-leaved forests and found that *S. obassia* possessed a relatively large number of intraspecific *cpDNA* variation with the nucleotide diversity value (π) of 1×10^{-3}. Comparing to this species which is endemic to Hokkaido-Japan and China, *S. sumatrana* maintains nucleotide diversity value three times higher ($\pi = 3.3 \times 10^{-3}$).

During the last glacial period (c. 20,000 years ago), western and northern Sumatra together with northern and eastern Borneo, and the Mentawai Islands existed as rainforest refugia (Thomas 2000; Gathorne-Hardy et al. 2002; Slik et al. 2003). The extant tropical forest in central and eastern Sumatra, therefore, appeared only recently after the last glacial period (no more than 20,000 years ago) and this may be the cause for why the existence of the *Styrax sumatrana* restricted only to the western coast of North Sumatran Province. The obtained pairwise F_{ST} values are presented in Tables 3. Based on the qualitative guidelines for interpretation of F_{ST} suggested by Wright (1978), a high genetic differentiation was found between Pakpak Bharat and two others population, while low genetic differentiation was found between Humbahas and Tapanuli Utara when both populations shared one similar haplotype, and there are no significant physical barriers among two areas. The differentiated Pakpak Bharat population can be explained by the existence of physical or geographical barriers. The significant Bukit Barisan mountain belt and the occurrence of Samosir Highland may act as a physical barrier altering or hindering gene flow between Pakpak Bharat and the remaining populations. In fact, Hamrick and Godt (2003) stated that the high genetic variation within populations and apparent population structuring between populations showed in this study result are typical of insect-pollinated woody species and indicates that gene flow is localized.

In conclusion, our results showed that each population maintained distinct genetic characteristic, therefore the conservation implication requires the preservation of all population as conservation unit. The strong genetic structuring also indicated how each population differentiated and therefore transferring genetic resources between population in conservation point of view, especially Pakpak Bharat to two others populations, should be avoided.

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The ethno-techno-conservation approach in the utilization of Black Fruit (*Haplolobus* sp.) by the Wandamen ethnic of Papua, Indonesia

ANTONI UNGIRWALU[1], SAN AFRI AWANG[2], PRIYONO SURYANTO[2], AHMAD MARYUDI[2,♥]

[1]Faculty of Forestry, Universitas Papua. Jl. Gunung Salju, Amban, Manokwari 98314, West Papua, Indonesia
[2]Faculty of Forestry, Universitas Gadjah Mada. Jl. Agro No. 1 Bulaksumur, Yogyakarta 55281, Indonesia. Tel.: +62-274-550541, ♥email: ahmad_maryudi@ugm.ac.id

Manuscript received: 26 May 2017. Revision accepted: 25 August 2017.

Abstract. *Ungirwalu A, Awang SA, Suryanto P, Maryudi A. 2017. The ethno-techno-conservation approach in the utilization of Black Fruit (*Haplolobus *sp.) by the Wandamen people of Papua, Indonesia. Biodiversitas 18: 1336-1343*. Identities and cultures are developed by societies through interaction with their environments. In caring for and using their environment, ecological knowledge is created. Deep knowledge of sustainable utilization and conservation of forest resources exist in Papua, but this traditional wisdom is poorly reflected in scientific literature. Such knowledge includes for example the adaptive traditional management of Black Fruit trees (*Haplolobus sp.*) by the Wandamen people of Papua, which is the subject of the case study reported in this paper. Our research focused on developing an understanding of how local knowledge about utilization, conservation, and protection of Black Fruit was constructed over time. It employed ethnoecology as the analytical lens. The study was conducted in Teluk Wondama District, West Papua. We found that the adaptive resource management of Black Fruit by the Wandamen is based on an approach which we have called "ethno-techno-conservation". This approach is an attitude of mind by which Wandamen communities manage their Black Fruit trees to meet the dual goals of fulfilling subsistence needs and conserving the resource. This adaptive strategy has evolved over time as a response to the dynamics of the environment and exemplifies the co-evolution of culture and environment that is a defining feature of the world we all inhabit. The traditional concepts and knowledge of the Wandaman elaborate conservation values in the utilization of the Black Fruit. These adaptive concepts and knowledge are codified in their beliefs, myths, and handed-down wisdom.

Keywords: Adaptive management, Black Fruit, ethno-techno-conservation, local wisdom, Wandamen

INTRODUCTION

Societies evolve over time; their interactions with the environment shape their identities. In many instances, their interactions with the environment result in the creation of traditional ecological knowledge (Liu et al. 2010). Traditional knowledge is rarely applied as a consideration in natural resource management at the national level. However, the pressing problems of environment depletion over recent decades have increasingly encouraged researchers and policy makers to seek alternatives to the conventional approaches to management of natural resources. In fact, there is a growing belief that the intimate synergies between local people and their environments are able to contribute positively to sustainable resource management (Stevens 1997; Kellert et al. 2000; Blaikie 2006). The involvement of local people is now seen as a key strategy in natural resource management (Maryudi et al. 2012; Mistry 2009). Adaptive traditional knowledge is increasingly valued; it is seen as having a potential to complement modern conservation approaches (Liu et al. 2010).

Worldwide, there has been a growing body of literature and studies on local ecological knowledge about the natural environment (Gerhardinger et al. 2009; Himmi et al. 2014; Iskandar et al. 2016; Tamalene et al. 2016). Nonetheless, there have been debates about the difficulties of integrating locally-based knowledge along with western knowledge as represented in the disciplines of anthropology, ethnobiology, biological conservation, and so on (Ludwig 2016). The common critiques are centered on how to improve those disciplines by incorporating historical and cultural perspectives, to broaden the naturalistic focus of purely ecological methodologies (Hobbs 2009). Hence, ethnoecology has been developed as a discipline that conceives of natural resources and their utilization as a human construct, a manifestation of knowledge systems and practices created by human-beings for survival (Campbell 2009). Researchers have increasingly proposed ethnoecology as an alternative approach to natural resource management, incorporating the perspectives of local communities, and applying sociocultural, and economic analyses to the ecosystems they inhabit and construct (Martin 2010).

Ethnoecological studies of adaptive resource management emphasize localities that have distinct biophysical and sociocultural characteristics (Ens et al. 2015; Naveh 2007; Musacchio 2009). In the Indonesian Province of West Papua, there exists a wide spectrum of the forms of sustainable utilization and conservation of forest resources. However, these are not well documented in the scientific literature. The study reported in this paper sought to examine one such under-studied case of adaptive resource management in Papua as exemplified in the traditional utilization and conservation of a species of tree known as Black Fruit (*Haplolobus* sp.), carried out by the

Wandamen people of Teluk Wondama District. Our research focused on the construction of local knowledge by the Wandamen of utilization, conservation, and protection of the natural resources of this Black Fruit species. It used ethnoecology as the analytical lens. The sustainable utilization of the fruit by the Wandamen communities is a manifestation of what we call "ethno-techno-conservation".

MATERIALS AND METHODS

Study area

The Wandamen are a people sharing a common ethnicity and language group who live in the coastal areas of the Doberai Peninsula – the Bird's Head (Indonesian: *Kepala Burung*) – of Papua; principally in Teluk Wondama District, West Papua, Indonesia (Figure 1). The study reported in this paper was conducted in this area of Teluk Wondama District, which lies between 134° 01' 49" and 134° 57' 51" E, and between 1° 58' 27" and 3° 00' 32" S. For the study, four villages in four different Sub districts were selected, i.e. Rado (Sub district of Wasior), Kaibi (Sub district of Wondiboi), Tandia (Sub district of Rasiey) and Sobey (Sub district of Teluk Duari). The villages were purposively selected, with the main consideration being the regular utilization of the Black Fruit (*Haplolobus* sp.) by their inhabitants.

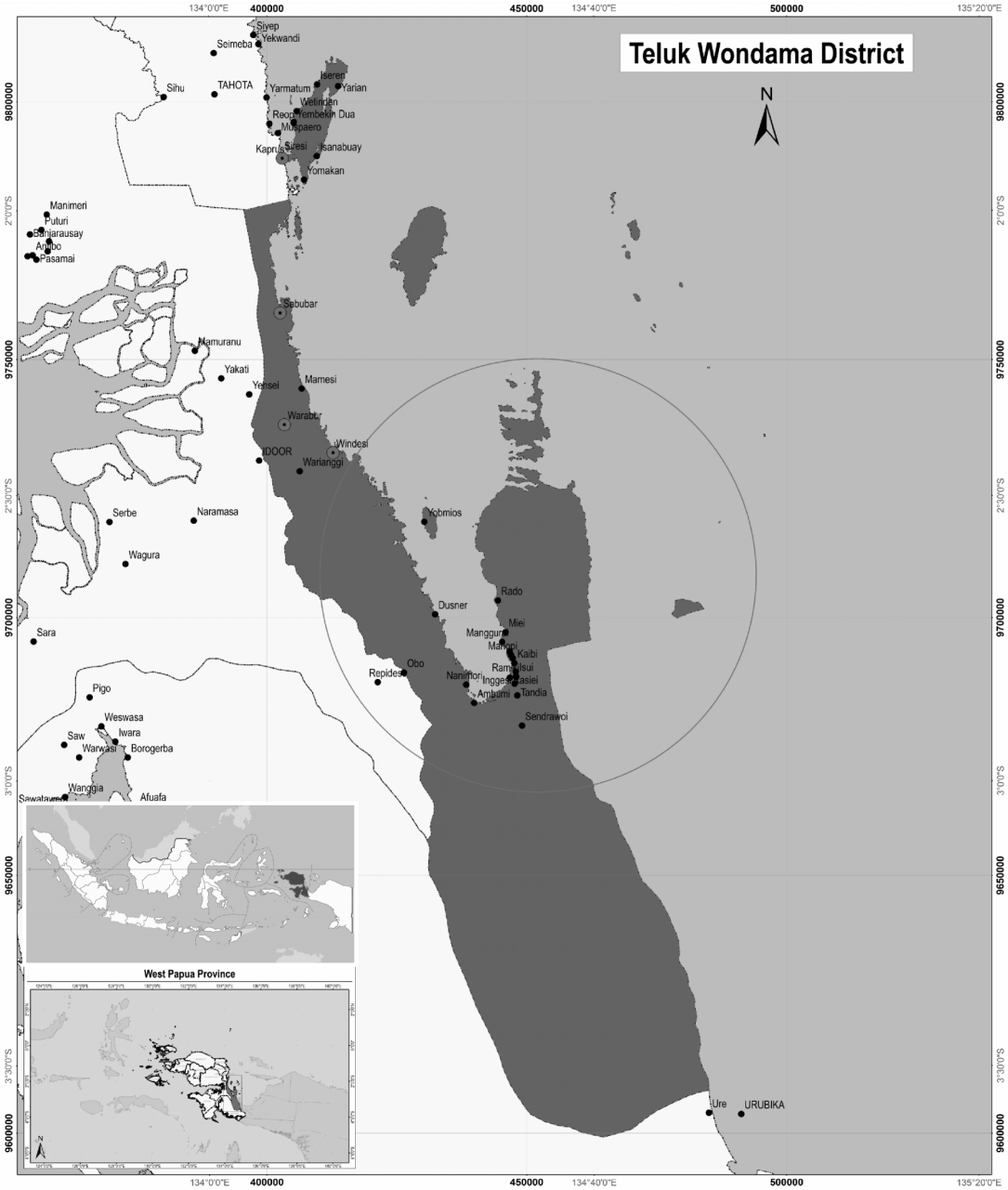


Figure 1. Location of study conducted in Teluk Wondama District, West Papua, Indonesia

Data collection

Our study employed an ethnoecological approach, which focuses on explaining the social interactions that influence natural resource management (Spradley 2006). It focused on local knowledge systems that inform the traditional concepts in utilizing and conserving the Black Fruit resource. Data and information were collected through the following methods: review of relevant literature, direct observation, and in-depth interviews with sampled respondents and with key informants (Prabowo et al. 2016; Setiawan et al. 2016). Observation and interviews were conducted from July to December 2015. Respondents (46 people) for interviews were selected from the four studied villages. The main consideration in selecting the respondents was their active participation in Black Fruit utilization. The snowball method of sampling (Bailey 1982) was used to select the key informants (3 informants/ village) for interviews.

Data analysis and interpretation

A constructivist method was adopted in the interpretation of the data. This involves a rational, empirical, synthesis of the different perspectives of the actors (interviewees and interviewers) taking into account both the subjective perceptions of the interviewees conditioned by their local (internal) knowledge systems and of the interviewers conditioned by their more global (external) knowledge systems (Stever 1986).

Our study combined both a consideration of the natural and of the social worlds of the Wandamen communities. An ecological methodology generally objectifies nature and environment as external to the social world; somewhat independent of the society itself (Barry 1999; Awang 2006). On the other hand, social constructivist methodologies attempt to get at an understanding of the social dynamics at play in the construction and utilization of knowledge and are based on a dialectic between the main players (Berger and Luckmann 1990), and on systems theory (Parsons 1961).

According to Odum (1992), a system is an entity with interrelated and interdependent objects; bounded, but integrating both external and internal dynamics. Within a constructivist analytical framework, the external dynamics involve a process of humans reaching out into the world through physical and mental activities, while the internal dynamics involve the internalization into the human system of the objects created by these human activities (Setiadi et al. 2006).

Ethno-science, which is part of ethnography, describes the interaction between knowledge of a local community and of nature, both biotic and abiotic aspects. Therefore, we used data derived from the cognition and linguistic processes of the local classification systems and carried out a semantic analysis (Spradley 2006). The ethnographic data we gathered about the Wandamen community, and the ecological data we collected about the Black Fruit were classified and integrated (Martin 2001). Simple descriptive statistics in accordance with the methods employed by Monteiro et al. (2006) and Tamalene et al. (2016) were used to interpret the information contributed by respondents regarding the way they construct local knowledge defining pre-harvest, harvest and post-harvest stages of Black Fruit utilization.

RESULTS AND DISCUSSION

Overview of Wandamen ethnic community

The Wandamen ethnic community is characterized by property rights and tenure of natural resources based on a patrilineal system (Malinowski 1948; Mansoben 1995). The origins of their systems of property rights and leadership can be tracked through myths and beliefs that spread over considerable time within the society. Etymologically, the word "*Wondama*" is derived from the language of the Wamesa sub-ethnic group: "*Won*" meaning "*a destined people*"; and "*Dama*" meaning "*a people inhabiting specific areas*". The people are part of the Melanesian race, inhabiting the region along the coast of Wondama Bay, which is characterized by varying topographic conditions, ranging from flat to mountainous. There are two main tribes, i.e. Wamesa and Soug, which respectively make up 90% and 10% of the population. The people mainly speak an Austronesian language, with differing dialects spoken by sub-cultures or sub-tribes. The Summer Institute of Linguistics (2006) recognizes seven dialects of the Wandamen language, namely: Windesi, Bintuni, Wamesa, Wasior, Ambumi, Dasaner (Dusner) and Mantion. Nonetheless, our research identified 22 micro-ethnic groupings, speaking 10 different strains/accents of the Wandamen Language (Table 1). Of the different, sub-ethnic groupings, only the Wamesa, possesses traditional knowledge concerning conservation and utilization of the Black Fruit.

Table 1. Ecological zones, sub-ethnic groupings, and dialects, of the Wandamen people, West Papua, Indonesia

Ecological zones	Sub-ethnic groupings	Dialects
Coastal and estuary area	Wamesa	Wamesa
	Windesi	Wamesa
	Wasior	Wamesa
	Tandia	Wamesa
	Werianggi	Wamesa
	Werabur	Wamesa
	Mamisi	Wamesa
	Dusner	Wamesa
	Miere	Miere
	Ambumi	Waropen Kay
	Kuri	Kuri
Coastal areas (islands)	Rumberpon	Wamesa
	Roswar	Roswar
	Roon	Roon
	Yoop	Yoop
Valley	Mairasi	Mairasi/Toro
	Sabubar	Soug
	Ambuar	Soug
	Iseren	Soug
	Yarmatum	Soug
	Wariab	Soug
	Syari	Soug

The ecology of the Black Fruit tree

Black Fruit (*Haplolobus* sp.) is a species that appears to be endemic to Melanesia, although there are members of the genus recorded in a few other parts of Malesia. It is found growing in the lowland of Papua to an altitude of about 850 m a.s.l. For decades, the Wandamen people have utilized Black Fruit as an alternative food source. Its extracted products can be directly consumed, or combined with sago in a prepared food known traditionally as *beriam tereu*. Black Fruit tree belongs to the family Burseraceae which includes the well-known canary nut trees (members of the genus *Canarium* L.). Black Fruit differs morphologically from the common canary (Tamalene et al. 2016). Its trunk can reach the height of 10-40 m with a diameter of 40-90 cm. Its bark is dark in color and it has buttress roots. Its leaves, without stipules, are red when young, and are compound with 4-6 leaflets oppositely arranged. The flowers are small and unisexual. Male flowers have 3-6 stamens, free from each other and independent of the petals. The fruits are ovally-shaped drupes, with 1-2 smoothly coated seeds.

Black Fruit grows in three different ecological zones: (i) natural forest, (ii) secondary forest and (iii) in gardens. These three zones are defined within the traditional knowledge system concerning utilization of the plant from pre-harvest, through harvest to post-harvest (Ungirwalu et al. 2016). Primary forest is generally undisturbed, but some parts in the area have been slightly altered by human activities such as food gathering and hunting that result in changes in land cover, species utilization, and ecosystem services (Thijs et al. 2015). In the primary forest, Black Fruit is rarely a dominant species. It is sporadically scattered with an average density of about five trees per hectare. It competes for spaces with other tree species, such as *Artocarpus integer* (Thunb.) Merr., *Lansium domesticum* Corrêa, *Litsea timoriana* Span., *Myristica fatua* Houtt, *Pometia acuminata* Radlk., *Syzygium malaccense* (L.) Merr. & L.M.Perry, *Canarium* sp., *Intsia bijuga* (Colebr.) Kuntze, and *Mangifera indica* L.

Secondary forest is a naturally regenerated forest following significant disturbance by human activities (Chokkalingam et al. 2000; Kanel and Shrestha 2001). The main human activity is the harvest of short-term crops for which local people carry out a slash and burn system. In the early stages, the intensification of Black Fruit utilization is done through planting a cluster of 5-8 trees per hectare. In the secondary forest, Black Fruit is found along with the following tree species: *Artocarpus altilis* (Parkinson ex F.A. Zorn) Fosberg, *Lansium domesticum* Corrêa, *Artocarpus heterophyllus* Lam., *Mangifera indica* L., *Horsfieldia irya* (Gaertn.) Warb., *Syzygium malaccense* (L.) Merr. & L.M.Perry, *Ficus septica* Burm.f., *Syzygium* sp., *Aceratium oppositifolium* DC.

Tree gardening is one of the oldest farming systems in the world (Gbedoman et al. 2016). In the Wandamen garden system, Black Fruit trees are planted close to settlement and are intensively nurtured. The density of Black Fruit in the garden system is 3-7 trees per hectare, sharing spaces with the following common tree species: *Lansium domesticum* Corrêa, *Mangifera indica* L., *Nephelium lappaceum* L., *Syzygium malaccense* (L.) Merr. & L.M. Perry, *Artocarpus altilis* (Parkinson ex F.A. Zorn) Fosberg, *Manilkara zapota* (L.) P.Royen, *Artocarpus integer* (Thunb.) Merr., *Cerbera floribunda* K.Schum., and *Durio zibethinus* L.

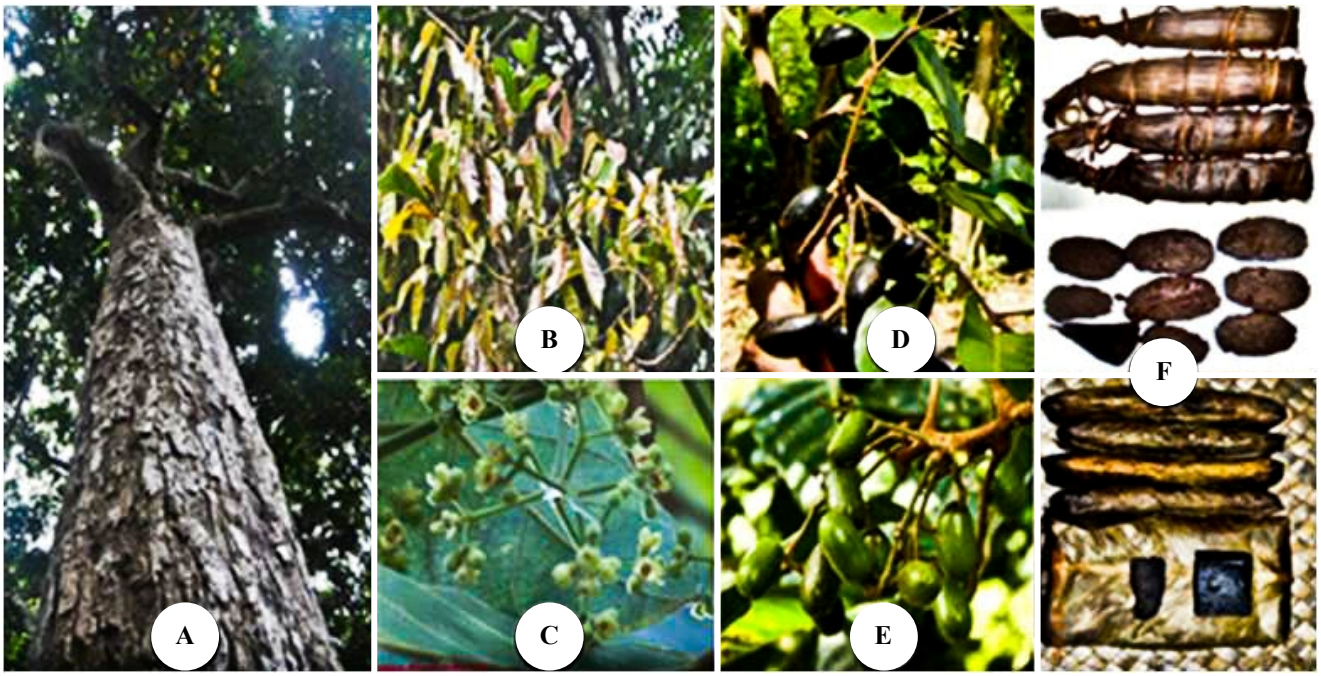


Figure 2. Morphological features of Black Fruit A. Trunk; B. Leaves; C. Flowers; D. Mature fruits; E. Unripe fruits; F. Product of Black Fruit combined with sago, *beriam tereu*

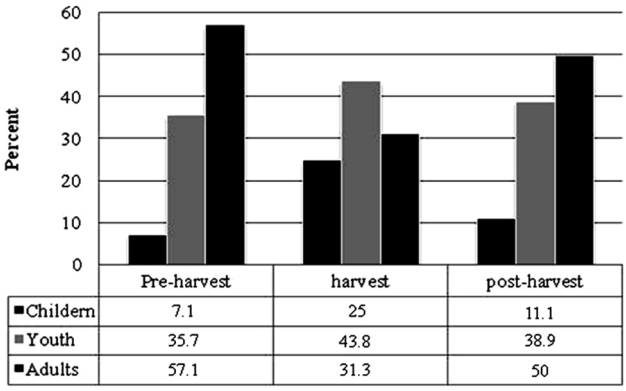


Figure 3. The proportion (%) of each of three age groups (children, youths, and adults) involved in pre-harvest, harvest and post-harvest activities in the Wandamen utilization of Black Fruit (*Haplolobus* sp.)

Local knowledge of Black Fruit utilization: Ethno-techno-conservation

Local Wandamen knowledge of Black Fruit is categorized into three phases: (i) *pre-harvest*, which includes activities to identify species, to determine the harvest time/ seasons, and to apply pest control techniques, (ii) *harvest*, which includes activities to collect mature fruits based on traditional knowledge and environment-friendly techniques, and (iii) *post-harvest*, which includes activities to extract the fruit and to make products such as the sago based product locally known as *beriam tereu*. Each stage involves division of labor based on the age groups of the people (Figure 3) and the creation of the ethno-techno-conservation approach.

Children (5-16 years of age) are rarely involved in pre-harvest (7.1%) as they have less-time and are underskilled for identifying species and mature fruits, as well as in creating tools for pest control. The pre-harvest activities are generally conducted by adults (above 30 years of age) as they have accumulated the necessary knowledge for executing this crucial task (57.1%). Youths are involved in pre-harvest activities to some extent (35.7%). Tamalene et al. (2016) argue that adults and the older generations in general play a crucial role in the transfer of knowledge concerning traditional conservation and utilization systems. During the pre-harvest activities, there is knowledge-transfer from adults to youths so that the latter will have adequate skills in the future. The knowledge is informally obtained through youths observing how the adults execute the activities in the field. In some cases, the adults also provide an explanation to the youths.

The pre-harvest activities are parts of a systematic approach based on cognition and perception of environmental processes. Ethno-techno-conservation during pre-harvest focuses on physical observation of the forest, of the phenology of the Black Fruit, and of the conservation of targeted trees from potential pests and diseases. Fruit production generally occurs once or twice a year. The traditional tools for pest control are (i) *Apiaimi* and (ii) *Kabareru. Apiaimi* is made of two pieces of Matoa boards (*Pometia* spp.) that are parallelly placed, and are bundled and hanged at one end close to the fruits. A rattan or plastic rope is attached at the other end; it is pulled from below to create noises to prevent pests (mainly bats) from eating the fruits. *Kabareru* is webbings of *Pandanus* leaves, made in the form of kites placed on the trees to create noises that prevent pests such as birds, bats and rodents. The kabareru is mounted on the tree when the fruit is ripe. When blown by the wind, this tool can create sounds used to ward off pests. This approach is locally-based, environment-friendly, and ensures fruit production without endangering the wildlife. It ensures the ecosystem balance and achieves synergies between humans and the environment.

Figure 3 shows that harvest activities are shared by the three groups, rather equally: children (25%), youth (43.8%), and adults (31.3%). This step requires efficiency and more labor. Children are intensively engaged in harvest activities, which are generally fun and require less-skills. Before the harvest, mostly women and children start with cleaning up the understory in close proximity to the targeted trees. The ritual of prayers follows, led by the clan leader. It is aimed to facilitate good harvests; there are myths that in the event of under-production inappropriate rituals must have been carried out. Kluckhohn dan Strodtbeck (1961) argue that the behavior of clan members are shaped by their leader under the patrilineal system. The culturally determined leadership system guides the direction of future generations (Mansoben 1995). The ethno-techno-conservation approach adopted during the harvest phase is reflected in the careful climbing of trees to be harvested, in which a Y-shaped pole is used to prevent destructive consequences to the environment. The method is different from the approach used by many local people in Papua who often cut branches and even fell the trees altogether simply to obtain the fruits of matoa (*Pometia* spp.).

As is the case in the pre-harvest phase, few children are engaged during post-harvest activities, which require specific skills and experiences. The activities are generally shared by the youths (38.9%) and adults (50%). The post-harvest phase, i.e. the making of *beriam tereu*, involves complex processes. It consists of the creation of a furnace, participation in traditional ceremonies, the preparation of a traditional batter from the fruit, and the cooking process. Through the process, youths learn from the adults, who in many instances are only managing the processes. The adults are mostly tasked with the traditional ceremonies. Interaction and solidarity are part of the cultural processes, as individuals come to depend on each other and on the environment in a spirit of cooperation and mutual respect (Koentjaraningrat 2009).

The design of the furnace for the cooking prevents unnecessary waste of food, which again reflects the ethno-techno-conservation approach. Small furnaces are made to satisfy the needs of small-groups, i.e. at a household level, while the larger ones are used for special ceremonies, such as weddings, engagements, ceremonies for a new-born baby, and the election and elevation of the clan leader. It

clearly represents the environmentally-minded wisdom of the community to conserve the local plants (Setiawan and Qiptiyah 2014, Wakhidah et al. 2017). The Wandamen people maintain a ritual approach to the processing of Black Fruit with Sago to make *beriam tereu*. They believe that the quality of the product symbolizes whether its production followed the correct procedures. "Good Sago" indicates that the ritual processes were in good order, and *vice versa*.

Discussion

This study has revealed that local people of Wandamen ethnicity construct the ethno-techno-conservation approach as an adaptive strategy in the management of their Black Fruit resource. The utilization of Black Fruit represents a unique synergy between local people and the natural resource through a traditional concept of conservation. This adaptive approach was created as a response to the dynamics of the environment. It exemplifies the co-evolution of culture and the environment (de Boo and Wiersum 2002). Local knowledge was constructed through internalization, externalization, and objectification of the natural phenomena, manifested in cultural rules and myths. During this co-evolution, ethno-techno-conservation was constructed in accordance with the following processes: stimulus, encouragement, and response (Kluckhohn and Stodbeck 1961; Koentjaraningrat 1974). The stimulus was provided by the phenomenon of Black Fruit being eaten by birds, which encouraged the people to find an explanation as to why the birds eat the fruit. It led to an understanding that the fruit is edible. The utilization of the fruit as a source for food was the response made by the people. The creation of the conservation concepts regarding the utilization of the fruit was a result of long-term processes within the development of the knowledge system. Previous generations developed logical understandings based on their cognition of nature around them, practiced this informal knowledge in their daily lives, and passed it on to the next generations.

The local knowledge of utilization and conservation of Black Fruit is codified in their beliefs, traditional rules, and culture, all expressed in their myths and shared wisdom. This wisdom is exemplified in their models for sustainable management of their natural resources (Sardjono 2004). It represents the behavior of a people responding to their environment, which reflects aspects of environmental determinism (Awang 2006). The adaptive utilization of Black Fruit by the Wandamen people takes into account conservation of the resource. The tools for utilization of the fruit, from pre-harvest to post-harvest phases, also represent adaptive technological skills, in responding to the circumstances of their environment.

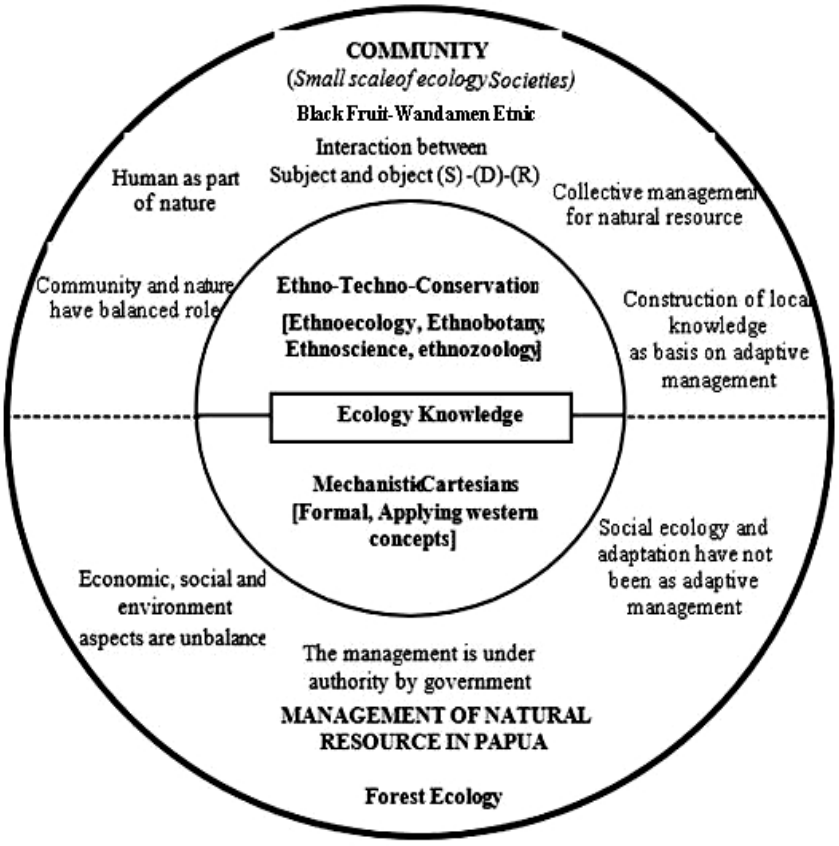


Figure 4. The connection between small-scale management of natural resources at local community level by the Wandamen people and the larger-scale management of resources in Papua as a whole

This study also illustrates that Wandamen local knowledge, actions, and practices of natural resource management are quite distinct from the common forest management approach in Papua (Figure 4). The ethno-techno-conservation approach for the utilization of Black Fruit clearly represents an adaptive strategy to satisfy the dual goals of addressing subsistence needs and of conserving the natural resource. However, this adaptive knowledge is often neglected in larger contexts. The government provides direction on how the resource should be managed through rules, regulations, and guidelines that often differ from local practices. Large-scale forest management approaches implemented in Papua tend to follow positivist-mechanistic paradigms focusing particularly on timber extraction and tend to neglect the needs and perspectives of communities already located in the forests. Foucault (2007) states that governments establish conditions in which they implement a regime of authority to control the subjectivity of local peoples. This often leads to conflicts between the government and local peoples. It is, therefore, crucial to consider the historical and political context of local communities whose lives are so intimately connected to the environments that sustain them. Decision making over natural resources should be linked to the social and cultural aspects of these local communities and to the traditional knowledge systems by which they have conserved down the ages their natural environment and drawn sustenance from it.

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Biodiversity of mangrove aquatic fauna in Purworejo, Central Java, Indonesia

WIRYANTO[1,2], SUNARTO[1,2], SLAMET MARDIYANTO RAHAYU[2,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, Indonesia

[2]Program of Bioscience, School of Graduates, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, Indonesia. Tel./Fax. +62-271-663375, ♥email: slamet.mardiyantorahayu84@gmail.com

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Abstract. *Wiryanto, Sunarto, Rahayu SM. 2017. Biodiversity of mangrove aquatic fauna in Purworejo, Central Java, Indonesia. Biodiversitas 18: 1344-1352.* Mangrove areas have important roles of ecologic, socio-economic, and socio-culture in ecosystem. Mangrove areas in Purworejo go into degradation as an impact of illegal logging, fishpond area expansion, settlements expansion and extension of agriculture area. All of the activities will influence the communities' structure of mangroves vegetation and aquatic fauna. The purposes of research are to observe (i) biodiversity of mangroves aquatic fauna; (ii) correlation between mangrove vegetation and aquatic fauna; and (iii) the influence of abiotic factors towards biotic condition in research sites. This research was conducted in June-September 2016 at 3 stations and was determined with purposive sampling method. The research stations are Gedangan (Station 1), Jatikontal (Station 2) and Ngentak (Station 3). Vegetation of mangrove and aquatic fauna were observed with method of line transect and sampling removal. Association between variables (vegetation and aquatic fauna) is analyzed with Pearson Correlation Test. Influence of abiotic factors (physics and chemicals) on biotic (vegetation and aquatic fauna) is analyzed with t-test and regression test. According to the research, 34 species of aquatic fauna are from mangroves area of Purworejo. Highest value of aquatic fauna is found in Station 3 (ID 2.50). Pearson correlation test between vegetation and aquatic fauna obtains r-value of 0,104. The t-test results in a significant difference (sig 0.000) between abiotic factors and biotic condition and shows the impact of changes on both. Further test with regression method also indicates a weak impact on changes in abiotic factors towards biotic condition (r^2 0.032).

Keywords: mangrove, biodiversity, aquatic fauna, Purworejo

INTRODUCTION

The mangroves ecosystem has important roles in ecosystem, especially in terms of ecological, environmental, biological, medical and environmental values (Baran 1999). Mangrove is known as a coastal ecosystem with the highest biological productivity (Alongi 2002). Mangrove forests have important ecological functions such as natural carbon absorber, pollutant remediation, abrasion prevention, intrusion and storm prevention. They can be as habitat for growth and development of diverse aquatic fauna.

The mangroves have an ability to increase product fishery activity which was reaching 23% from normal value (Anneboina and Kumar 2012). Mangrove also played a vital role to defend coastal area from abrasion by giving stability to the sediment with their bonding roots. Mangrove is a pollutant filter of the sea; it maintains the water quality and clarity; and also traps the sediment from land (Monsef and Smith 2008).

The roots of *Acanthus ilicifolius* can be used as medicine since it has the content of an ethyl acetate, ethanol, and methanol. Their contents have an antimicrobe effect on *Vibrio harveyi*, Aeromonas hydrophila and *Escherichia coli* (Sreenivasa et al. 2015). An extract of leaves and bark of Sonneratia alba was an antibacteria for pathogen such as *Bacillus cereus, Staphylococcus aureus, E. coli, Salmonella typhimurium* and *Klebsiella pneumoniae*. An extract of roots and barks of *Avicennia lanata* was an antibacteria for pathogen likes *E. coli, S. aureus* and *K. pneumoniae* while an extract of leaves and barks of *Ceriops tagal* have similar utilization as antibacteria *for S. aureus* (Eldeen et al. 2015).

Purworejo District is part of Central Java Province, Indonesia which is located at south coast of the island. Mangroves areas on Purworejo are located in sub-districts of Purwodadi, Ngombol, and Grabag (Local Government of Purworejo 2011). Mangrove areas on Purworejo are degrading as the effect of illegal logging, fishponds expansion, expansion of settlements and extension of agriculture area.

The pond areas in Purworejo have increased from 136 hectares (2009) to 250 hectares in 2015 (Regional Development Planning Board of Purworejo 2016). Logging and land conversion act are the major factors of mangroves area degradation in Purworejo. Reduction of mangrove area will impact on changes in abiotic factors which can influence aquatic fauna life in association with mangrove vegetation.

Indonesia has the largest area of mangroves. However, the areas are reduced to 1.5 million hectares since 1980 (Spalding et al. 2010). Mangrove degradation was driven by rapid growth of the local population and their economy which trigger input of pollutant from various anthropogenic

activities (Dsikowitzky et al. 2011). The mangrove degradation in Purworejo was aligned with historical research by Ilman et al. (2016). The research found out that an aquaculture development (tambaks) was main factor of degradation, added by a change of land use into a palm oil plantation. According to Giri (2008) in Asia, a land conversion into agriculture and aquaculture reduced 12% of mangrove area.

A community of mangrove fauna is divided into two main groups: the terrestrial fauna and the aquatic fauna. The group of terrestrial fauna generally occupies higher part of mangroves, for example, insects, snakes, primates, and birds. This group doesn't have specific adaptability to survive on mangroves. This group will pass most of their life cycle outside the marine water but still hunt for the marine fauna, as their main food sources, taking advantage of low tide time. The groups of aquatic fauna consist of two types, (i) the type living in the water column, including fish and shrimp; (ii) the type living on the substrates either in hard or soft type, including crabs, clams and another type of invertebrates (Bengen 2001).

The mangrove degradation will reduce an ecosystem service related to a human welfare and sustainability of mangrove ecosystem itself (Oudenhoven et al. 2015). Continuous degradation of mangroves in Purworejo will cause reduction on biodiversity organisms. Until today, research about biodiversity of aquatic fauna in mangrove area of Purworejo District is still rare. Actually, in research about biodiversity, a correlation between vegetation and aquatic fauna and the influence of abiotic factor was important to be conducted.

The objectives of the research are to observe (i) biodiversity of mangroves aquatic fauna, (ii) correlation between mangrove vegetation and aquatic fauna, and (iii) the influence of abiotic factors towards biotic condition in research sites.

MATERIALS AND METHODS

Study area

This research is conducted at mangrove area of Purworejo District, Central Java, Indonesia. The research Station is determined by purposive sampling method. Station 1 is located in Gedangan Village and Station 2 is located in Jatikontal Village, both are in Purwodadi Subdistrict and near settlements and beach tourism site of Jatikontal. Station 3 is located near paddy field area of Ngentak Village, Ngombol Subdistrict. The entire mangrove is located in the riverbanks of Pasir River, that empties into the estuary of Bogowonto River in the Indian Ocean.

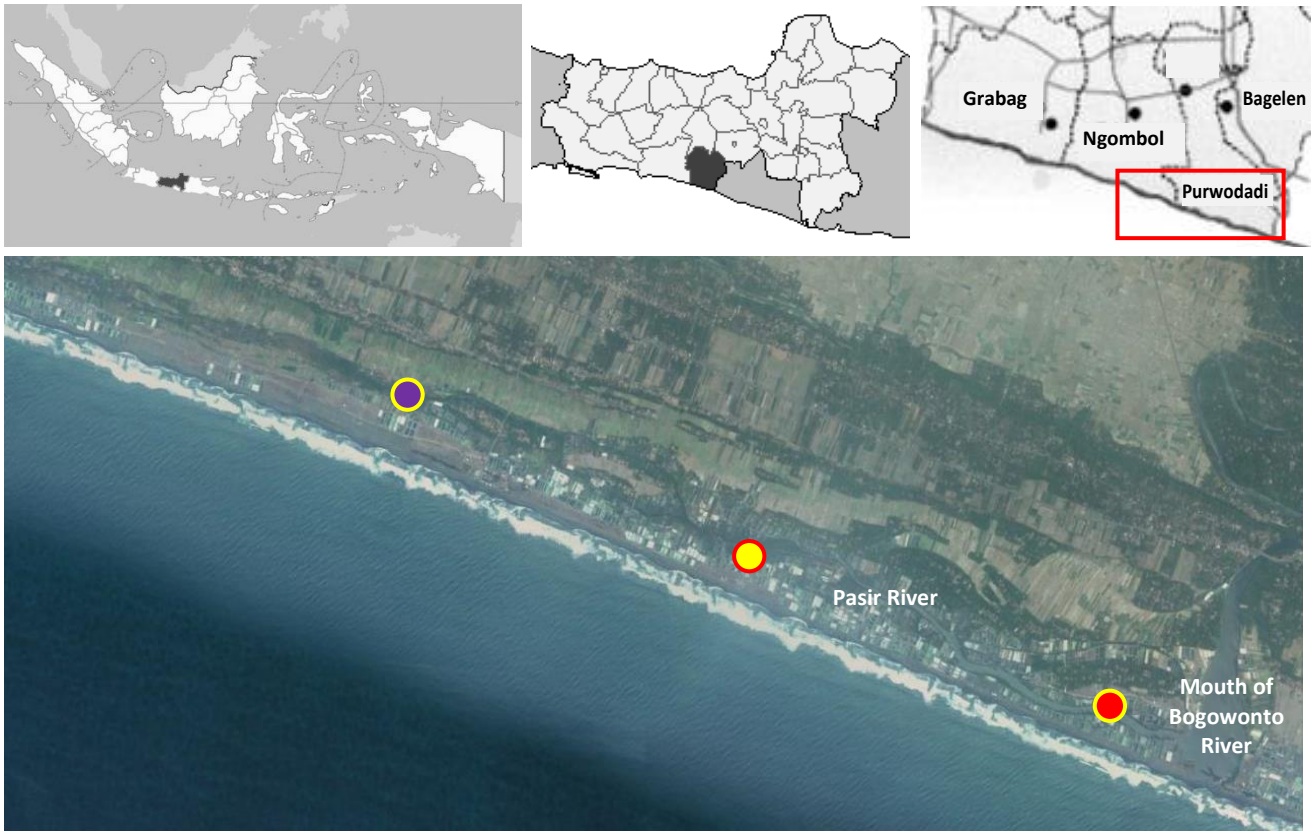


Figure 1. Study site of mangrove aquatic fauna in Subdistrict of Purwodadi, District of Purworejo, Central Java, Indonesia. Note: Station 1, red circle (07°50'16,0"S-110°00'33,2"E); Station 2, yellow (07°52'54.3"S-109°59'31.4"E); Station 3, purple (07°52'16,5"S-109°58'20,4"E)

Materials

The research equipment is wooden pegs, measuring roll meter, identification guidelines of vegetation and aquatic fauna of mangrove, thermometer, DO-meter, pH-meter, refractometer, aquatic fauna capturing tool (*pintur* and net trap), icebox, digital camera, stationary and laptop. The materials of research are papers and plastics, aquadest and alcohol. Diversity of mangrove vegetation is observed with transect line plots method, which is in accordance with the guidelines in Decree of Minister of Environmental Number 201 Year 2004. Type of aquatic fauna is observed with removal sampling method.

Procedures

Observation of mangrove vegetation biodiversity

Transect is assembled in each station, and it consists of 20 plots, and each of plot is divided again into subplots. Minimum length suitable for mangrove vegetation analysis is 300 m (Onrizal 2008). The size of plants and subplots are: herbs (sprouts' size < 1.5 m) using subplot size of 2 m x 2 m, shrubs (height = 1.5 m and diameter of the rod < 10 cm) using subplot size of 5 m x 5 m; and trees (trees with diameter of rod = / > 10 cm) using subplot size of 10 m x 10 m. Identification of mangrove vegetation refers to Noor et al. (2012).

Observation of mangrove aquatic fauna

Catching aquatic fauna is done with traditional capturing tool. This implementation is intended to optimize catching results so that a more diverse aquatic fauna can be collected. Identification of aquatic fauna refers to Ng and Sivasothi (2001).

Measuring of abiotic factors

The measurement of water salinity is conducted with hand refractometer instrument. The measurement of pH level is conducted with pH meter. The measurement of water temperatures is conducted with water thermometer. The measurement of dissolved oxygen values is conducted with DO-meter. Soil textures are determined with feeling method, namely doing the massaging and to feel the soil samples using bare fingers to specify substrates condition (Hardjowigeno 1989).

Data analysis

Species density

Species diversity has been analyzed refer to Indriyanto (2012):

$$K = \frac{\text{sum of individuals}}{\text{total area of sample plots}}$$

Diversity indices

Analysis of diversity has been conducted for mangrove vegetation and aquatic fauna which had been caught with capturing tool. The biodiversity is calculated with Diversity Index of Shannon Wiener which is formulated as follows:

$$H' = -\sum \frac{ni}{N} \ln \frac{ni}{N}$$

Note:
H' : the diversity index of Shannon Wiener
ni : sum of individual of species in all individuals
N : total numbers of individuals

Results of Shannon Wiener diversity index calculation should be categorized below (Odum and Barrett 2009):

H' < 1 : Low diversity, low abundance, low of communities stability
1 ≤ H' ≤ 3 : Moderate diversity, moderate abundance, moderate of communities' stability.
H' > 3 : High diversity, high abundance, high of communities stability

Correlation between mangrove vegetation and aquatic fauna

Correlation analysis between researched variable (aquatic fauna and mangrove vegetation) is conducted with Product Moment Pearson Test. The analysis is intended to determine level of relationship between variables.

Influence of abiotic factors on mangrove vegetation and aquatic fauna condition

The variables of research are categorized into independent and dependent variable. In this research, the independent variables are abiotic factors such as temperatures, pH, salinity, dissolved oxygen (DO) and substrates texture. The dependent variables are vegetation of mangrove and aquatic fauna. Influence of independent variable toward dependent variable values is analyzed with T-Test and Regression Test. All of the statistics matters are performed with the assistance of SPSS 20 software.

RESULTS AND DISCUSSION

The diversity of mangrove aquatic fauna

Aquatic fauna is observed in mangrove area of Purworejo District, and it consists of 19 families and 34 species (Table 1, Figure 2). *Mugil cephalus* is a common species found in all stations and belongs to Family of Mugilidae. This condition is possible since the characteristic of mobility of *M. cephalus* which prefers to clump in surface area of water column. Family Mugilidae is classified as a group of fish with excellent adaptation ability. Thus, they would be found in almost all type of waters, especially in estuary, sub-tropic and tropical ocean, such as Indo-Pacific and South China Sea until Australia (Carpenter and Niem 1999).

Scylla serrata (mud crabs or mangrove crabs) and *Uca rosea* (rose fiddler crabs) are discovered in all research station. This condition is influenced by the type of substrates, a soft sandy loam. Crabs are categorized as invertebrates and considered as aquatic fauna of mud substrates in mangrove ecosystem. According to Supriharyono (2007), aquatic fauna of mangrove

ecosystem is divided into two main groups: burrowing species and epifauna (species which have life cycle in surface of the substrates). Crabs are categorized as epifauna, especially genus *Uca* and Family Portunidae. Kathiresan and Bingham (2001) explained that the distribution and the presence of crabs in mangrove ecosystem may be affected by some environmental factors including mangrove litter, substrates character and salinity.

Faunus ater has a highest density in Station 1 and II. This species is a kind of macrozoobenthos which have ability to adapt in Pasir River by immersing themselves into waters, having low mobility and ability to catch their prey, and also having a long life cycle and a relatively high density in specific area (Pong-Masak and Pirzan 2006). Key factors of life sustainability on aquatic fauna are the ability to adapt in an environment with specific character (Strong et al. 2008), high reproduction ability and the absence of predator species (Marwoto and Istianingsih 2014).

Channa striata have highest density in Station 3. This condition is influenced by adaptation ability of the species in mangrove ecosystem. Station 3 is located in relatively far away from settlement. Thus, Station 3 will have a relatively low interfere of anthropogenic activities, especially from the hunt of fish, such as *C. striata*. The density of *C. striata* is also affected by the existence of scrubs (lower plants) which is an ideal habitat for this fish species.

The diversity index of aquatic fauna in Station 1, 2 and 3 could be categorized as moderate. Values of each diversity index are 2.30 (1), 2.22 (2) and 2.50 (3). This condition indicates a moderate complexity of aquatic fauna community, as an effect of good interaction between species. Biodiversity can be implemented to measure communities' stability. Definition of communities' stability is an ability of a community to protect their own stability by themselves.

Polychaeta is known as common group in mangroves. But, this group will never be found in Java Island. Polychaeta is usually found in mangroves area outside Java Island.

Table 1. Mangrove aquatic fauna observed in mangrove area of Purworejo District, Central Java, Indonesia

Class	Family	Species	Density (individuals/m^2)		
			Station 1	Station 2	Station 3
Actinopterygii	Gobiidae	*Periophthalmus novemradiatus*	0.290	0.028	-
		Boleophthalmus boddarti	0.118	0.017	-
	Clariidae	*Clarias batrachus*	0.006	0.005	0.008
	Channidae	*Channa striata*	0.012	0.010	0.079
	Bagridae	*Mystus nigriceps*	0.010	0.010	0.011
	Cichlidae	*Oreochromis mossambicus*	0.009	0.013	-
		Oreochromis niloticus	0.008	0.007	0.021
	Mugilidae	*Mugil cephalus*	0.036	0.074	0.010
	Osphronemidae	*Trichogaster trichopterus*	-	-	0.063
	Anabantidae	*Anabas testudineus*	-	-	0.049
	Synbranchidae	*Monopterus albus*	-	-	0.017
	Anguillidae	*Anguilla bicolor*	-	-	0.012
Malacostraca	Penaeidae	*Penaeus monodon*	0.015	0.013	-
		Penaeus merguiensis	0.009	0.005	-
		Penaeus sp.	-	-	0.019
	Alpheidae	*Alpheus microrhynchus*	0.006	-	0.014
		Alpheus euphrosyne	0.008	-	-
	Palaemonidae	*Macrobrachium equidens*	-	-	0.028
	Portunidae	*Scylla serrata*	0.020	0.008	0.007
		Scylla paramamosain	0.010	-	-
	Ocypodidae	*Uca annulipes*	0.080	0.013	-
		Uca crassipes	0.010	0.005	-
		Uca paradussumieri	0.020	0.007	-
		Uca rosea	0.017	0.007	0.008
		Uca tetragonon	0.013	0.011	-
		Uca vocans	0.026	0.009	-
		Uca vomeris	0.060	0.012	-
	Sesarmidae	*Chiromantes eumolpe*	0.008	0.011	0.009
		Metopograpsus sp.	-	0.014	0.011
		Perisesarma bidens	0.009	0.009	-
		Sarmatium germaini	-	-	0.012
Gastropoda	Pachychilidae	*Faunus ater*	0.441	0.216	-
	Potamididae	*Cerithidea obtusa*	0.045	-	-
Bivalvia	Corbiculidae	*Polymesoda expansa*	0.069	-	-
	Diversity index of Shannon Wiener		2.30	2.22	2.50

The diversity of mangrove vegetation

Identification of mangrove vegetation determines the existence of 15 families and 18 species in the mangrove area of Purworejo District, according to Noor et al. (2012) (Table 2, Figure 3). Family Rhizophoraceae, Sonneratiaceae, Arecaceae, and Asteraceae are discovered in all of the research stations. Family Rhizophoraceae consists of 2 species, i.e., *Rhizophora mucronata* and *Rhizophora stylosa*. Family Sonneratiaceae consists of 2 species, i.e. *Sonneratia alba* and *Sonneratia caseolaris*. Family Arecaceae consists of only single species, *Nypa fruticans*. Family Asteraceae is similar with Arecaceae which is one species only, *Wedelia biflora*. Some of the species are defined as mangrove species, based on their ability and conformity to adapt to a specific environmental condition of the mangrove ecosystem. The specific condition could be described as specific condition of abiotic which is dominated by sandy loam substrates.

Definition of density is sum of individuals of organisms per total research area. Tomlison (1994) defines mangrove density as sum of trees of specific type of mangrove in specific monitoring areas. Density of species is associated with distance of tree, sum of individuals, species of mangrove plants and the extents of research area. The value of density ratio will depend on sum of observed individuals which formed positive correlation in between. In this research, the value of mangrove density on specific phases of plant varies in each station (Table 3).

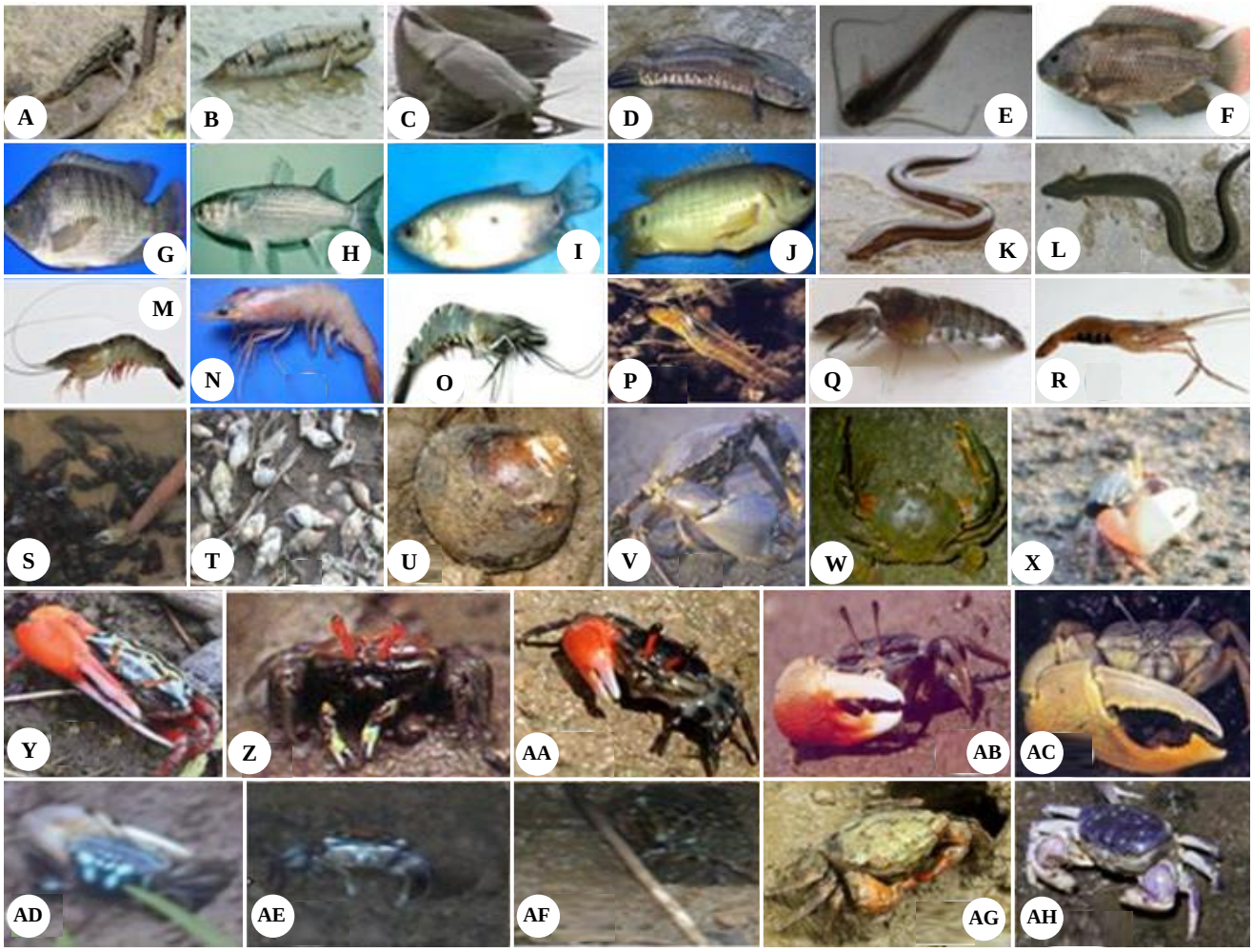


Figure 2. Diversity of aquatic fauna in Mangrove Area of Purworejo District, Central Java, Indonesia. Note: A. *P. novemradiatus*, B. *B. boddarti*, C. *C. batrachus*, D. *C. striata*, E. *M. nigriceps*, F. *O. mossambicus*, G. *O. niloticus*, H. *M. cephalus*, I. *T. trichopterus*, J. *A. testudineus*, K. *M. albus*, L. *A. bicolor*, M. *P. monodon*, N. *P. merguiensis*, O. *Penaeus* sp., P. *A. microrhynchus*, Q. *A. euphrosyne*, R. *M. equidens*, S. *F. ater*, T. *C. obtusa*, U. *P. expansa*, V. *S. serrata*, W. *S. paramamosain*, X. *U. annulipes*, Y. *U. crassipes*, Z. *U. paradussumieri*, AA. *U. rosea*, AB. *U. tetragonon*, AC. *U. vocans*, AD. *U. vomeris*, AE. *C. eumolpe*, AF. *Metopograpsus* sp., AG. *P. bidens*, AH. *S. germaini*

Table 2. List of discovered plants species in mangrove area of Purworejo, Central Java, Indonesia

Family	Species	Station
Acanthaceae	*Acanthus ilicifolius*	1, 2
Arecaceae	*Nypa fruticans*	1, 2, 3
Asclepiadaceae	*Calotropis gigantea*	1, 2
Asclepiadaceae	*Gymnanthera paludosa*	1, 2
Asteraceae	*Wedelia biflora*	1, 2, 3
Convolvulaceae	*Ipomoea pescaprae*	1, 2
Cyperaceae	*Scirpus* sp.	1
Malvaceae	*Hibiscus tiliaceus*	1, 2
Molluginaceae	*Sesuvium portulacastrum*	3
Passifloraceae	*Passiflora foetida*	2
Poaceae	*Setaria sphacelata*	3
Pteridaceae	*Acrostichum speciosum*	3
Rhizophoraceae	*Rhizophora mucronata*	1, 3
Rhizophoraceae	*Rhizophora stylosa*	2
Rubiaceae	*Morinda citrifolia*	2
Sonneratiaceae	*Sonneratia alba*	1
Sonneratiaceae	*Sonneratia caseolaris*	2,3
Verbenaceae	*Stachytarpheta jamaicensis*	2

Table 3. Density of species in mangrove area of Purworejo, Central Java, Indonesia

Station	Species	Density (ind./ha)		
		Trees	Shrubs	Herbs
1	*R. mucronata*	400	7260	71125
	N. fruticans	20	40	500
	H. tiliaceus	5	160	125
	S. alba	15	-	-
	G. paludosa	-	-	45375
	A. ilicifolius	-	-	20625
	I. pescaprae	-	-	29500
	C. gigantea	-	-	375
	Scirpus sp.	-	-	16625
	W. biflora	-	-	4250
	Sum total	440	7460	188500
2	*N. fruticans*	185	60	500
	R. stylosa	15	860	8500
	S. caseolaris	210	1380	21500
	H. tiliaceus	-	100	-
	M. citrifolia	-	20	-
	G. paludosa	-	-	44500
	A. ilicifolius	-	-	26375
	S. jamaicensis	-	-	30125
	I. pescaprae	-	-	13750
	P. foetida	-	-	3500
	C. gigantea	-	-	2125
	W. biflora	-	-	1125
	Sum total	410	2420	152000
3	*S. alba*	190	340	15125
	S. caseolaris	105	640	6375
	N. fruticans	25	140	250
	R. mucronata	-	40	-
	A. speciosum	-	-	27250
	S. portulacastrum	-	-	62625
	W. biflora	-	-	4125
	S. sphacelata	-	-	30875
	Sum total	320	1160	146625

In herbs phase, the highest density value of mangrove plants is *R. mucronata* which is obtained from Station 1 (71125 ind./ha). *R. mucronata* is purposely planted in Station 1 as parts of mangrove rehabilitation. Similar with herbs phase, *R. mucronata* becomes the species with highest density on shrubs phase (7260 ind./ha). *R. mucronata* is usually sprouted in a clump, either near or on the riparian zones. This species has an ability to bloom all over the year and germinate on the parental plants. The plants will be released as propagules which stick to the substrates as new plants. *R. mucronata* prefers an inundation area as suitable habitat, similar to the condition of Pasir River.

Similar to other phases, phase of tree on mangrove plants results *R. mucronata* as species with highest density in Station 1 (400 ind./ha). This condition is influenced by regeneration type of *R. mucronata* which happened in seeds form with the help of tidal waves. Tidal waves will facilitate the deployment of seeds throughout the mangrove areas. The seeds have roots in both ends which uses as the fastening tool on substrates on low tide. This condition will enable the plants to grow upright. Supardjo (2008) described that *R. mucronata* is a kind of pioneer plants with ability to grow on soft mud.

Its adaptability on brackish water with extreme salinity influences the density value of *R. mucronata.* The flora communities in mangrove forest have undergone an adaptation and specialization process as natural mechanism to survive in an environment which has high salinity levels (Kustanti 2011). The adaptability of mangrove plants is by removing the salt excess inside their tissues (Indriyanto 2012). Thus, they could grow better in mangrove ecosystem. Highest value of density on *R. mucronata* correlates with reproductive ability. This species has propagules with compliant shapes and attributes to survive in extreme condition. The propagules have a big and elongated shape, are easy to spread by sea-waves and have plentiful reserves of food.

Biodiversity of mangrove is assessed with diversity index of Shannon Wiener. Analysis of biodiversity is used to measure the stability of community which has a definition as ability of specific community to protect themselves in harmony against the occurrence of disruption of their components. Diversity index is also used to measure an abundance of species based on the numbers of species and sum of individuals in specific location. Variation in community has positive correlation with the numbers of species. Diversity index is significantly influenced by numbers of species and sum of individuals. According to the research, value of diversity index in each station varies (Figure 3).

Diversity index of mangrove vegetation on herbs phase can be categorized as moderate. This condition is obtained in all stations. Highest value of biodiversity is observed in Station 2 (1.84), and the lowest is in Station 3 (1.49). It means that the ecological pressure is on moderate level in research location. The observation in Station 2 gives result that 10 species of mangrove plants are found on herbs phase. The species consist of *S. caseolaris, R. stylosa, N. fruticans, G. paludosa, A. ilicifolius, S. jamaicensis, I.*

pescaprae, *P. foetida*, *C. gigantea*, and *W. biflora*. While, the observation in Station 3 results 7 species of mangrove plants on herbs phase, namely: *S. alba*, *S. caseolaris*, *N. fruticans*, *A. speciosum*, *S. portulacastrum*, *W. biflora*, and *S. sphacelata*.

Diversity index of mangrove vegetation on shrubs phase is classified as low level in Station 1 and II (H' value: 0.14 and 0.95) while in other station (Station 3), it is classified as moderate level (1.06). Four species are obtained in Station 3, including *S. alba*, *S. caseolaris*, *R. mucronata*, and *N. fruticans* while only three species are obtained in Station 1 namely *R. mucronata*, *N. fruticans*, and *H. tiliaceus.*

Diversity index of mangrove vegetation on trees phase are categorized as low level in all station. The highest diversity index is obtained in Station 3 (0.87) and the lowest one is in Station 1 (0.39). It means that the ecological pressure is on high level, it was an effect of many environmental problems (probably from anthropogenic activities). Observation of Station 3, result in 3 species of mangrove plants on the tree phase, consisting of *S. alba*, *S. caseolaris*, and *N. fruticans.* The abundance levels of each species are relatively in similar values. Observation of Station 1, gives result in 4 species of mangrove plants on trees phase, consisting of *R. mucronata*, *S. alba*, *N. fruticans*, and *H. tiliaceus.* The abundance level of each species in this Station is significantly different.

Relation between vegetation and aquatic fauna of mangrove

Correlation test between diversity index of vegetation and aquatic fauna obtains a weak negative correlation between both of the variables on all stations (R:-0.104). These results mean a weak and probably no significant correlation between vegetation and aquatic fauna in research location. The increase of aquatic fauna diversity will reduce the vegetation diversity (Figure 4). This condition is influenced by the existence of the herbivore and the climber species which consuming and damaging mangroves. The species categorized as herbivore and climbers are for example *Metopograpsus* sp., *C. eumolpe*, and *S. germaini*.

Zakaria and Rajpar (2015) explain that Marudu Bay mangrove habitats harbored a diversity of fauna species including 22 aquatic invertebrate species (encompassing 11 crustacean species, six mollusk species, and four worm species) and 36 fish species. The difference results about the vegetation and aquatic fauna between Purworejo and Marudu mangrove are due to different location and vegetation structure there.

Diversity of aquatic fauna illustrated that mangrove area in Purworejo serves as a nursery ground for various juvenile fish communities. It may be because these areas are rich in invertebrate assemblages such as crustaceans and mollusks. In addition, the extensive root systems of mangroves create habitat heterogeneity and complexity, offering suitable foraging sites for juvenile fishes and protecting them from predators by reducing their visibility. Habitat heterogeneity and complexity is a major factor that influences fauna diversity (Kovalenko 2012).

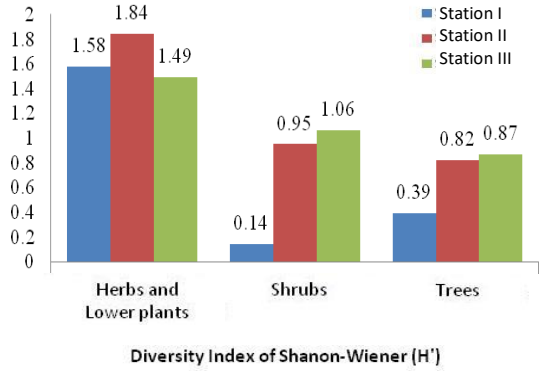


Figure 3. Diversity index of mangrove vegetation in mangrove area of Purworejo, Central Java, Indonesia

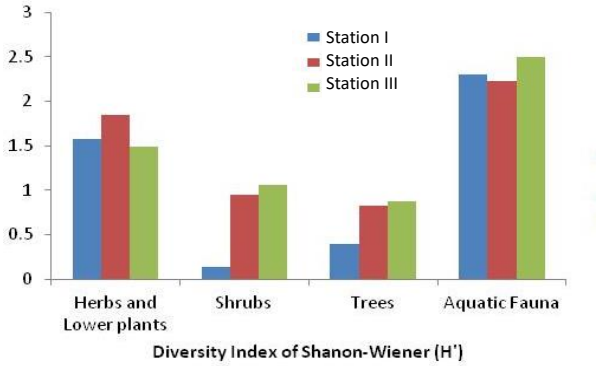


Figure 4. Diversity index of vegetation and aquatic fauna in mangrove area of Purworejo, Central Java, Indonesia

Influential value of abiotic factors towards vegetation and aquatic fauna

Regression analysis of abiotic factors to biodiversity of mangrove vegetation

The regression test placed diversity index of mangrove vegetation (in all phase) as dependent variable (y). The independent variables include temperatures (x_1), pH (x_2), dissolved oxygen (x_3) and salinity (x_4). According to the analysis, R^2 value is 0.129. It shows a weak effect of independent variables to dependent variable. The impact value is 12.9%, and the rest is influenced by other factors which are included as research subjects.

Research on mangrove ecophysiology shows the adaptation on the intertidal environment. The environment has a heterogenic salinity, filled with water and lack of nutrients. Mangrove is not affected significantly by a change of salinity or an extreme salinity (Naidoo 2016). Mangrove is a halophyte facultative which undergo their lifecycle in a saline condition (Krauss and Ball 2013).

The changes of abiotic values give only very weak impact on diversity of mangrove vegetation. By the way, this condition is happened only in specific location but for

all phase of plants. The impact value is probably changed in other location or on specific phase of plants as an effect of different dependence of each phase on abiotic condition.

$$Y = a + b_1x_1 + b_2x_2 + ... + b_nx_n$$

Description:

Y is diversity index of mangrove vegetation on all phases of plants

x_1 is temperatures, x_2 is pH, x_3 is DO, and x_4 is salinity.

a and b are constants of regression

The regression analysis is determined by the equation

$$Y = 3.826-0.052(x_1)-0.234(x_2) + 0.072(x_3) + 0.034(x_4)$$

Regression analysis on abiotic factors against diversity index of aquatic fauna

The regression test places diversity index of aquatic fauna as dependent variable (y). The independent variables include temperatures (x_1), pH (x_2), dissolved oxygen (x_3) and salinity (x_4). According to the analysis, R^2 value is 0.032, and it shows a weak effect of independent variables to dependent variable. The impact value is 3.2%, and the rest is influenced by other factors which are included as research subjects. The changes of abiotic values give only a very weak impact on diversity of aquatic fauna. The impact is weaker than similar abiotic factors on vegetation as the effect of mobility of aquatic fauna.

$$Y = a + b_1x_1+...+b_nx_n$$

Y is diversity index of aquatic fauna

x_1 is temperatures, x_2 is pH, x_3 is DO, and x_4 is salinity.

a and b are constants of regression

The regression analysis is determined by the equation

$$Y = 7.058-0.203(x_1) + 0.174(x_2)-0.163(x_3) + 0.084(x_4)-0.270(x_5)$$

According to the research, 34 species of aquatic fauna are from mangroves area of Purworejo, and the result that the highest value of aquatic fauna is in Station 3 (ID 2.50). Pearson correlation test between vegetation and aquatic fauna shows that R-value is 0.104. The T-test determines a significant difference (sig 0.000) between abiotic factors and biotic condition which also shows the impact of changes between them. Further test with regression method also indicates a weak impact on changes in abiotic factors towards biotic condition (R^2 0.032).

Mangrove rehabilitation efforts were done properly, especially in relation to maintaining of the aquatic fauna diversity (macrobenthos). Research from Bosire et al. (2004) gave a result that an increase of macrobenthic diversity happened as the effects of reforestation on the over-exploited mangroves. The step should be aligned with reduction of pollutant which considered to give similar impacts on increasingly of macrobenthic diversity (Leung and Cheung 2017).

Based the results of this study, it is concluded that Purworejo mangrove area must be protected sustainably to protect its diverse aquatic fauna species for future generations.

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Short communication: Diversity of tropical plants and their attractant properties for subterranean termite *Coptotermes curvignathus*

YULIATI INDRAYANI[1,♥], MUSRIZAL MUIN[2,♥♥], TSUYOSHI YOSHIMURA[3,♥♥♥]

[1]Faculty of Forestry, Universitas Tanjungpura. Jl. Imam Bonjol Pontianak 78124, West Kalimantan, Indonesia. Tel./Fax. +62-561-767673, ♥email: mandaupermai@yahoo.com

[2]Faculty of Forestry, Universitas Hasanuddin. Jl. Perintis Kemerdekaan km 10, Makasar 90245, South Sulawesi, Indonesia. ♥♥email: musrizal@unhas.ac.id

[3]Research Institute for Sustainable Humanosphere, Kyoto University. Gokasho, Uji, Kyoto 611-0011, Japan. ♥♥♥email: tsuyoshi@rish.kyoto-u.ac.jp

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Abstract. *Indrayani Y, Muin M, Yoshimura T. 2017. Short communication: Diversity of tropical plants and their attractant properties for subterranean termite* Coptotermes curvignathus. *Biodiversitas 18: 1353-1357.* The effectiveness of bio-based attractant from different leaves of tropical plants on subterranean termites *Coptotermes curvignathus* was examined under laboratory conditions. Five kinds of leaves of tropical plant such as cajuput (*Melaleuca leucadendron* L.), cinnamon (*Cinnamomum burmannii* (Nees & Th. Nees) Nees ex Blume, clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry), bay (*Syzygium polyanthum* Wight) and basil (*Ocimum sanctum* L.) were serving as termite feeding bio-based attractants for *C. curvignathus*. Bio-based attractants were obtained by cross cut dry leaves into smaller particles (dry weight 200 g) and dipped in 70% ethanol solution for 3 x 24 hrs. The extracts were then subjected to a rotary evaporator at 60 ºC until the extract changed to gel condition. 50 workers and five soldiers of *C. curvignathus* were tested in a no-choice test bioassay to determine their preferred bio-based attractant under the concentration of 10% and 50%. No-choice bioassays chambers were assembled by connecting two clear plastic glasses (bottom diameter 5 cm, top diameter 7 cm) with clear plastic hoses (inner diameter 3 mm, outer diameter 5 mm). Clear plastic hoses connectors allowed termites to move freely between glasses. A laboratory bioassay determined that the response of subterranean termite *C. curvignathus* on clove leaf was greater than other five leaves as indicated by termite moving time, and did not differ significantly between concentrations. No direct relationships were noted between the speed of the first termite moving time and number of termites recruited to the bio-attractant chamber.

Keywords: Bio-attractant, *Coptotermes curvignathus*, diversity, subterranean termite, tropical leaves plant

INTRODUCTION

Subterranean termites are one of the most important termite pests in Indonesia even in the world. Subterranean termites have been found with a widespread global distribution (Henderson 2008; Rust and Su 2012). Methods have been widely used for subterranean termite control, such as bait technologies and liquid termiticides (Henderson, 2001). Liquid termiticides for a building are applied by drilling holes into the walls and slabs (Rambo 1985). In general, liquid termiticides were categorized into two groups. First is repellent that serves as a barrier between termites in the soil and the structure above ground; second is non-repellent which acts to kill termites upon contact (Gahlhoff and Koehler 2001; Kard 1998, 2000; Thorne and Breisch 2001). However, liquid termiticides have several limitations such as (i) it only affects termite population that is located in the treated areas, (ii) termites exposed to the non-repellent termiticides might die before returning to its nest to spread the toxicant to other nest mates (Forschler 1994; Su and Scheffrahn 1988, 1990; Su et al. 1993), (iii) the active ingredients in liquid termiticides will be degraded over time in soil and have to be reapplied (Mauldin et al. 1987).

Although liquid termiticides application is still the most widely used method for subterranean termite control, new methodologies are required to overcome some of their limitations. One of those new methodologies has been the termite bait systems. The main concept of bait system is the slow killing action property of the toxicant in affecting the termites. These bait systems are designed to allow the worker termites to feed the toxicant and live long enough to return to the nest to spread it to other nest members (Dow AgroSciences 1999).

Like liquid termiticides, bait systems also have their limitations. One of those limitations is the presence of competing for food resources. Termites have many food resources in the field, such as building structures or natural food sources. If natural food sources are more attractive than the bait, termites might not be recruited to the bait. As Waller et al. (1999) stated that one of the factors affecting termite recruitment is chemical directional cues to the location of the bait, this chemical is known as an attractant. Therefore, it is indispensable to find proper attractive food sources for bait matrix. Termite colony depend on worker termites for its food supply. Thus, a bait should be formulated with an attractant to recruit the worker termites as much as possible to the bait.

In principle, termite will follow a concentration gradient of the attractant material to find food sources in the bait matrix. So far, attractants utilized in the bait system consist of synthetic chemicals, and there has been no report on the effect of synthetic attractants to the environment. Thus, we think it is necessary to develop a bio-based attractant from nature. From this study, we hope to develop a technique to ensure termites to locate baits and answer the question on how termites find food sources in nature.

The objective of this research was to investigate the effectiveness of the bio-based attractant from five different leaves of tropical plants on subterranean termite (*Coptotermes curvignathus* Holmgren, 1913) in the laboratory condition.

MATERIALS AND METHODS

Termites

A number of 50 workers and five soldiers subterranean termites were obtained from secondary forest, Pontianak, West Kalimantan, Indonesia in June 2016. Termites were maintained in the plastic chamber with rubber wood (*Hevea brasiliensis*) at 28-30ºC and 70-80% relative humidity for one month before used in the bioassay. Only healthy termites used for experiments.

Attractant preparation

Five kinds of leaves, cajuput leaf, cinnamon leaf, clove leaf, bay leaf and basil leaf were collected from an area in Pontianak City, West Kalimantan, Indonesia in March-April 2016. Before being used in experiment, the leaves were air dried inside a room for three days. Dry leaves were then cross cut into smaller particles (with a total dry weight of 200 g) and dipped in 70% ethanol solution for 3 x 24 hrs. The extracts were then subjected to a rotary evaporator at 60 ºC until the extract changed to gel condition.

Termite bioassay

Bioassay chambers

Consumption bioassays were performed in no-choice bioassays chambers. Tests chamber was used to evaluate termite moving time and number of termites recruited to bio-attractants upon the presence of a competing food source.



Figure 1. No choice-test bioassay

No-choice bioassay

No-choice bioassays chambers were assembled by connecting two clear plastic cups (bottom diameter 5 cm, top diameter 7 cm) with a clear plastic hose (inner diameter 3 mm, outer diameter 5 mm). The plastic hose acts as a connector that allowed termites to move freely between glasses. Both plastic cups were filled with 50 g moist sand, and the surface of the sand was covered with a plastic sheet (Figure 1).

A sheet of cellulose paper with a diameter of 4 cm containing an attractant (concentration of 10% and 50%) was placed on top of the sand substrate in one of the plastic cups, while a same size cellulose paper that was added with distilled water was put on the sand substrate in the other plastic cup. In each experiment, 50 workers and five soldiers of *C. curvignathus* were subjected into the plastic cup containing the sheet of cellulose paper with distilled water. Termite moving activities were then monitored for 8 hours. Bait efficacy is determined by the number of termites that are moving to the attractant-supplemented cellulose paper. Each treatment consisted of three replicates.

Identification of chemical components using GC-MS

Five leaves crude extract was analyzed using a Shimadzu Gas Chromatography-Mass Spectrometry (GC-MS-QP2010) with a Flame Ionization Detector (FID) using fused silica capillary column type phase Rtx-5MS (60 m by 0.25 mm ID) with helium as carrier gas at a flow rate of 0.85 mL per minute. The column temperature was programmed initially at 50ºC for 10 min, then increased 3ºC per minute to 280ºC and was kept isothermally for 1 min. The temperature of injector port and interface of mass spectrometry (MS) was 280ºC. The total ion chromatogram obtained as auto integrated by Chemstation software and the compounds were identified by comparison with Wiley 9th edition NIST 11 Mass Spectral Library (Indrayani et al. 2016).

Data analysis

Data were statistically analyzed by Variable Analysis in a factorial design, and Tukey Analysis was used for comparison of the mean values (Gaspersz 1994).

RESULTS AND DISCUSSION

Termite moving time

In this experiment, a significantly faster termite movement to the bio-based attractant chamber was noted in both clove leaf treatment concentration (10% and 50%), which were under 1.5 minutes. The termite movement time in the clove leaf treatment was significantly lesser than that of all other leaves (Tukey's test: $P < 0.05$) (Figure 2). In treatments other than clove leaf, the termite moving moment was noted after 7 to 14 minutes for the concentration of 10% and 4 to 11 minutes for the concentration of 50%, except that of the cinnamon leaf treatment which was at 1.58 minutes. There were also significantly lesser moving times of termites in the concentration of 50% than that in the concentration of 10%

for all kind of leaves except for clove leaf (Tukey's test: $P < 0.05$). The least moving time of termite observed in the clove leaf treatment was presumably because clove leaf contains a higher level of eugenol among the five leaves. Eugenol has been reported to successfully recruit fruit flies (Stark et al. 2000). GC-MS analysis showed relative percentage (%) of eugenol compound in each leaf extracts used in this study: clove leaf 7.92%, cinnamon leaf 4.64%, cajuput leaf 3.36%, basil leaf 2.24% and bay leaf 0% (Figure 4.A-E). GC-MS analysis results of the clove and cajuput leaves in this experiment were the same as those in the previous experiment (Indrayani et al. 2016). Consistent with its zero-eugenol content, basil leaf showing the latest termite moving time of all leaf extract treatment which was after 14 minutes. Control treatment in this study, i.e. without any bio-attractant addition, showed the slowest termite moving time (108.47 minutes).

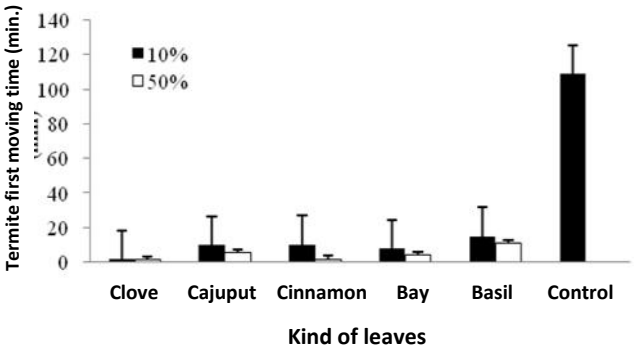


Figure 2. Termite first moving time (minutes) entering attractant chamber

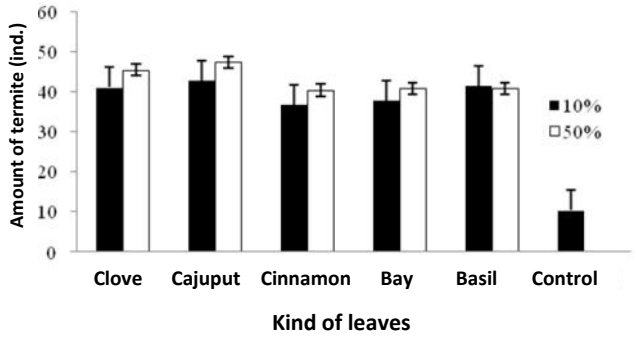


Figure 3. Number of termite entering attractant chamber after 8 hours observation

Number of termites entering attractant chamber

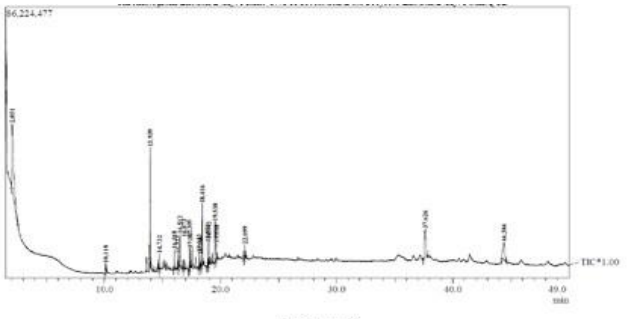
Our study showed that there was no significant difference in the number of termites recruited to the attractant chamber in all leaves treatment for both concentrations of 10% and 50 % (Tukey's test: $P < 0.05$). This result indicates that there is no direct relationships between termite moving time and the number of termites recruited in bio-based attractants. Figure 3 illustrates the number of termites accumulated inside the attractants chambers after 8 hours observation. From the initial amount of 50 worker termites introduced into the starting chamber, the average numbers of termites found to be recruited to the leaf-based attractant chambers was approximately 40 individuals at the concentration of 10% and about 43 individuals at the concentration of 50%. As a reference, the number of termites that moved towards the control chamber containing a sheet of cellulose paper with distilled water was only about 10 individual termites.

These results revealed that termites could detect chemicals in the substrates and move towards chambers containing bio-based attractants. Furthermore, these results also indicated that eugenol compounds in the leaf extracts have the potential to attract termites to find the bait matrix. These results are similar to those reported by Waller et al. (1999) in which a greater number of worker termites was recruited to chambers containing sucrose and yeast than those that remained in the water chamber. A similar finding was also found by Sajap et al. (2005) who worked with noviflumuron as the bait active ingredient to attract *Coptotermes gestroy*.

In conclusion, this study highlights that extracts of plant leaf used as bio-based attractants for termite *C. curvignathus* were found to be effective in the laboratory test. Laboratory bioassay showed that among the five plant leaves observed in this study, the greatest response of the subterranean termite regarding their first moving time could be found on clove leaf-based attractants, regardless of the attractant concentrations. In addition, no direct relationships were noted between the speed of termite moving time and the number of termites located in bio-based attractants. Further works are still required, in particular, concerning the effectiveness of eugenol as an attractive compound for the development of a termite bait system.

ACKNOWLEDGEMENTS

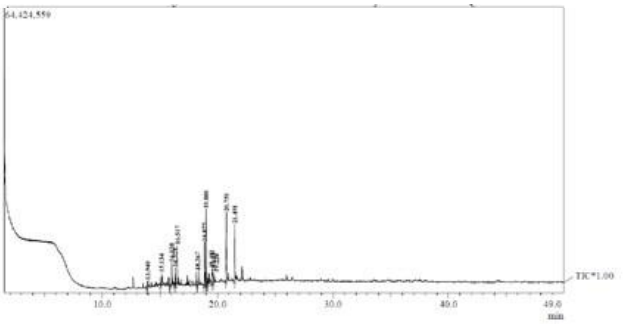
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Peak Report TIC

Peak#	R.Time	Area	Conc%	Name
1	2.051	245731140	35.46	Propane, 2-fluoro- (CAS) 2-Fluoropropane
2	10.118	6675181	0.96	Cyclohexene, 1-methyl-4-(1-methylethenyl)- (CAS) 1-P-MENTHA-1,8-DIENE
3	13.939	54851031	7.92	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol
4	14.732	7604224	1.10	Benzene, 1,2,3-trimethoxy- (CAS) 1,2,3-Trimethoxybenzene (CAS) Methylsyr
5	16.018	11823600	1.71	Guaiol
6	16.323	8642122	1.25	Guaiol
7	16.512	31218595	4.51	.beta.-Eudesmol
8	16.873	6006466	0.87	2,5-Dimethyl-3,4,6-trimethoxybenzaldehyde
9	17.309	12082544	1.74	Benzyl benzoate
10	17.367	8425715	1.22	2',3',4' Trimethoxyacetophenone
11	18.133	7379705	1.06	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) METHYL 14-METHYL-P
12	18.258	9057546	1.31	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
13	18.416	33139356	4.78	Hexadecanoic acid (CAS) Palmitic acid
14	18.873	10859092	1.57	1,4-Naphthalenedione, 5,8-dihydroxy-2-methoxy- (CAS) 1,4-Naphthoquinone
15	18.992	12688220	1.83	4H-1-benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-methyl-6-(3-me
16	19.538	54309086	7.84	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid
17	19.648	14844139	2.14	9-Octadecenoic acid (Z)- (CAS) Oleic acid
18	22.099	9028883	1.30	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate
19	37.626	85837182	12.39	Olean-12-en-28-al, cyclic 1,2-ethanediyl mercaptal (CAS)
20	44.384	62744333	9.05	
		692948160	100.00	

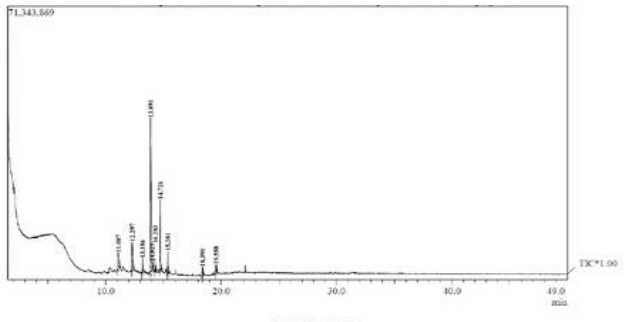
Figure 4.A. GC-MS analysis result of clove leaf



Peak Report TIC

Peak#	R.Time	Area	Conc%	Name
1	13.940	6799739	3.36	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol
2	15.134	9188052	4.54	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS) ar-Curcumene
3	16.020	12085258	5.97	Guaiol
4	16.325	6859341	3.39	alpha.-Gurjunene
5	16.517	22725186	11.22	Guaiol
6	18.267	7149964	3.53	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
7	18.877	21016358	10.38	1,4-Naphthalenedione, 5,8-dihydroxy-2-methoxy- (CAS) 1,4-Naphthoquinone
8	19.001	27491771	13.58	4H-1-benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-methyl-6-(3-me
9	19.226	11411867	5.64	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
10	19.481	16323310	8.06	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
11	19.645	9624680	4.75	9-Octadecenoic acid (Z)- (CAS) Oleic acid
12	20.751	28102959	13.88	1-Phenyl-3-(p-chlorophenyl)-1,3-propandione
13	21.491	23687037	11.70	2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- (CAS) Pi
		202465522	100.00	

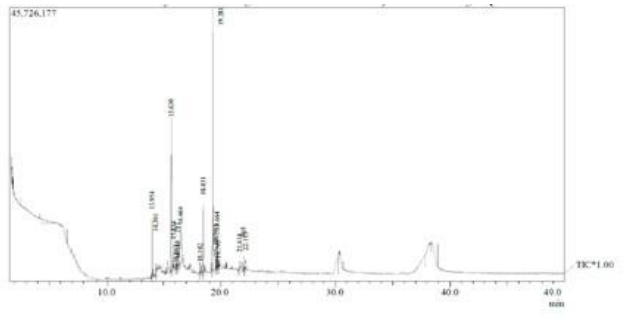
Figure 4.B. GC-MS analysis result of cajuput leaf



Peak Report TIC

Peak#	R.Time	Area	Conc%	Name
1	11.087	15996227	7.27	Phenol, 2-methoxy- (CAS) Guaiacol
2	12.297	21404599	9.73	2-Methoxy-4-methylphenol
3	13.186	9538226	4.34	2,5-Dimethoxytoluene
4	13.891	115439902	52.47	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
5	14.025	4974816	2.26	Phenol, 2-methoxy-4-propyl- (CAS) 5-PROPYL-GUAIACOL
6	14.283	4918670	2.24	Benzene, 1,2-dimethoxy-4-(2-propenyl)- (CAS) Methyleugenol
7	14.726	31568104	14.35	Benzene, 1,2,3-trimethoxy- (CAS) 1,2,3-Trimethoxybenzene (CAS) Methylsyr
8	15.381	9816310	4.46	Benzene, 1,2,3-trimethoxy-5-methyl- (CAS) Toluene, 3,4,5-trimethoxy-
9	18.390	3092094	1.41	Hexadecanoic acid (CAS) Palmitic acid
10	19.558	3270520	1.49	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate
		220019468	100.00	

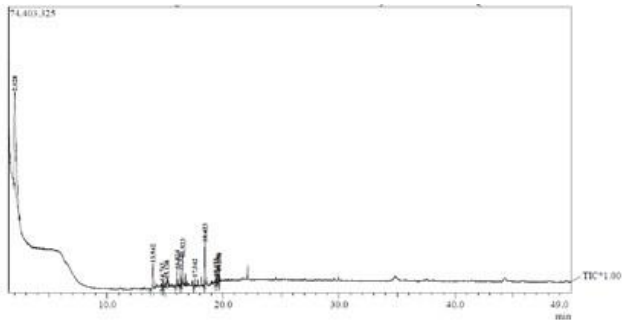
Figure 4.C. GC-MS analysis result of basil leaf



Peak Report TIC

Peak#	R.Time	Area	Conc%	Name
1	13.954	16056687	4.64	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol
2	14.301	2241022	0.65	Benzene, 1,2-dimethoxy-4-(2-propenyl)- (CAS) Methyleugenol
3	15.630	67599859	19.54	Dodecanoic acid (CAS) Lauric acid
4	15.834	7458324	2.16	Naphthalene, 1,4,6-trimethyl- (CAS) 1,4,6-Trimethylnaphthalene
5	16.067	2347607	0.68	Naphthalene, 1-methyl-7-(1-methylethyl)- (CAS) 1-Methyl-7-isopropylnaphth
6	16.158	2502578	0.72	
7	16.217	20564062	5.94	.alpha.-D-Glucopyranoside, methyl (CAS) Methyl .alpha.-D-glucopyranoside
8	16.464	61637733	17.81	QUINIC ACID
9	18.142	2257546	0.65	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate
10	18.431	21125977	6.11	Hexadecanoic acid (CAS) Palmitic acid
11	19.281	103677477	29.96	9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate
12	19.551	7302834	2.11	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid
13	19.592	4221713	1.22	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate
14	19.664	12583255	3.64	9-Octadecenoic acid (Z)- (CAS) Oleic acid
15	19.795	2673803	0.77	Heptadecanoic acid, ethyl ester (CAS) Ethyl n-heptadecanoate
16	21.614	2310229	0.67	7-Hexadecenoic acid, methyl ester, (Z)-
17	21.965	5503764	1.59	19-Norpregn-4-ene-3,20-dione, 10-vinyl- (CAS) 19-METHYLENE-PROGEST
18	22.113	3970265	1.15	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate
		346034735	100.00	

Figure 4.D. GC-MS analysis result of cinnamon leaf



Peak Report TIC

Peak#	R.Time	Area	Conc%	Name
1	2.028	262719687	61.44	1-Propanethiol (CAS) Propanethiol
2	13.942	27957003	6.54	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (CAS) Aceteugenol
3	14.743	6998353	1.64	Benzene, 1,2,3-trimethoxy- (CAS) 1,2,3-Trimethoxybenzene (CAS) Methylsyr
4	15.138	26242038	6.14	Calarene
5	16.024	11582378	2.71	Guaiol
6	16.325	6610703	1.55	10-epi-.gamma.-eudesmol
7	16.523	24160318	5.65	Elemol
8	17.542	4625197	1.08	3-Eicosyne (CAS)
9	18.423	26521467	6.20	Hexadecanoic acid (CAS) Palmitic acid
10	19.372	7785155	1.82	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol
11	19.536	12576961	2.94	9-Octadecenoic acid (Z)- (CAS) Oleic acid
12	19.658	9842143	2.30	Octadecanoic acid (CAS) Stearic acid
		427621383	100.00	

Figure 4.E. GC-MS analysis result of bay leaf

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Use of DNA barcode in the identification of catfishes (Siluriformes: Ariidae) from Malaysia

MOHD LUTFI ABDULLAH, SITI AZIZAH MOHD NOR, DARLINA MD. NAIM♥
School of Biological Sciences, Universiti Sains Malaysia. 11800 Penang, Malaysia. Tel./Fax.: +60-04 653 4056, ♥email: darlinamdn@usm.my

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Abstract. *Abdullah ML, Nor SAM, Naim DMd. 2017. Use of DNA barcode in the identification of catfishes (Siluriformes: Ariidae) from Malaysia. Biodiversitas 18: 1358-1366.* The genus Ariidae contains many valuable fish species threatened by overfishing, but knowledge on distribution and threats is still limited due to taxonomic ambiguities. The aim of this study was to apply DNA barcoding techniques to establish a resource of DNA for identification of Ariidae species in Malaysia. A 621 bp of mitochondrial cytochrome oxidase subunit I (COI) gene was utilized to resolve phylogenetic relationships and molecular taxonomy of eight presumed Malaysian Ariid species. We found the monophyly of most species was well established with a mean Kimura-2 parameter (K2P) interspecies distance of 9.6% except for two species, *Arius venosus*, and *Nemapteryx caelata* that have very low interspecies genetic distance. The BLAST result shows only two species matched the presumably eight identified fish species. Such discrepancies could arise as a result of misidentifications or errors in GenBank database input, hybridization or incomplete lineage sorting. We suggest the use of DNA barcoding is integrated into the workflow during taxonomic studies as it could significantly increase knowledge about species distributions.

Keywords: Ariidae, COI, DNA barcoding, phylogenetic, conservation

INTRODUCTION

Ariidae are distributed in marine, brackish and fresh waters of warm temperate and tropical regions of the world. Most are confined to coastal and marine habitats, but some were also found in fresh water rivers, streams, and lakes. Ariidae are locally abundant in mangrove areas, large rivers and turbid waters, as well as in fully marine waters (to 150 m) and clear fresh waters (Kailola 2004; Marceniuk and Menezes 2007). There are approximately 25 species of 11 genera reportedly found in Malaysia (Yusri et al. 2010). Most ariids have high economic value due to their large size, local abundance, hardiness and flesh quality (Carpenter and Niem 1999). They are captured using a variety of gears such as demersal trawl, gill nets, and seines and are sold fresh, smoked and brined (Carpenter and Niem 1999). In Malaysia, the Department of Fisheries (2006, 2007) reported that approximately 11,645 tons of ariids species were harvested in 2006, but the number was increasing to 12,657 tons in 2007. From the 12,657 tons captured in 2007, 7,515 tons were from the west coast, 928 tons from the east coast and 4,214 tons from Sarawak, Sabah and the Federal Territory of Labuan. The increment of yearly ariids captured of about 9% inferred that they have a high demand value in the market.

Analogous to the increasing demands of fish products in recent years giving rise to the flow of more players into this business. This has encouraged economic deception that involves misbranding and false labelling of costly fish species with lower priced species (Rasmussen et al. 2009; Miller and Mariani 2010). Furthermore, such misidentification has also raised food safety concern such as cases of toxic puffer fish samples that mislabelled as 'headless monkfish' or other harmless products (Cohen et al. 2009). Fisheries conservation and sustainability have also been neglected to the point of commercializing overexploited species (Jacquet and Pauly 2008). There are many cases of substitution of closely related species from other countries or continent among commercialized fish due to an ambiguous nomenclature. Some species are grouped into a single name such as tuna (Lowenstein et al. 2009) while there are also singular species with multiple nomenclatures (Barbuto et al. 2010).

Indeed, understanding the taxonomic details of a species is central to the development of successful management strategies for sustainable fisheries resources (Abu Bakar and Md. Naim 2014). For centuries, taxonomic identification of fishes is mainly morphologically based. However, there are limitations to relying primarily on morphological-based characterization when attempting to identify animal species during various stages of their development or when examining fragmentary or processed remains. In other words, morphological distinction alone is inadequate for species identification or for elucidating valid phylogenetic relationships (Grewe et al. 1993; Felsenstein 2001).

Taxonomic ambiguity exists for several fish/genera including ariid family. Although the morphological characteristics have been outlined and used, however, the technique has been considered inconsistent and of limited information (Marceniuk and Menezes 2007). To illustrate, Kailola (2004) have recognized 23 valid genera of ariids which later revised by Marceniuk and Menezes (2007) who successfully recognized 26 valid genera. Afterward, recent application of molecular techniques aid in the revision of

the ariids taxonomy. Interestingly, the study done by Betancur and Acero (2007) are incongruent with the classification suggested by both Kailola (2004) and Marceniuk and Menezes (2007). Obviously, such complexity within Ariidae requires a tool which can help to solve the taxonomic problems.

Due to the above taxonomic uncertainty, we utilize the mitochondrial DNA cytochrome oxidase subunit I (COI) gene to investigate the phylogenetic relationships among the Malaysian Ariidae using a DNA barcoding technique, which was declared as one of the increasingly important taxonomic tools for species detection (Hebert et al. 2009). The COI is one of the most conserved protein-encoding genes in the mitochondrial genome, hence, it is used to catalog animal biodiversity (Hebert et al. 2010; Ward et al. 2009). The principal aims of this study are to examine the ability of the COI gene as a DNA barcode for ariids species and to genetically identify the ariids species from Malaysia. It is anticipated that this research will provide a baseline genetic data that are required for planning the management strategy for fisheries resource in Malaysia.

MATERIALS AND METHODS

Sample collection

A total of 45 individuals that represent eight species of Ariidae namely *Netuma thalassina* (n=9), *Plicofollis argyropleuron* (n=5), *Plicofollis polystaphylodon* (n= 5), *Nemapteryx caelata* (n=9), *Hexanematichthys sagor* (n=5), *Arius maculatus* (n=5), *Osteogeneiosus militaris* (n=5), and *Arius venosus* (n=2) were collected from six different locations; four in Peninsular Malaysia (PM) - (Merbok, Tanjong Dawai, Telok Bahang and Hutan Melintang) and two in East Malaysia (Sandakan and Tawau in Sabah) (Figure 1). All species collected were identified by referring to FAO identification sheet (Fisher and Bianchi 1984; Carpenter and Niem 1999). From each specimen, approximately 5-10 g of tissues were cut and stored in 75% ethanol. Samples were stored at room temperature prior to DNA extraction.

DNA isolation and sequencing

Total genomic DNA from tissue was extracted by using AquaGenomic Solution Protocol Kits (MultiTarget Pharmaceuticals) following manufacturer protocols with some modification of the original protocol in order to improve the yield and quality of the extracted DNA. Polymerase chain reaction (PCR) was used to amplify the target region of the cytochrome oxidase subunit I (COI) gene in the mtDNA genome of all species sampled. Each PCR amplification was performed in the total volume of 25 µL of PCR mixture consisting of 18.75 µL ultrapure water, 2.25 µL 10X PCR buffer, 3.0 µL $MgCl_2$, 0.25 µL of each primer (FishF1 - 5'TCAACCAACCACAAAGACATTGGCAC3', FishR1 - 5'TAGACTTCTGGGTGGCCAAAGAATCA3') (Ward et al. 2009), 0.5 µL of dNTP, 0.1 µL of Taq polymerase, and 1.5 µL of DNA template. The amplification condition consisted an initial step for 2 min at 95°C followed by 35 cycles of 0.5 min at 94°C, 0.5 min at 54°C, and 1 min at 72°C, followed by a final incubation at 72°C for 10 min. The PCR products were visualized on an agarose gel and the most intense bands were selected for purification according to the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA). Purified PCR products were then sent for sequencing using an ABI3730 sequencing machine provided by First Base Laboratories (Taiwan) and Center for Chemical and Biology, Universiti Sains Malaysia, Penang, Malaysia.



Figure 1. The location of catfishes samples collected from Malaysia

Data analysis

Five individuals of each species were selected for phylogenetic analysis except *Netuma thalassina, N. caelata* and *A. venosus*. *N. thalassina* represented by nine individuals from two different populations; Sandakan (n=5) (East Malaysia) and Hutan Melintang (n=4) (Northern Peninsular Malaysia). *N. caelata* were represented by nine individuals from two different populations in Northern Peninsular Malaysia; Merbok (n=5) and Tanjong Dawai (n=4), while *A. venosus* represented by only 2 individuals. Sequences were aligned and visualized using ClustalW in MEGA ver. 5 (Tamura et al. 2011). The resulting sequences for each individual were then aligned using CLUSTAL W ver. 1.6 (Thompson et al. 1994) and were manually checked and trimmed in the BIOEDIT ver 7.0.9 sequence-editing program (Hall 1999); alignments were subsequently revised by eye in an effort to maximize positional homology. The final alignment that consisted of 621 base pairs was then imported into Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast) to ensure the identity of the samples. Conspecific COI sequences of three species namely *N. thalassina* (JN242655, EF609288, HQ149892, EF607327, EU014255), *A. maculatus* (HQ009490, FJ403390) and *O. militaris* (EF609566, EF609564, EF609562) that were available in GenBank of Barcode of Life Database (BOLD) were also included in the analysis and compared with the aligned sequences.

Out of 25 ariid species reported in Malaysia, only four species have their COI sequences available in GenBank. The four species with their respective sequence are as follows; (i) *Arius arius* (EU148551, EU148549, EU148552, EU148550, EU148548), (ii) *Arius jella* (FJ265865), (iii) *Arius subrostratus* (EU148555, EU148556, FJ624208) and (iv) *Bagre marinus* (GU225557, GU225558, GU225559, GU225560). With that, a total of 71 sequences were included in the analysis (Table 1). The phylogenetic relationship tree was constructed under Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithm. *Plotosus lineatus* (EU148554) of Plotosidae was selected as an outgroup because morphologically it shows a close relationship to ariids. Genetic distances within and between species calculated based on Kimura-2 parameter (Kimura 1980). In a case for overlooked species, we employed a sequence divergence of 3.5% as a screening threshold as recommended by Hebert et al. (2004). This is a conservative threshold that approximates 10 times the average within-species COI sequence variation that is typical for marine fishes (Ward et al. 2005; Steinke et al. 2009).

RESULTS AND DISCUSSION

BLAST analysis

The BLAST result shows that only *N. thalassina* and *O. militaris* showing similarity with the species presumably identified. The remaining six species namely *P. argyropleuron, P. polystaphylodon, N. caelata, H. sagor, A. maculatus* and *A. venosus* did not match with species presumably identified (Table 2).

Maximum Likelihood and Maximum Parsimony analysis

Maximum likelihood (ML) and Maximum Parsimony (MP) constructed under the Kimura 2-parameter shows almost the same topology, thus only ML tree will be discussed. The tree bifurcated into two main clades. All the Ariidae species were grouped in the main clade, with the outgroup (Plotosidae) formed the basal root. On the upper part of the tree, all the sequences of *O. militaris*, including conspecific sequences retrieved from GenBank were grouped together forming their own clade with 98% support. Two sequences of *A. maculatus* obtained from GenBank (accession number: FJ403390 and HQ009490) however does not clustered together with the presumed *A. maculatus* collected in this study, but placed together with the group of *Arius arius* retrieved from GenBank (Figure 2). Except *for A. venosus* which placed in a clade of *N. caelata*, the rest of the individual (*N. caelata*, *N. thalassina*, *P. argyropleuron, P. polystaphylodon* and *H. sagor*) were placed within their own group with a high support, thus forming a clear and observable species basis clade. This pattern also showed by GenBank sequences of *Arius subrostratus* and *Bagre marinus*, which also group together, forming their own subclade with 99% support respectively. However, two individual sequences retrieved from GenBank (*N. thalassina,* accession number: JN242655 and EF607327) were not placed in their own subclade, but they were located between *A. maculatus* and *N. caelata* group (Figure 2).

Genetic distance

The genetic distance (Table 3) within and between species (including GenBank sequences) was calculated based on Kimura-2 parameter (K2P). Intraspecific genetic distances showed a small difference, ranged from 0.000 to 0.042 (Table 3). The genetic distances between species ranged from the lowest (0.004) between *A. venosus* and *N. caelata* to the highest (0.169) between *O. militaris* and *Bagre marinus*. A slightly higher interspecific genetic distances (0.037) was also observed between *A. maculatus* and *A. arius*. The genetic distances between *A. jella* cannot be calculated since it represented by only one individual.

Discussion

The placement of *A. venosus* together with *N. caelata* clade in Maximum Likelihood (Figure 2) and the low genetic distances (0.004) (Table 3) between both species has raised a question either *A. venosus* and *N. caelata* is actually the same species, but has reported as a different species, or presumably *A. venosus* is actually *N. caelata*, but misidentified as *A. venosus* due to morphological complexity.

Accordingly, the morphology of two individual of *A. venosus* was relooked. Wongratana and Bhatia (1974) have highlights the similarities of morphological characteristic

between *A. venosus* and *N. caelata*, which may lead to misidentification. Wongratana and Bhatia (1974) reported that both species can be differentiated by looking at their head shield and palatal teeth. *N. caelata* is described to have strongly rugose and granulated head shield. Its supra-occipital process is short, as long as broad at base, with the median keel and its hind end concave. While their teeth in jaws small and fine, those in upper jaw (premaxillary teeth) in a long and narrow band, eight to ten times longer than broad; palate teeth in one patch on each side, small and fine, front margins of patches longest and convex, separated from each other and from jaw teeth by a space somewhat less than their width (Figure 3).

Table 1. Presumptive species identification of catfishes collected samples from wild and samples obtained from GenBank

Presumptive species identification	No. of specimen	Source	Collection site	Accession number
N. thalassina	5	Wild	Sandakan	-
	4	Wild	H. Melintang	-
	5	GenBank		JN242655 EF609288 HQ149892 EF607327 EU014255
P.argyropleuron	5	Wild	Merbok	-
P.polystaphylodon	5	Wild	Tawau	-
N. caelata	4	Wild	Merbok	-
	5	Wild	T. Dawai	-
H. sagor	5	Wild	H. Melintang	-
A. maculatus	5	Wild	Merbok	-
	2	GenBank		HQ009490 FJ403390
O. militaris	5	Wild	Teluk	-
	5	GenBank	Bahang	EF609566 EF609564 EF609562
A. venosus	2	Wild	H. Melintang	-
Arius arius	5	GenBank		EU148551 EU148549 EU148552 EU148550 EU148548
Arius jella	1	GenBank		FJ265865
Arius subrostratus	3	GenBank		EU148555 EU148556 FJ624208
Bagre marinus	4	GenBank		GU225557 GU225558 GU225559 GU225560
Plotosus lineatus	1	GenBank GenBank		EU148554

Note: H. Melintang = Hutan Melintang, T. Dawai = Tanjong Dawai

On the other hand, as recorded by Wongratana and Bhatia (1974), *A. venosus* is described to have head shield almost smooth; median fontanelle narrow and short, reaching to median keel of supra-occipital process, which is slightly longer than broad, its side borders straight and slightly convergent. While their premaxillary tooth-band rather broad and slightly arched, four to five times longer than broad; teeth on palate fine, only one large sub-triangular group on each side, greatest length of each group about equal to curved front base, outer edge concave, the inner convex; both groups separated from each other and from jaw teeth by a space about 1/3 the breadth of jaw band (Figure 3).

However, our observation cannot clearly distinguish between their head shield (Figure 4). While, the observation on the palatal teeth has caused our decision to fall in the FAO guide described by Wongratana and Bhatia (1974) (Figure 5).

Table 2. The top three BLAST result of Malaysian ariids with respective score and maximum identity

Presumably identified species	Top three BLAST result	Max score	Query coverage	E value	Max identity
N. thalassina	*N. thalassina*	1177	100%	0.0	99%
	N. thalassina	1177	100%	0.0	99%
	N. thalassina	1171	100%	0.0	99%
O. militaris	*O. militaris*	1171	100%	0.0	99%
	O. militaris	1171	100%	0.0	99%
	N. thalassina	906	100%	0.0	92%
P.argyropleuron	*N. thalassina*	910	99%	0.0	92%
	N. thalassina	910	99%	0.0	92%
	N. graeffei	910	99%	0.0	92%
P.polystaphylodon	*N. thalassina*	933	99%	0.0	93%
	N. thalassina	933	99%	0.0	93%
	N. thalassina	921	99%	0.0	92%
N. caelata	*A. manillensis*	998	100%	0.0	95%
	A. manillensis	998	100%	0.0	95%
	A. manillensis	998	100%	0.0	95%
H. sagor	*N. thalassina*	846	99%	0.0	90%
	N. thalassina	846	99%	0.0	90%
	N. thalassina	840	99%	0.0	90%
A. maculatus	*N. thalassina*	1010	100%	0.0	95%
	N. thalassina	1010	100%	0.0	95%
	N. thalassina	1004	100%	0.0	95%
A. venosus	*A. manillensis*	992	100%	0.0	94%
	A. manillensis	992	100%	0.0	94%
	A. manillensis	992	100%	0.0	94%

Note: *N. graeffei = Neoarius graeffei*

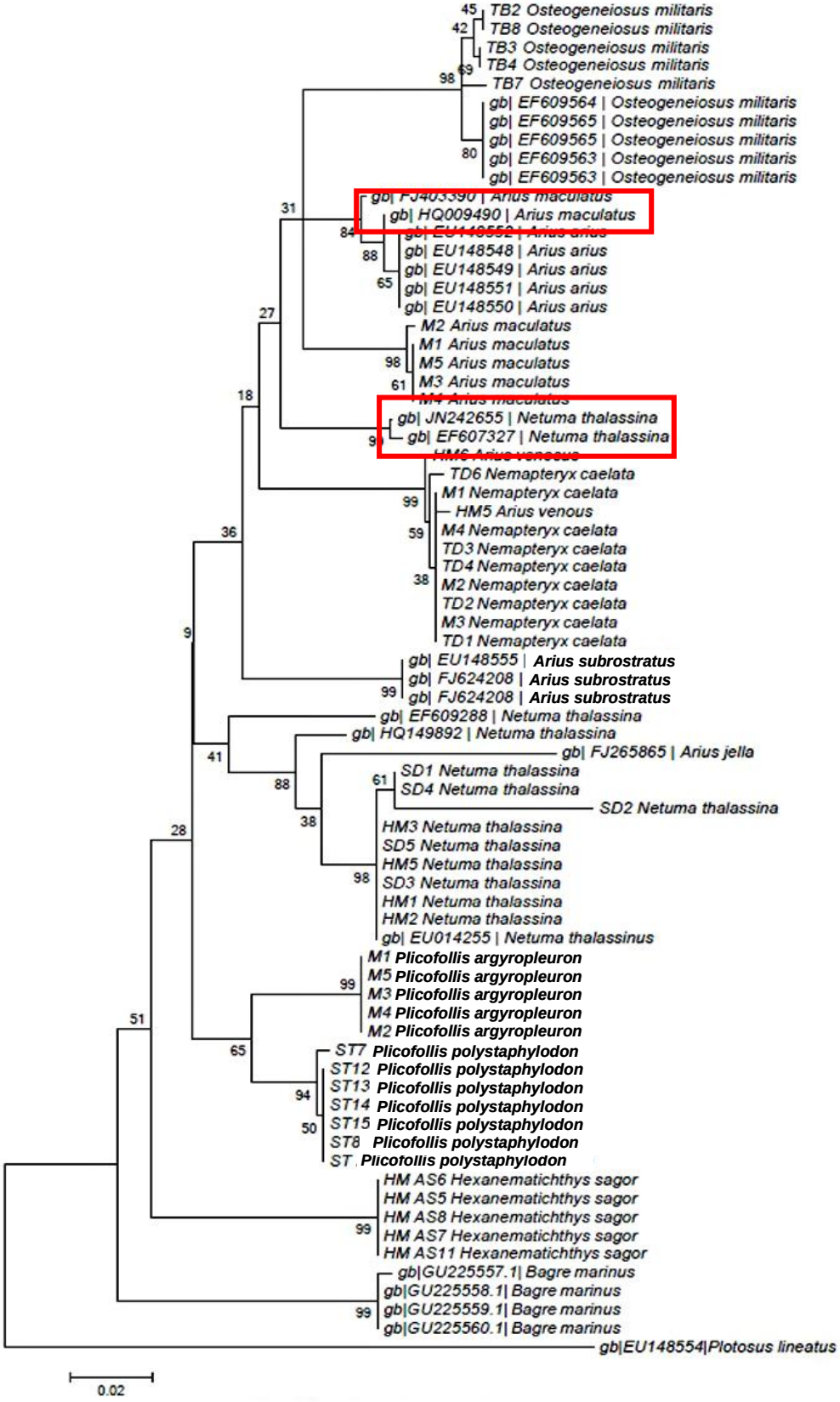


Figure 2. Maximum Likelihood (ML) tree of Ariidae from Malaysia and conspecific sequences retrieved from GenBank for COI. M=Merbok TD=Tanjong Dawai HM=Hutan Melintang SD= Sandakan ST=Tawau gb=GenBank. Four sequences in the box show the *Netuma thalassina (*accession number: JN242655, EF607327) and *Arius maculatus* (accession number: FJ403390, HQ009490) retrieved from GenBank that were placed out from their group.

Table 3. Estimation of evolutionary divergence over sequence pairs between groups within Malaysian Ariidae. The asterisk (*) shows a presumably *Arius venosus*. Bold indicates the high intraspecific genetic distances within *N. thalassina*.

	1 n = 10	**2 n=5**	**3 n=7**	**4 n=9**	**5 n=2**	**6 n=3**	**7 n=15**	**8 n=5**	**9 n=5**	**10 n=5**	**11 n=1**	**12 n=4**
O. militaris	0.007											
A. arius	0.068	0.000										
A. maculatus	0.068	0.037	0.021									
N. caelata	0.105	0.072	0.073	0.001								
*A. venosus**	0.109	0.076	0.077	0.004	0.007							
A. subrostratus	0.099	0.076	0.076	0.089	0.088	0.000						
N. thalassina	0.109	0.094	0.092	0.110	0.109	0.114	**0.042**					
P. argyropleuron	0.114	0.088	0.097	0.109	0.110	0.089	0.093	0.000				
P. polystaphylodon	0.096	0.087	0.089	0.092	0.092	0.079	0.086	0.045	0.001			
H. sagor	0.144	0.118	0.131	0.123	0.122	0.105	0.113	0.101	0.105	0.000		
A. jella	0.155	0.123	0.124	0.146	0.145	0.150	0.081	0.132	0.123	0.158	n/c	
B. marinus	0.169	0.141	0.143	0.142	0.137	0.132	0.128	0.127	0.115	0.130	0.159	0.002

Table 4. Estimates of evolutionary divergence over sequence pairs between groups of Malaysian ariids species. Bold indicates the intraspecific genetic distance when two GenBank's *N. thalassina* sequences (accession number: JN242655, EF607327) excluded.

	1 n = 10	**2 n=5**	**3 n=7**	**4 n=9**	**5 n=2**	**6 n=3**	**7 n=15**	**8 n=5**	**9 n=5**	**10 n=5**	**11 n=1**	**12 n=4**
O. militaris	0.007											
A. arius	0.068	0.000										
A. maculatus	0.068	0.037	0.021									
N. caelata	0.105	0.072	0.073	0.001								
*A. venosus**	0.109	0.076	0.077	0.004	0.007							
A. subrostratus	0.099	0.076	0.076	0.089	0.088	0.000						
N. thalassina	0.109	0.094	0.092	0.110	0.109	0.114	**0.026**					
P. argyropleuron	0.114	0.088	0.097	0.109	0.110	0.089	0.093	0.000				
P. polystaphylodon	0.096	0.087	0.089	0.092	0.092	0.079	0.086	0.045	0.001			
H. sagor	0.144	0.118	0.131	0.123	0.122	0.105	0.113	0.101	0.105	0.000		
A. jella	0.155	0.123	0.124	0.146	0.145	0.150	0.081	0.132	0.123	0.158	n/c	
B. marinus	0.169	0.141	0.143	0.142	0.137	0.132	0.128	0.127	0.115	0.130	0.159	0.002

Note: *presumably *Arius venosus*

The BLAST result of both *N. caelata* and *A. venosus* also do not contribute any sign to solve this problem. BLAST analysis of both species shows similarity to the Philippines ariid *Arius manillensis*, with the maximum identity of 95% and 94% (Table 2). *A. manillensis* is first reported by Valenciennes (1840) in brackish and fresh water of Philippine. This species is described to have palatal teeth in two ovate patches, placed far forward which was clearly distinguishable with *A. venosus* and *A. caelata* (Carpenter and Niem 1999). For clarification, we decided to expand our Maximum Likelihood analysis and included five sequences of *A. manillensis* retrieved from GenBank. Interestingly, we found that *A. manillensis* is clearly distinguishable different species (Figure 6) and that solves the confusion raised by BLAST result (Table 2). However, it does not solve our doubt on do we misidentified *N. caelata* as *A. venous*, or *A. venous* and *N. caelata* is maybe the same species but reported as a different species: cryptic/synonymous.

Since our sample size of *A. venosus* in this study is very low (two individuals only) it does not give a strong support to conclude that *N. caelata* and *A. venosus* are the same species, but reported as different species (misidentified species). Thus, it leads to the decision that we were confused by the complexity of morphological characteristic of both species which lead us to misidentification. However, the decision is not final. Further study with large sample size of *A. venosus* will give a clearer picture of its status.

The high intraspecific genetic distance of *N. thallasina* (Table 3) is due to the sequences analyzed were obtained from many sources. As in Maximum Likelihood (Figure 2) tree, two sequences of *N. thallasina* retrieved from GenBank (accession number: JN242655 and EF607327) were placed out of other *N. thallasina* clade and eventually show more close relation to *A. maculatus*. With that, we hypothesized that the higher intraspecific genetic distances of *N. thallasina* is contributed by diversity of the two

sequences included in the analysis. To clarify this hypothesis, we expand our genetic distance analysis without those two sequences (accession number: JN242655, EF607327). The analysis was based on Kimura 2-parameter model with 71 nucleotide sequences involved. As expected, the genetic distances within *N. thalassina* were reduced to 0.026 when both sequences, JN242655 and EF607327 were excluded (Table 4). This has proved our hypothesis that the higher diversity within *N. thalassina* was contributed by the diversity of sequences obtained from GenBank. Since all the GenBank's *N. thalassina* analyzed in this current study were originated from wide range of country (JN242655, South China Sea; EF609288, Australia; EF607327, China; HQ149892, Iran; EU014255 India), lack of consistency between different country during species identification could lead to unexpected figure in analysis.

Arius arius and *A. maculatus* have shown very close interspecific relationship by having a fairly low genetic distance (0.037) (Table 3). Both species also formed sister clade in Maximum Likelihood and Maximum Parsimony analysis. The close relationship between these two species is probably contributed by their similarity in morphological characteristics. Both species could be found in estuaries and inshore waters (Fisher and Bianchi 1984; Carpenter and Niem 1999). *Arius arius* is described to have teeth on palate in two oval-elliptical patches, one on each side, placed well forward in palate, and usually parallel to each other. While *A. maculatus* is described to have teeth on palate in two oval patches, one on each side placed well back in palate, with their long axes tend to converge posteriorly. Other shared morphological characteristics between both species are their coloration. Both species are bluish brown above and white below, fins yellow, dorsal, caudal and pectoral fins with dark margins, and adipose fins with large black spot. Carpenter and Niem (1999) have also highlighted the misidentification between both species.

This study provides a good support for the taxonomic status of Malaysian Ariidae, which previously studied based on morphological characteristic. On the other hand, this study also shows that DNA barcode is very effective in detection of misidentification caused by traditional identification method. Additionally, the low intraspecific genetic distances and high interspecific genetic distances between species yielded based on cytochrome oxidase subunit I proved that DNA barcoding can be utilized as a useful tool in facilitating the molecular phylogenetic and population genetic field. Thus the work on establishing DNA barcode for all species of Ariidae must be continued.

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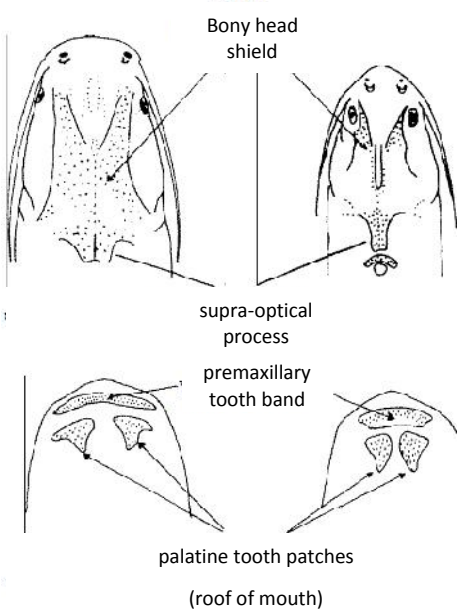


Figure 3. Differentiation between head shield and palatal teeth of both *Nemapteryx caelata* (left) and *Arius venosus* (right). Image modified from Wongratana and Bhatia (1974)



Figure 4. The head shield of *Nemapteryx caelata* (left) and *Arius venosus* (right) collected in this study

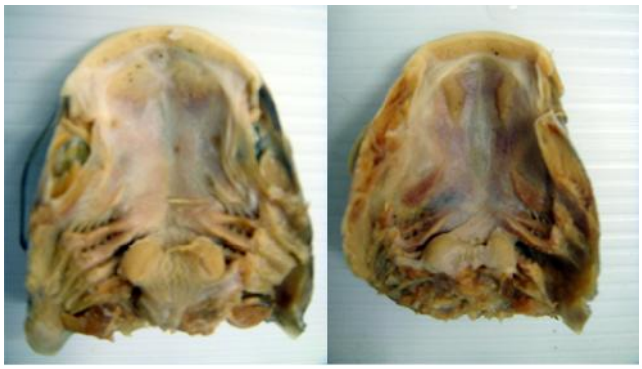


Figure 5. The palatal teeth of *Nemapteryx caelata* (left) and *Arius venosus* (right) collected in this study

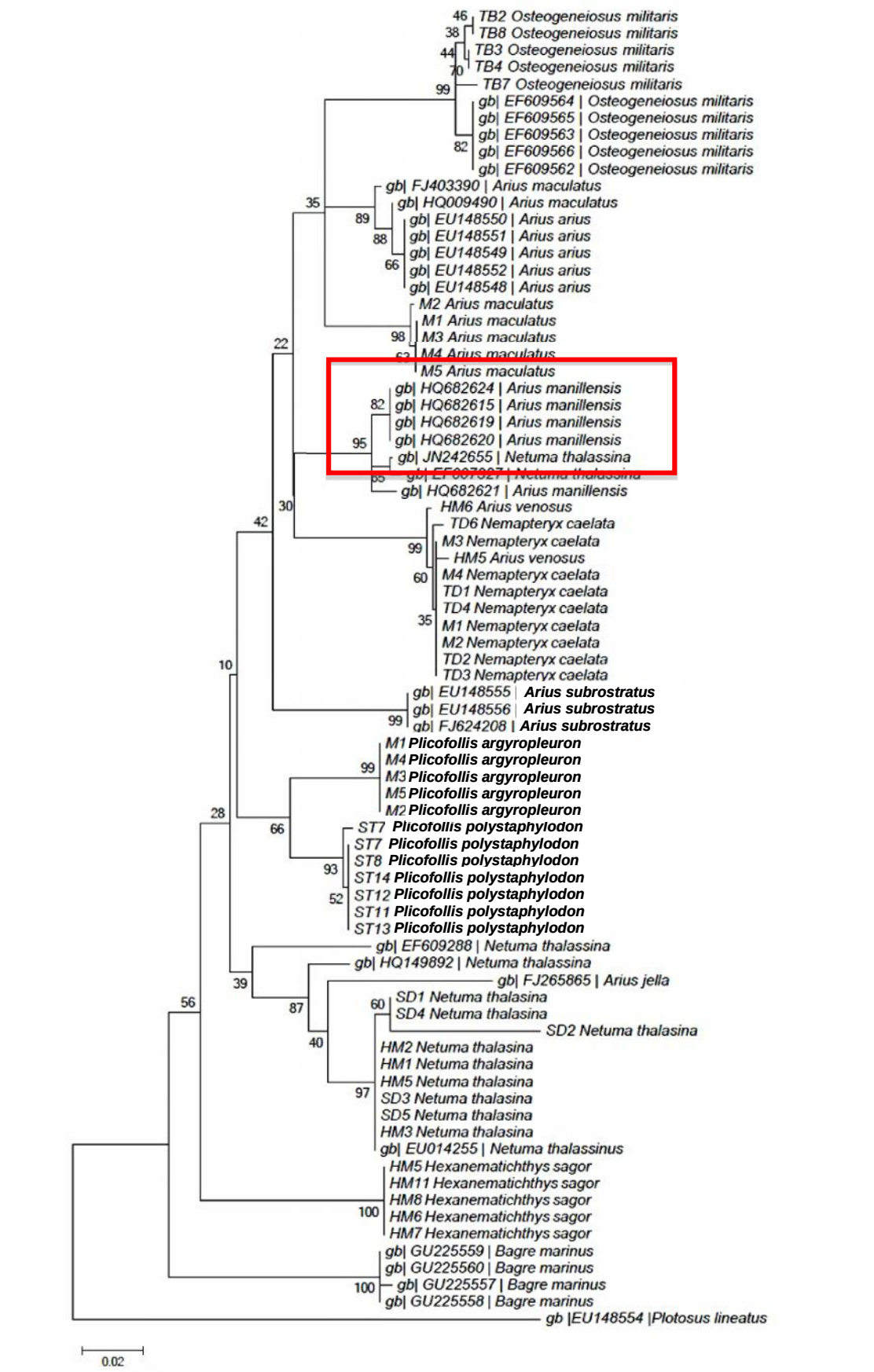


Figure 6. Phylogenetic tree among Ariidae species in Malaysia constructed under Maximum Likelihood (ML) algorithm including five sequences of *A. manillensis* obtained from GenBank

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Identification and characterization of morphological diversity of Lemba (*Curculigo latifolia*) in East Kalimantan, Indonesia

INCE RADEN♥, CANDRA CATUR NUGROHO, SYAHRANI
Faculty of Agriculture, Universitas Kutai Kartanegara. Jl. Gn. Kombeng 27, Tenggarong, Kutai Kartanegara 755 3, East Kalimantan, Indonesia.
♥email: irad_unikarta@yahoo.com.

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Abstract. *Raden I, Nugroho CC. Syahrani. 2017. Identification and characterization of morphological diversity of Lemba (*Curculigo latifolia*) in East Kalimantan, Indonesia. Biodiversitas 18: 1367-1376.* Lemba (*Curculigo latifolia*) is Kalimantan's land prospective for commercial cultivation because its leaf fiber has been traditionally utilized as fiber source for ulap doyo woven cloth; its fruit as sweetener; and its root as medicinal herb. To this days, only a few of research has been conducted on lemba in East Kalimantan or even in Indonesia, including studies on lemba plant phenotype suitable for weaving material. Thus, a research about identification and morphological characterization of lemba cultivars is necessary to identify and characterize lemba plant in East Kalimantan to reveal phenotypic variability in both quantitative and qualitative traits that can be utilized as the source of germplasm for the plant breeding program. This study was carried out in three Sub-districts, i.e.: Tenggarong Sub-district (Kutai Kartanegara District), Kota Bangun Sub-district (Kutai Kartanegara District), and Jempang Sub-district (West Kutai District). Data collection was done by observation, and measurement of quantitative and qualitative traits of lemba plant. Descriptive analysis was applied on the morphological traits data, while correlation analysis was performed to reveal relationship between the qualitative and quantitative morphological traits by using software Minitab v.14.12. Phenotypic variability was determined based on the standard deviation. Principal component analysis and cluster analysis of the data were performed by using software SPSS v.11.6. The variance value of the 33 observed lemba plant accessions from the three Sub-districts indicated that the phenotypic variability of the qualitative traits was narrow while that of the quantitative traits was broad. There are three principal components that account for the observed variability with a cumulative variance of 96% which is obtained from 19 observed traits. Cluster analysis at similarity 95% indicates that there are five lemba accessions groups based on only qualitative, only quantitative, or based on combined qualitative and quantitative traits.

Keywords: Cluster analysis, *Curculigo latifoliadoyo*, characterization, phenotypic variability

INTRODUCTION

East Kalimantan is rich with biodiversity, one of them is lemba (*Curculigo latifolia*). Lemba is an endemic plant of Kalimantan that so far only naturally grow in the wild and has not been cultivated. Besides in Kalimantan, various variants of the plant can also be found in Sumatra, Bangka, Papua, and Java. Moreover, the plant is reported to be distributed in the Western Africa (marasi or lemba), India, Burma (Myanmar), Thailand, Malaysia (Perak, Pahang, Sarawak, and Sabah), and the Philippines (Palawan, Balabac, and Samar). Lemba is a Kalimantan's landrace plant that is high potential for cultivation because of its high economic value and commercial prospect owing to its diverse purposes. A study by Kocyan (2007) reported that there are more than 20 species from the family of Hypoxidaceae, and among those species, *Curculigo* and *Hypoxis* were the most important genera of the family.

Genus *Curculigo* is distributed in the tropical Africa and rainforest of Asia, especially in Malaysia and Singapore. According to Ismail et al. (2010), there are four important species of the genus namely *C. latifolia*, *C. capitulations*, *C. racemes*, and *C. orchioides*. In Malaysia, *C. latifolia* Dryand is an important medicinal plant (Farzinebrahimi et al. 2013). There, the plant propagates by rhizome and abundantly found in highland areas with an altitude between 1500-2000 m asl., and generally grow in slope and forest area. In Kalimantan and Malaysia, fibers from lemba leaves are utilized for making fishing net, rope, and material for weaving doyo. Shaari (2005) stated that lemba leaves and flowers could be used to treat high fever, while lemba flower and rhizome potion has been used to treat stomachache and frequent urinating. Ahmad and Holdsworth (1994) reported that lemba rhizome as topical ointment to treat cut wound. Wiart (2000) reported that lemba rhizome extract was able to inhibit hepatitis B virus. Yamashita et al. (1990) identified and characterized curculin sweet proteins with taste-modifying activity from *C. latifolia*. These proteins are 500 times sweeter than sucrose (Kant 2005; Masuda and Kitabatake 2006) and can be used as a low-calories sweetener for diabetic and obese people (Ismail et al. 2010). In Eastern Kalimantan, lemba leaf fiber is used for traditional practice of weaving ulap doyo cloth. According to ulap doyo weavers, to produce one woven cloth with a length of about 1.5 m, it requires about two spools of yarn. A spool of yarn was made from about thirty *senik*, and a *senik* is produced from 30-36 lemba fresh leaves. Thus, it requires 1800-2160 lemba leaves to weave one sheet of cloth. The demand on lemba plant as raw material for ulap doyo woven cloth is high, but the availability of the material is low because no one is cultivating the plant. Generally, lemba plant used for

material for the weaving by people of Dayak Benuaq tribe are lemba biang and lemba pentih.

Identification and characterization of lemba plant are necessary steps in developing new superior lemba varieties with high productivity and other important traits with a goal to increase plant quality and competitiveness (Carsono 2008; Barnum 2005; Sari 2013). Identification step aims to identify taxonomical characteristics of various individuals and to classify each into a particular taxon (Mayr and Ashlock, 1999; Litbang Pertanian 2004). Identification can be made using several approaches i.e. (i) identification based on morphological and agronomical characteristics; (ii) identification based on cytology; (iii) identification based on DNA or molecular pattern (Swasti 2007; Jamsari 2008).

Low diversity of vegetative-propagating lemba plant is one of the obstacles encountered in lemba plant breeding. Narrow genetic diversity hinders the selection process thereby resulting in a low selection efficiency (Sigrist et al. 2011). Conversely, wide genetic diversity in germplasm is a determining factor in developing a superior plant variety (breeding) (Pribadi 2009; Singh et al. 2012). Until now, only a few of research has been conducted on lemba in East Kalimantan or even in Indonesia, including studies on lemba plant phenotype suitable for weaving material. People in Kutai Kartanegara to these days have not cultivated lemba. Therefore, a study on identification and morphological characterization of lemba cultivars need to be done. This study aimed to identify and characterize lemba plant in East Kalimantan to reveal phenotypic variability in both quantitative and qualitative traits that can be utilized as the source of germplasm for the plant breeding program.

MATERIALS AND METHODS

Study area

The study was conducted in July - December 2016 in Tenggarong Sub-district and Kota Bangun Sub-district, Kutai Kartanegara District, East Kalimantan Province, Indonesia; and in Jempang Sub-district, West Kutai District, East Kalimantan Province, Indonesia (Figure 1). The area, altitude, and soil type in those study area are shown in Table 1.

Procedures

Data collection

Data collection was done by interview, exploration and direct measurement on quantitative and qualitative traits of lemba plant in three sub-districts i.e., Tenggarong (TGR), Kota Bangun (KOBA), and Jempang (JMP) (Cunningham 2001; Newing et al. 2011). An interview was carried out to gather information from the locals on the utilization of naturally occurring wild lemba as material for weaving. Direct observation and measurement of the object of the study were conducted to characterize lemba plant according to Developing Crop Descriptor Lists (BI 2007). The characterization included 12 qualitative and quantitative traits.

Observed variables

Variables observed and measured in lemba plant were qualitative and quantitative traits which are shown in Table 2. Observation and measurement of those traits were done on mature lemba plant. Data scoring system for each trait is provided in Table 2.

Data analysis

Data obtained in this study were accordingly subjected to descriptive analysis, correlation analysis, principal component analysis, and cluster analysis. Descriptive analysis was used to evaluate morphological characteristics. Correlation analysis was employed to examine the relationship between morphological characteristics in both quantitative and quantitative traits. Correlation analysis in this study was performed by using software Minitab version 14.12. Principal component analysis was used to highlight the diversity in the observed variables or technique to determine plant traits that have significantly contributed to a particular variation, thus pointing out the character diversity (Afuape et al. 2011). In this study, Principal Component Analysis was performed using software SPSS version 11.6. Cluster analysis was utilized to group objects based on their characteristics. The cluster analysis used Euclidean distance as the criterion and generated dendrogram that shows a relationship between germplasms. The analysis was a hierarchical clustering since the clustering was carried out structurally based on character similarity and the number of desired clusters was not known. The cluster analysis was performed using software SPSS 11.6.

RESULTS AND DISCUSSION

Morphological characteristics of lemba germplasm

The characterization used in this study employed an agronomical-morphological approach (Swasti 2007; Jamsari 2008); Bajracharya et al. 2006). Morphological characteristics observation on seven quantitative variables of 33 lemba accessions revealed that some lemba had a short leaf sheath and a short petiole (Figure 2a) while some others have a long leaf sheath and petiole (Figure 2b). Lemba accession possessing the shortest leaf sheath and the shortest petiole was TGR2 (25 mm) and JMP6 (52 mm), respectively. Meanwhile, accession with the longest leaf sheath and petiole was KOBA5 (298 mm) and TGR4 (510 mm), respectively. Leaf dimension analysis revealed that JMP12 accession has the shortest leaf length (775 mm), while KOBA11 has the longest leaf (1195 mm). Observation on lemba leaf fiber identified that the accessions with the lowest and the highest fiber length to width ratio was KOBA4 (810.67) and KOBA12 (1165.3), respectively.

This morphological characterization is beneficial to highlight the variations and identify germplasms for plant breeding purpose. Morphological character variations of germplasm are particularly important for plant breeding (Tresniawati and Randriani 2011; Swasti 2007). The result of quantitative observation on the seven traits of lemba plant is shown in Table 4.



Figure 1. Study location in East Kalimantan Province, Indonesia. 1. Tenggarong Sub-district, Kutai Kartanegara, 2. Kota Bangun Sub-district, Kutai Kartanegara, 3. Jempang Sub-district, West Kutai.

Table 1. Area, altitude, and soil type in the study locations

Sub-district	District	Area (km^2)	Altitude (m asl.)	Soil type
Tenggarong	Kutai Kartanegara	398.10	10	Organosol glei humus
Kota Bangun	Kutai Kartanegara	1.143.74	9	Organosol glei humus
Jempang	Kutai Barat	744.47	10	Organosol glei humus

Note: Bappeda Kukar (2007); Suryadi (2014); BPS Kubar (2015); BPS Kukar (2016)

Table 2. Data scoring system for lemba plant qualitative and quantitative traits

Variables	Score	Characteristic
Qualitative traits		
Leaf sheath shape	3	Flat and short
	5	Flat and open
	7	Wide and open
Leaf sheath color	1	Green yellow
	2	Yellow green
	3	Green
	4	Bluish green
	5	Dark green
Leaf sheath arrangement	1	Alternate (only one leaf emerges from each stem node)
	2	Opposite (a pair of leaves emerge is situated opposite to each other in the same node ring)
	3	Sub-opposite (leaves from the neighboring node that are not far apart are arranged in opposite direction)
	4	Verticillate (there are more than two leaves arranged in the same stem node)
Petiole color	1	Green
	2	Yellow
	3	Brown
	4	Bluish green
Leaf surface	1	Glabrous
	2	Pubescent
	3	Rugose
	4	Tomentose
Leaf blade color	1	Green yellow
	2	Yellow green
	3	Green
	4	Bluish green
	5	Dark green
Leaf margin	1	Entire
	2	Serrate
	3	Doubly serrate
	4	Dentate
	5	Crenate
	6	Repand
Leaf base	1	Accuminate
	2	Acute
	3	Cuneate
	4	Obtuse
	5	Rounded
	6	Truncate
Leaf tip	1	Accuminate
	2	Acute
	3	Obtuse
	4	Rounded
	5	Truncate
	6	Retuse
	7	Mucronate
Stem surface	1	Glabrous
	2	Costale
	3	Sulcate
	4	Winged
	5	Pubescent
	6	Spiny
	7	Showing the remaining of leaves
Stem color	1	Green
	2	Greyish green
	3	Dark Green
	4	Yellowish brown
	5	Redish brown
	6	Grey
Leaf fiber	1	Very fine
	3	Fine
	5	Intermediate
	7	Rough
	9	Very rough
Quantitative traits		
Leaf sheath length	1	Very short (< 5cm)
	3	Short (6-10 cm)
	5	Intermediate (11-13 cm)
	7	Long (14-16 cm)
	9	Very long (> 16 cm)
Petiole length	1	Very short (≤ 10 cm)
	3	Short (10-20 cm)
	5	Intermediate (21-30 cm)
	7	Long (31-40 cm)
	9	Very long (> 40 cm)
Leaf blade length	1	Short (< 75 cm)
	3	Intermediate (75-85 cm)
	5	Long (86-100 cm)
	7	Very long (> 100 cm)
Leaf blade width	1	Short (< 5 cm)
	3	Intermediate (6-10 cm)
	5	Long (11-15 cm)
	7	Very long (> 15 cm)
Stem Diameter	1	Small (< 2 cm)
	3	Medium (2-4 cm)
	5	Large (> 4 cm)
Plant height	3	Short (< 50 cm)
	5	Intermediate (50-100 cm)
	7	Long (> 100 cm)
Leaf fiber length to diameter ratio	0	< 1000
	1	> 1000

Note: BI (2007) (Data scoring in this study was modified according to the necessity)



Figure 2. Lemba leaf sheath and petiole traits. Plant with (A) short and (B) long leaf sheath and petiole

Table 4. Quantitative traits of 33 lemba accessions

Accession	Leaf sheath length (mm)	Petiole length (mm)	Leaf blade length (mm)	Leaf blade width (mm)	Stem diam. (mm)	Plant height (mm)	Fiber length to diameter ratio
TGR1	30	100	780	100	29.5	988	1001.00
TGR2	25	98	880	92	26.3	1100	1026.60
TGR3	28	100	820	98	28.2	930	1033.00
TGR4	160	510	1040	140	50.1	1732	813.00
TGR5	140	491	1030	132	45.6	1701	946.60
TGR6	133	131	880	107	33.1	1241	1108.00
TGR7	135	129	875	105	33.1	1241	1118.67
TGR8	50	182	1030	117	29.1	1312	1145.30
TGR9	55	191	1040	117	29.3	1331	1161.30
KOBA1	140	483	1020	130	44.8	1643	894.67
KOBA2	132	130	870	106	32.5	1212	1104.00
KOBA3	50	180	1010	115	28.4	1270	1125.30
KOBA4	220	252	845	113	24.2	1347	810.67
KOBA5	298	390	921	110	39.1	1641	884.00
KOBA6	199	285	962	131	33.2	1481	881.33
KOBA7	63	128	856	111	18.1	1067	1030.67
KOBA8	72	78	782	64	19.1	924	1002.67
KOBA9	122	252	1050	110	41.1	1453	885.33
KOBA10	60	154	921	101	26.8	1163	1028.00
KOBA11	129	240	1195	150	32.7	1606	934.67
KOBA12	96	196	991	74	17.6	1307	1165.30
JMP1	104	213	902	123	44.3	1243	868.00
JMP2	103	164	898	121	44.6	1192	1038.67
JMP3	72	98	782	124	39.8	953	1014.60
JMP4	72	174	892	164	45.7	1162	1041.30
JMP5	78	174	894	141	34.4	1168	1064.00
JMP6	77	52	779	108	30.4	897	1001.30
JMP7	112	298	862	94	31.5	1298	677.30
JMP8	52	472	936	93	31.8	1498	937.30
JMP9	42	351	878	81	42.5	1300	845.33
JMP10	141	453	936	93	44.1	1561	921.33
JMP11	62	84	908	98	31.9	886	1173.33
JMP12	103	208	775	84	25.3	1105	766.67
Mean	101.7	225.5	916.4	110.5	33.6	1271.3	983.3

Note: TGR = accession found in Tenggarong, KOBA = accession found in Kota Bangun, JMP = accession found in Jempang

Correlation analysis

Correlation analysis of the quantitative data revealed that there was a highly significant positive correlation between the leaf sheath length and the petiole length (0,476) and plant height (0.617). Furthermore, the analysis indicated another significant positive correlation between the petiole length and the leaf blade length (0.491), stem diameter (0.563), and the plant height (0.886). Other significant positive correlations were shown between the leaf blade length and the width (0.452) and the plant height (0.743); between the leaf blade width and the stem diameter (0.551); and between the stem diameter and the plant height (0.500). The leaf width also exhibited a positive correlation with the plant height (0.361). Whereas significant negative correlations were shown between the leaf fiber length to width ratio and the petiole length (-0.562), the leaf sheath length (-0.428) and the plant height (-0.399) (Table 5).

Two characters are positively correlated when one character increases quantitatively as the other character increases, or one character decrease when the other decreases. Positive correlations between the leaf sheath length and the petiole and between the leaf sheath length and the plant height meant that the longer the leaf sheath, the longer the petiole length and the plant height. Similarly, the longer the petiole, the longer the leaf blade, the wider the stem diameter and the higher the plant. Accordingly, the positive correlation between the stem diameter and the plant height, and between the stem diameter. Conversely, a negative correlation such as those between the leaf fiber length to diameter ratio and the leaf sheath length, the leaf width and the plant height, respectively, meant that the higher the ratio, the shorter the sheath, the narrower the width and the shorter the plant.

Analysis of phenotype variance

Determination of phenotypic variability criteria was done by comparing the phenotypic variance with the standard deviation. A Phenotypic variability is wide if the phenotypic variance is bigger than or equal to 2 times the standard deviation (σ2f ≥ 2 Sd σ2f). A phenotypic variability is considered narrow if the phenotypic variance is smaller than 2 times the standard deviation (σ2f < 2 Sd σ2f) (Anderson dan Bancroft, 1952). Phenotype variability is a multiplication between genotypic and phenotypic variability (Allard 1960; Falconer and Mackey 1996). A higher genotypic variability allows a better chance for improvement through breeding method.

Variances in qualitative and quantitative traits of observed lemba plants from the three Sub-districts showed a narrow phenotypic variability in the qualitative traits and a wide phenotypic variability in the quantitative traits (Table 6). Quantitative traits are highly influenced by environment and multiple genes (Acquaah 2007; Makmur, 1992). Furthermore, Mangoendidjojo (2003) stated that different in environmental condition raise the chance of the emergence of variation that determines plant appearance. The apparent variation is not necessarily passed down to next progeny. Nevertheless, a broad phenotypic variability is an essential requirement for an effective selection program as it provides an array of genotype/accession options to be utilized as germplasm source. The variability of lemba plant qualitative traits was considered narrow. Crowder (1993) stated that qualitative traits are discreet and to an inconsiderable degree influenced by environment.

Table 6. Variability of qualitative and quantitative traits

Trait	σ^2 ± SD	Criterion
Qualitative		
Leaf sheath shape	0	Narrow
Leaf sheath color	0.27 ± 0.52	Narrow
Leaf sheath arrangement	0	Narrow
Petiole color	0	Narrow
Leaf surface	0	Narrow
Leaf color	1.96 ± 1.4	Narrow
Leaf margin	0	Narrow
Leaf base	0	Narrow
Leaf tip	0	Narrow
Stem surface	0.33 ± 0.57	Narrow
Stem color	1.27 ± 1.13	Narrow
Leaf fiber fineness	0.98 ± 0.99	Narrow
Quantitative		
Leaf sheath length (mm)	3419.1 ± 58.473	Wide
Petiole length (mm)	17483 ± 132.22	Wide
Leaf length (mm)	9214.8 ± 95.994	Wide
Leaf width (mm)	452.19 ± 21.265	Wide
Stem diameter (mm)	70.821 ± 8.4156	Wide
Plant height (mm)	56442 ± 237.57	Wide
Leaf fiber length to diameter ratio	15102 ± 122.89	Wide

Note: σ^2 = variance; SD = standard deviation

Table 5. Correlation analysis among quantitative characteristics of 33 lemba accession

Variable	LSL	PL	LL	LW	SD	PH	FR
LSL	1.000	0.476**	0.205^{ns}	0.242^{ns}	0.293^{ns}	0.617**	-0.428*
PL	-	1.000	0.491**	0.193^{ns}	0.563**	0.886**	-0.562**
LL	-	-	1.000	0.452**	0.286^{ns}	0.743**	0.060^{ns}
LW	-	-	-	1.000	0.551**	0.361*	-0.010^{ns}
SD	-	-	-	-	1.000	0.500**	-0.311^{ns}
PH	-	-	-	-	-	1.000	-0.399*
FR	-	-	-	-	-	-	1.000

Note: LSL = Leaf sheath length, PL = petiole length,LL = leaf length, LW = leaf width,SD = stem diameter,PH = plant height,FR = leaf fiber length to diameter ratio, ** = significantly different α = 1%, * = significantly different α = 5%,ns = not significant.

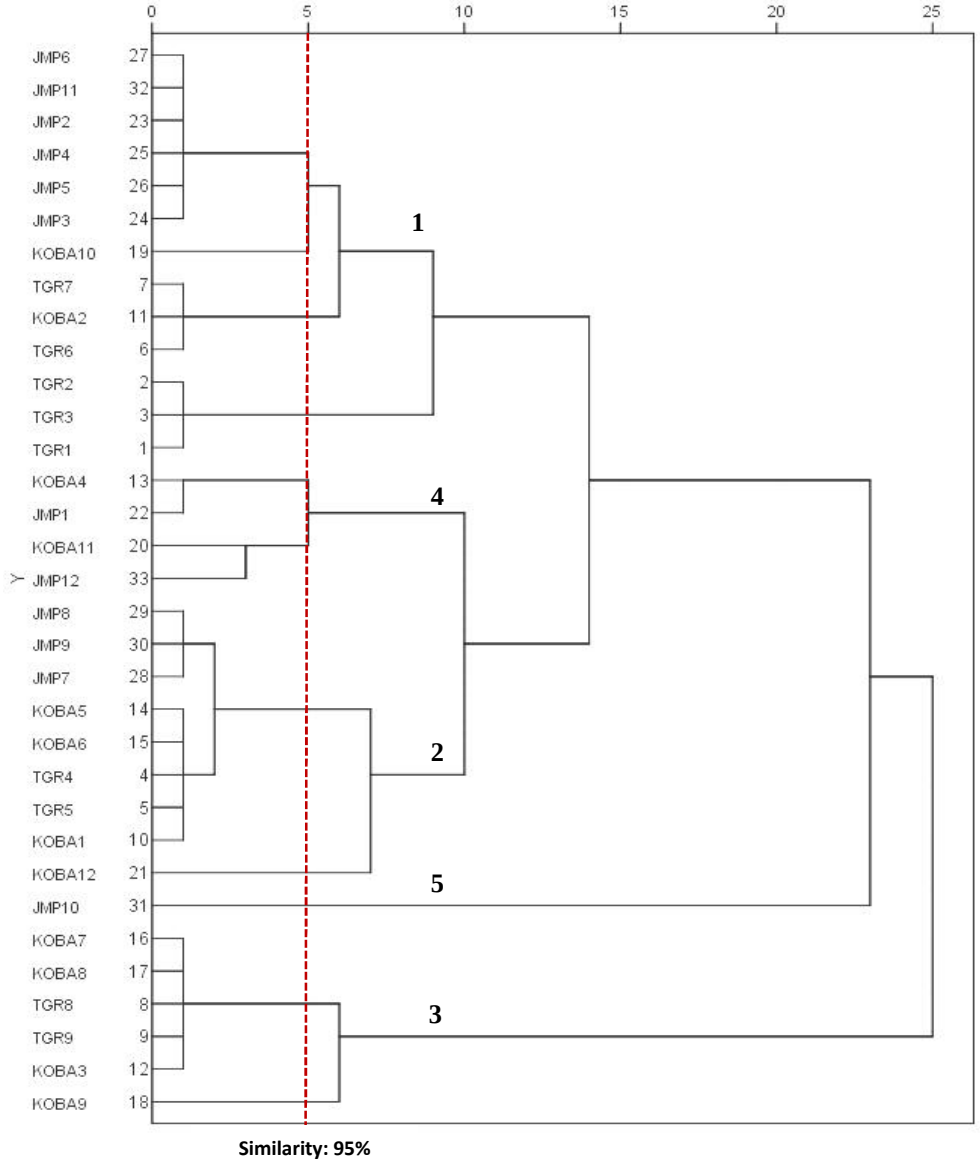


Figure 3. Dendrogram of 33 lemba accessions based on their qualitative traits

Principal Component Analysis

In this study, three principal components that account for the variability were identified. The cumulative variance was 96% which is obtained from 19 observed traits. A principal component was determined based on total initial eigenvalue (Mattjik and Sumertawijaya 2011; Tresniawati and Randriani 2011). Principal component analysis result showed that morphological traits in principal component 1 that affect the variability the most are leaf color, leaf fiber fineness, leaf sheath length, petiole length, leaf blade length, leaf blade width, stem diameter, and plant height. Morphological traits that have the most impact on principal component 2 are leaf sheath color, stem surface, and stem color. Meanwhile, traits that are highly affecting in the principal component 3 are leaf sheath shape, leaf sheath arrangement, petiole color, leaf surface, leaf margin, leaf base, leaf tip, and leaf fiber length to diameter ratio (Table 7).

Cluster analysis

Cluster analysis in this study used Euclidean distance and generated dendrograms that showed phylogenetic relationship between lemba germplasms. Classification based on morphological characteristics is important in plant breeding, especially to highlight variations and the relationship between germplasm accessions (Tresniawati and Randriani 2011). Accessions that share many similarities means that those accessions have a close relationship. Conversely, accessions that have many dissimilarities or differences are indicative of distant kinship (Jan et al. 2012).

Table 7. Observation on three principal components of lemba accessions at a cumulative variance of 96%.

Principal component	Observed character
KU1	Leaf blade color (LC),Leaf fiber fineness (LF),Leaf sheath length (LSL),petiole length (PL), leaf length (LL),leaf width (LW),stem diameter (SD),and plant height (PH).
KU2	Leaf sheath color (LSC), stem surface (SS), and stem color (SC).
KU3	Leaf sheath shape (LSS),Leaf sheath arrangement (LSA),petiole color (PC),leaf surface (LS),leaf margin (LM),leaf base (LB),leaf tip (LT),leaf fiber length to diameter ration (LFR).

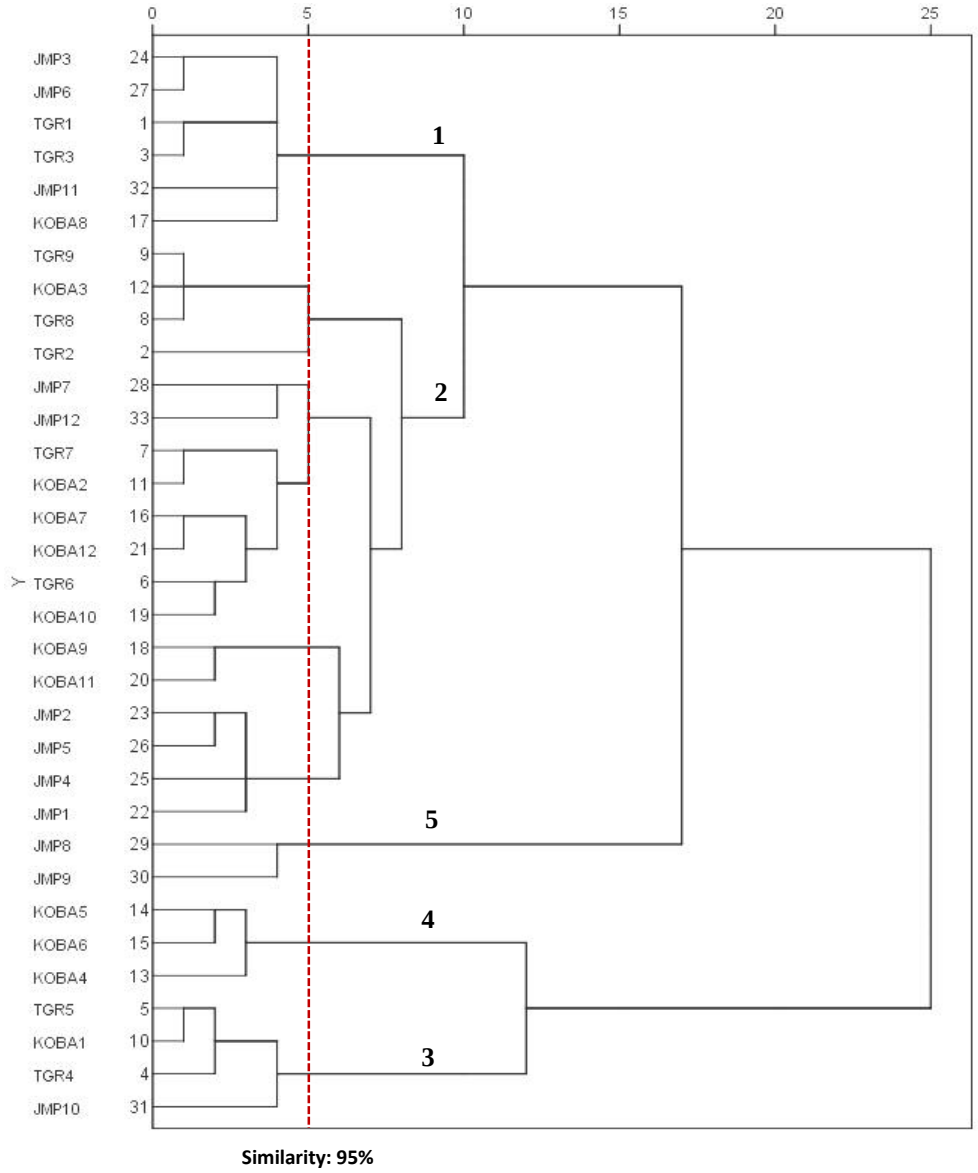


Figure 4. Dendrogram of 33 lemba accessions based on their quantitative traits

Cluster analysis result based on qualitative traits at 95% similarity revealed that there are five lemba accession groups. Group 1 consists of TGR1, TGR2, TGR3, TGR6, TGR7, KOBA2, KOBA10, JMP2, JMP3, JMP4, JMP5, JMP6, and JMP11 accessions; group 2 consists of TGR4, TGR5, KOBA1, KOBA5, KOBA6, KOBA12, JMP7, JMP8, and JMP9 accessions; group 3 which consists of TGR8, TGR9, KOBA3, KOBA7, KOBA8, and KOBA9 accessions; group 4 includes KOBA4, KOBA11, JMP1, and JMP12 accessions; and group 5 which consists of JMP10 accession (Figure 3).

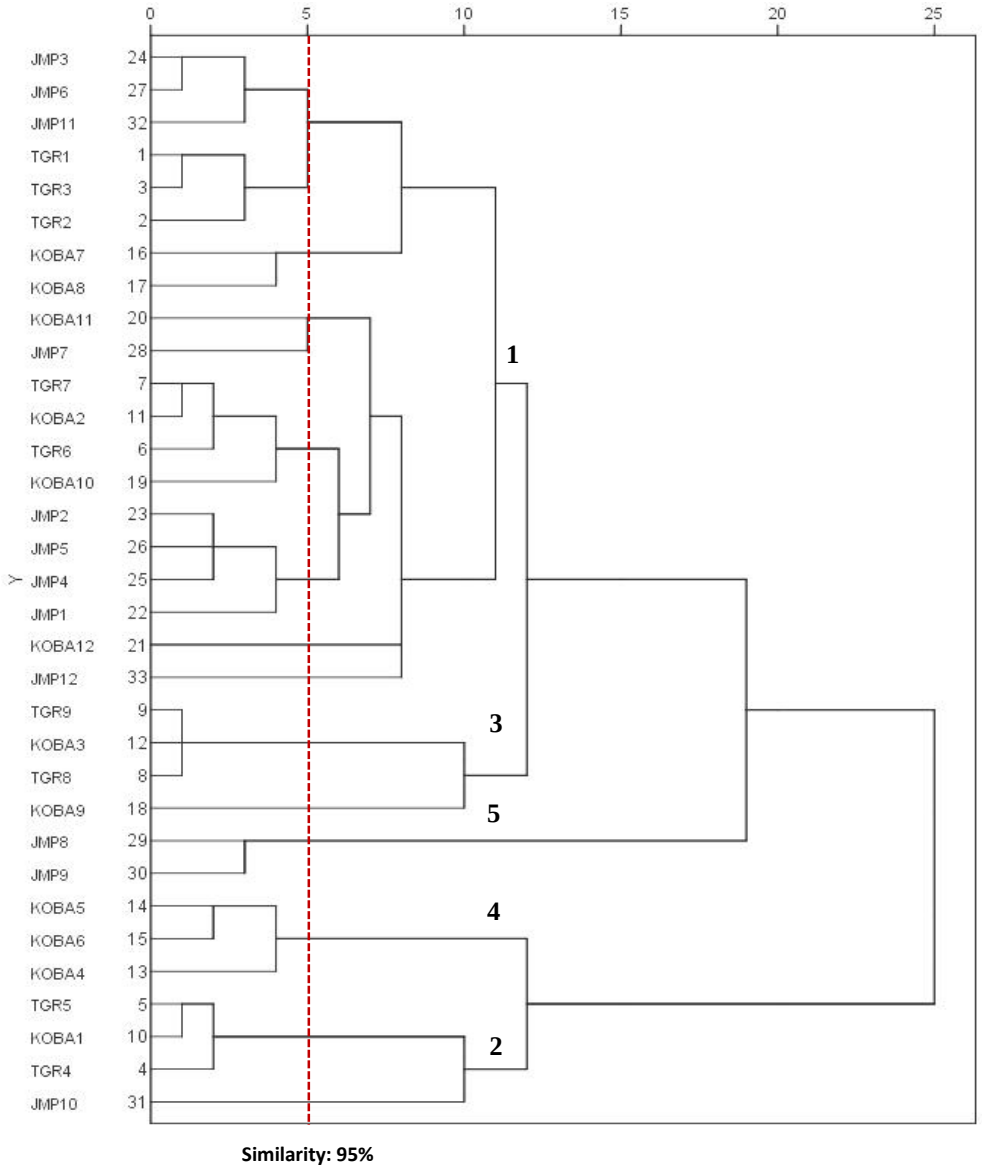


Figure 5. Dendrogram of 33 lemba accessions based on their combined qualitative and quantitative traits

Cluster analysis result based on quantitative traits at 95% similarity indicated five lemba plant accession groups. Group 1 consists of TGR1, TGR3, KOBA, JMP3, JMP6, and JMP11; group 2 consists of TGR2, TGR6, TGR7, TGR8, TGR9, KOBA2, KOBA3, KOBA7, KOBA9, KOBA10, KOBA11, KOBA12, JMP1, JMP2, JMP4, JMP5, JMP7 and JMP12 accessions; group 3 consists of TGR4, TGR5, KOBA1, and JMP10 accessions; group 4 consists of KOBA4, KOBA5, and KOBA6 accessions; and group 5 consists of JMP8 and JMP9 accessions (Figure 4).

Cluster analysis result based on combined qualitative and quantitative traits at 95% similarity indicated that there are five lemba plant accession groups. Group 1 consists of TGR1, TGR2, TGR3, TGR6, TGR7, KOBA2, KOBA7, KOBA8, KOBA10, KOBA11, KOBA12, JMP1, JMP2, JMP3, JMP4, JMP5, JMP6, JMP7, JMP11 and JMP12 accessions; group 2 consists of TGR4, TGR5, KOBA1, and JMP10 accessions; group 3 consists of TGR83 TGR9, KOBA3, and KOBA9 accessions; group 4 consists of KOBA4, KOBA5, and KOBA6 accessions; and group 5 consists of JMP8 and JMP9 accessions (Figure 5). According to Maji and Shaibu (2012), variables or observed traits that are tightly linked to the same group allows accessions to be divided into several existing relationships, and this interrelation is necessary to understand the behavior of complex traits of plant and allows the future development of the plant breeding program.

As a conclusion, the variance value of the 33 observed lemba plant accessions from the three Sub-districts points out that the phenotypic variability of qualitative traits was narrow while the that of the quantitative traits was broad. There are three principal components that account for the observed variability with a cumulative variance 0f 96%

which is obtained from 19 observed traits. Cluster analysis at similarity 95% indicates that there are five lemba accessions groups based on only qualitative, only quantitative, or based on combined qualitative and quantitative traits.

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Screening and characterization of endophytic fungi as antagonistic agents toward *Fusarium oxysporum* on eggplant (*Solanum melongena*)

FAJAR RAHMAH NURAINI, RATNA SETYANINGSIH♥, ARI SUSILOWATI
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Kentingan Surakarta 57126, Central Java, Indonesia. Tel/Fax. +62-271-663375 ♥email: ratnasetya@yahoo.co.id

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Abstract. *Nuraini FR, Setyaningsih R, Susilowati A. 2017. Screening and characterization of endophytic fungi as antagonistic agents toward Fusarium oxysporum on eggplant (*Solanum melongena*). Biodiversitas 18: 1377-1384. Fusarium oxysporum* is a soil borne pathogenic fungus that causes wilt disease in members of the family Solanaceae including the eggplant (*Solanum melongena* L.). One approach to resolving the problem of wilt disease in eggplant is to find endophytic microbes with antagonistic activity against *F. oxysporum*. The study reported here aimed to isolate such endophytic fungal antagonists from growing eggplants, to determine their antagonistic mechanisms, and to identify them. Samples of pathogenic fungi from diseased plants, assumed to be *F. oxysporum*, were obtained from the Laboratory of Plant Pests and Diseases of the Faculty of Agriculture, Universitas Sebelas Maret Surakarta. These were used to evaluate the antagonistic potential of endophytic fungi obtained from healthy eggplants in Dawung Village, Matesih, Karanganyar, Central Java. Specimens of various plant parts were collected from the healthy eggplants. The surfaces of these samples were sterilized for four minutes to remove contaminants, and then crushed excisions were cultured on a potato dextrose agar (PDA) medium. Antagonistic tests between endophytic and pathogenic fungi used the agar plug diffusion technique. Identification of fungi isolates was carried out on the basis of morphological characteristics. Six endophytic fungi isolated had antagonist activity against *F. oxysporum*. The antagonistic mechanism of FEB1, FEB2, FEB5 and FED1 was competition; FED2 was antibiosis, and FED3 was parasitism. Based on their morphological characteristics, FEB2, FEB5 and FED3 were identified as *Helicomyces* spp.; FEB1 was a *Rhizopus* sp.; FED1 was a *Mucor* sp.; and FED2 was a species of *Penicillium.*

Keywords: *Solanum melongena*, *Fusarium oxysporum*, endophytic fungi, antagonistic

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a vegetable crop that can be grown in the tropics and subtropics (Daunay and Janick 2007). The nutritional content of the fresh fruit is good: it is low in calories, fat, and sodium; and contains a favorable balance of protein, starch, fiber and additional nutrients such as potassium, magnesium, folic acid, vitamin B6, and vitamin A (Erica 2011). Extracts from the skin of the purple eggplant have the effect of lowering blood sugar levels (Aer et al. 2013). Anticancer activity has been identified in hepatocellular carcinoma, owing to the presence of steroidal alkaloids and sterol glycoside compounds (Shabana et al. 2013). Based on data from the Central Bureau of Statistics (2015), eggplant vegetable crop production in Indonesia has fluctuated little over the period 2011 to 2014; the production decreased from 519,481 tons in 2011 to 518,827 tons in 2012 but increased to 557,053 tons in 2014.

One of the diseases that attack eggplant is wilt disease caused by a soil-borne pathogen, namely the fungus *Fusarium oxysporum* (Fo). The symptoms include the plant looking wilted and yellowish, and its vascular tissue developing a brown color (Yildiz et al. 2012). Plant productivity declines. An effective, economical and practical, way to control wilt disease has not been discovered. Crop rotation, use of disease resistant varieties, sterilizing or solarizing the soil, and use of fungicides have all been recommended at times to deal with the disease (Agrios 1988; Dikilitas and Kocyigit 2010; Yildiz et al. 2012). *Fusarium oxysporum* (Fo) produces chlamydospores which can last a long time in the soil even when extreme environmental conditions constrain other microorganisms (Agrios 1988). Therefore, Fo is a fungal pathogen of eggplant that is difficult to control. One potential approach to addressing the problem of wilt disease in eggplant is to identify and use endophytic microbial isolates with activity antagonistic to the development of the pathogen.

Endophytes are microbes that inhabit higher plants. Endophytes are often considered as sources of novel metabolites with potential for medical, agriculture, and/or industrial exploitation. Natural products from endophytic microbes have been observed to inhibit or kill a wide variety of harmful diseases-causing agents, including bacteria, fungi, viruses and protozoa that affect humans, animals, and plants (Strobel and Daisy 2003). Endophytic fungi have proved to be the most promising. Many have been accessed as sources of bioactive compounds that can be used for treatment of a number of diseases (Kumar et al. 2014). Many plants in nature appear to be symbiotic with fungal endophytes. These endophytic fungi have a high diversity, with various impacts on plants (Rodriguez et al. 2009). The purposes of the study reported in this paper were to obtain endophytic fungi having antagonistic

activity against Fo from eggplant, to determine their antagonistic mechanisms, and to identify them.

MATERIALS AND METHODS

Sampling area and materials

Endophytic fungi were isolated from healthy eggplant crops derived from Dawung Village, Matesih, Karanganyar, Central Java, Indonesia. *Fusarium* (Fo) isolates pathogenic towards eggplant were obtained from the Laboratory of Plant Pests and Diseases of the Faculty of Agriculture, Universitas Sebelas Maret Surakarta. These isolates of the pathogen (Fo) derived from diseased eggplants in Karanglo Village, Matesih. The medium used to test antagonism of the endophytic fungal isolates again Fo was potato dextrose agar (PDA).

Procedures

Sampling of healthy eggplant crop as a source of endophytic fungi

Three eggplant plants were sampled from three different random points in crops in Dawung Village. Specimens of roots, stems, and leaves were taken from the plants. The abiotic factors measured at the site were pH of the soil, air temperature, soil temperature, soil humidity, and air humidity.

Isolation and purification of endophytic fungi

Specimens of the plant parts were cleaned under running water and cut into ± 5x5 cm sections. The surfaces of the specimens were sterilized by soaking in an emulsion of 70% ethanol for 4 minutes; NaClO 5.25% for 4 minutes; and sterile distilled water three times each for 1 minute. Samples, 1 cm^2 in size, excised from the specimens were then crushed and plated out on PDA media. Separated fungal colonies that grew were purified on new PDA medium.

Identification of Fusarium oxysporum *(Fo) isolates*

Eight isolates of the pathogenic fungi from eggplants with symptoms of *Fusarium* disease, coded C1, C2, C3, C4, C5, C6, C7 and C8, were identified macroscopically and microscopically for the presence of *Fusarium oxysporum* (Fo) based on Gandjar et al. (1999) and Watanabe (2002).

Endophytic fungi screening using agar plug diffusion method

The screening method used for assessment of antagonism was a modification of the agar plug diffusion method (Sharma et al. 2011; Balouiri et al. 2015). For the test of antagonistic activity of the endophytic fungi against the Fo pathogenic fungi, both were grown and incubated for 3 days. Samples, 6 mm in diameter, from both the Fo isolate and one of each of the endophyte isolates were then placed on a new PDA medium. The two isolate samples were separated by a distance of 3 cm on the medium. Cultures were then incubated for 7 days at the temperature of 25°C.

The percentage of growth inhibition of the Fo pathogenic fungi were categorized as follows; <30% = low antifungal activity, 30 - <50% = moderate antifungal activity, 50 - <70% = high antifungal activity, and ≥70% = very high antifungal activity (Živković et al. 2010; Khrueayu and Pilantanapak 2012). The percentage inhibition was calculated based on the formula of Kurnia et al (2014).

$$I = \frac{r_1 - r_2}{r_1} \times 100\%$$

Where*:*

I = the percentages inhibition (%)

r_1 = the radial growth of the Fo pathogen away from endophyte (mm)

r_2 = the radial growth of the Fo pathogens toward endophyte (mm)

Observation of antagonistic mechanisms

Inhibition mechanisms of endophytic fungi against the Fo pathogenic fungi were observed macroscopically and microscopically. The macroscopic observation was conducted by observing the interaction between endophytic fungi and Fo in a Petri dish. The mechanism of inhibition that occurred could be identified based on Baker and Cook (1983). Microscopic observation was conducted to detect the meeting between the hypha of the endophytic fungi with the pathogen, or else to detect changes in the hypha of the pathogen due to the influence of the active compounds produced by endophytic fungi. The way it worked was that a part of each PDA medium that had been inoculated with both endophytic fungi and pathogenic fungi isolates was cut and dripped with lactophenol. Afterward, the preparations were observed under a microscope (Kurnia et al. 2014).

Morphological characterization of potential endophytic microbes

Endophytic fungi were characterized macroscopically and microscopically. Fungal isolates were characterized based on Watanabe (2002).

RESULTS AND DISCUSSION

Sampling locations of healthy eggplant crops

The total area of Dawung is ± 256.6040 Ha. Dawung Village includes areas that are located in the uplands (± 370 masl) with rainfall ± 2,000 mm/year (Dawung Village Monograph Data 2013). Dawung Village has soil and air temperatures between 25-33°C, soil and air humidity around 64-88%, and soil pH ranging from 6.8 to 7.4 (Table 1). The temperature range in Dawung Village where the samples were taken supports the growth of eggplant crops, since eggplants are commonly grown in a temperature range of 22-30°C (Andersen 2011), a humidity of 85-90% (Delahaut and Newenhouse 1997), and a soil pH

of 6.0 to 7.0 (Andersen 2011). Fo has an optimum temperature of growth about 25°C while its optimum pH for growth is about 8 (Fayzalla et al. 2008). Temperatures in Dawung Village support the growth of Fo but the neutral soil pH condition is perhaps less than optimal for the growth of Fo.

Endophytic fungal isolates

Samples of the healthy eggplant crop being used, were four months old. The eggplant crops at that age are usually in good condition, not too young or too old. From the specimens taken from plant parts, six endophytic fungi isolates were obtained (Table 2).

Isolates of *Fusarium oxysporum* (Fo)

The identification of the putative *Fusarium* isolates obtained from the diseased field showed that the C2 isolate was, in fact, *Fusarium oxysporum*. Its macroscopic characters included circularly shaped colonies, flat elevation, colony edges, purple-colored smooth colony profile, aerial mycelia in moderate numbers and white color. The diameter of the colony reached 60.0 mm on the 7th day and the growth rate reached 8.6 mm/day (Figure 1A). In addition to the macroscopic characters, its microscopic characters included elongated and curved macroconidia with a slightly tapered tip. The macroconidia had 4 cells sized 30.3 µm x 3.6 µm (Figure 1C and 1D). Microconidia had 1-2 cells with or without septa and size of 11.6 µm x 3.2 µm (Figure 1B and 1E). The C2 isolate had two kinds of chlamydospores namely intercalary chlamydospores and terminal chlamydospores. The diameter of the chlamydospores was 5.6 µm (Figure 1F and 1G).

Activities of endophytic fungi as antagonistic agents

From the screening results, six endophytic fungal isolates with antagonistic potential were obtained. The percentage inhibition at 7 dai (days after inoculation) for these six isolates was more than 50%. Therefore, they were classified in the category of high antifungal activity (Table 3).

Isolate FED1 had a very high antifungal activity while isolates FEB1, FEB2, FEB5, FED2, and FED3 were categorized as having high antifungal activity. Overall, the percentage inhibition by endophytic fungi increased up to 7 dai. Six of the endophyte isolates exhibited visible signs of antagonistic mechanisms such as antibiosis, parasitism, and competition (Figure 2). and

Isolates FEB1, FEB2, FEB5, and FED1were antagonistic towards Fo by a competition mechanism. Those four isolates competed with Fo for food resources and space as indicated by the dominance of endophytic fungi in the Petri dishes (Figures 2B-E). Fo nutritional needs were disrupted by the mechanism of competition, causing a decline in the germination percentage of its spores by as much as 20-30% (a phenomenon described by Berlian et al. 2013). The FED2 isolate achieved inhibition by an antibiosis mechanism as indicated by the zone of inhibition in Figure 2F. The FED3 isolate acted as a parasite of Fo because its hyphae grew over the Fo hyphae (Figure 2G).

According to Dolakatabadi et al. (2012), endophytic fungi can form hooks around the hyphae of fungal pathogens before penetration, or sometimes can directly penetrate into the area of the fungal pathogens. Kurnia et al. (2014) explains that the hyphae of fungi pathogens undergo malformations into spiral, curved, irregular shapes, and experience a shortening, due to the exposure to the active compounds produced by endophytic microbes.

Figure 3A shows normal hypha that grew straight lengthwise. On the other hand, FEB1 hyphae were wrapped around the Fo hyphae, then directly penetrated into the Fo area (see Figure 3B). Isolates FEB2 and FED1 formed a hyphal structure that looked like a hook and wrapped around the Fo hypha (Figure 3C and 3E). FEB5 and FED3 hypha ensnared Fo (3D and 3G). Fo hypha underwent shortening, breakage, and crimping, presumably due to active compounds produced by FED2 (Figure 3F).

Table1. The abiotic factors at the sampling site

Parameter	Sampling time		
	Morning	Afternoon	Evening
Soil temperature (°C)	25.5	31.3	26.0
Air temperature (°C)	28.1	32.5	26.8
Soil humidity (%)	84.0	74.3	80.0
Air humidity (%)	84.3	64.7	87.5
Soil pH	6.8	6.8	7.0

Tabel 2. Endophytic fungi isolated from *Solanum melongena*

Part of plant	Codes given to identify the endophyte isolates	No. of isolates
Roots	FEA1	1
Stems	FEB1, FEB2, FEB3, FEB4, FEB5	5
Leaves	FED1, FED2, FED3	3
Total isolates		9

Table 3. Percentage growth inhibition of *Fusarium oxysporum* C2 resulting from antagonism by endophytic fungi isolated from *Solanum melongena*

Isolate	Percentage of inhibition (%)					
	2 dai	3 dai	4 dai	5 dai	6 dai	7 dai
FEA1	6.3	7.3	13.2	26.7	33.6	40.8
FEB1	24.2	38.4	57.7	61.8	68.0	68.6
FEB2	15.3	46.8	59.2	63.8	69.0	68.5
FEB3	3.5	5.9	22.0	37.5	40.0	48.1
FEB4	5.0	4.2	10.3	25.5	39.0	43.2
FEB5	11.7	24.4	44.4	55.6	60.1	62.9
FED1	24.7	41.9	63.2	71.8	75.6	73.4
FED2	9.2	14.8	25.4	36.0	43.5	50.2
FED3	9.3	10.5	23.5	42.0	47.9	50.3

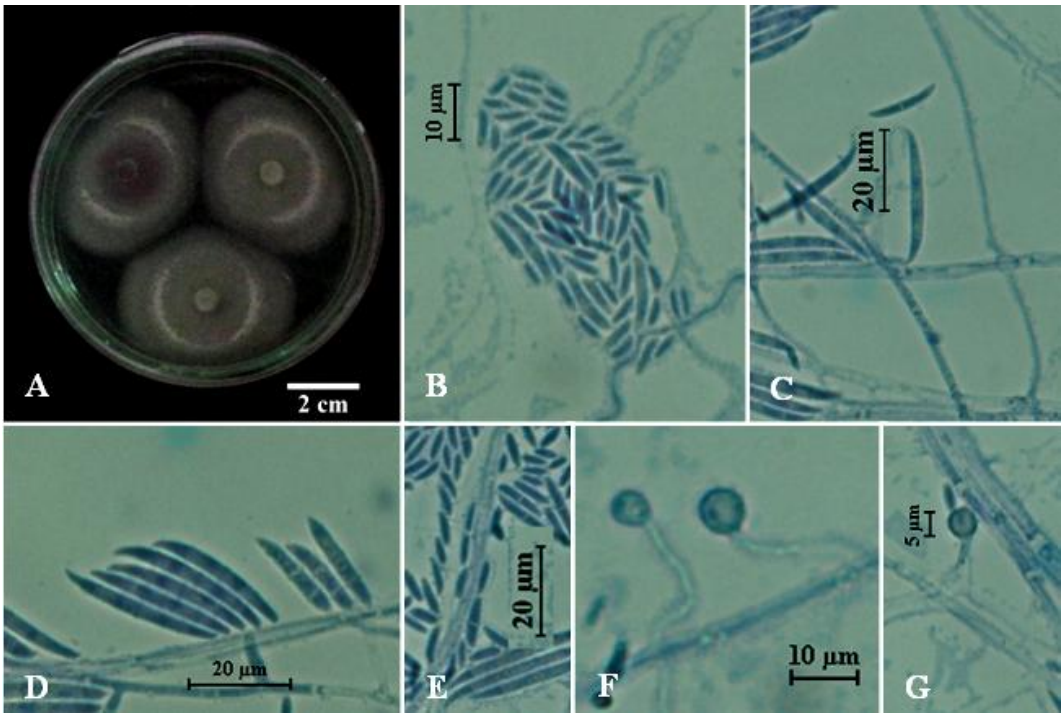


Figure 1. *Fusarium oxysporum* C2 (Fo); (A) colony on PDA medium incubated 7 days in the dark, (B and E) microconidia, (C and D) macroconidia and (F and G) chlamydospores.

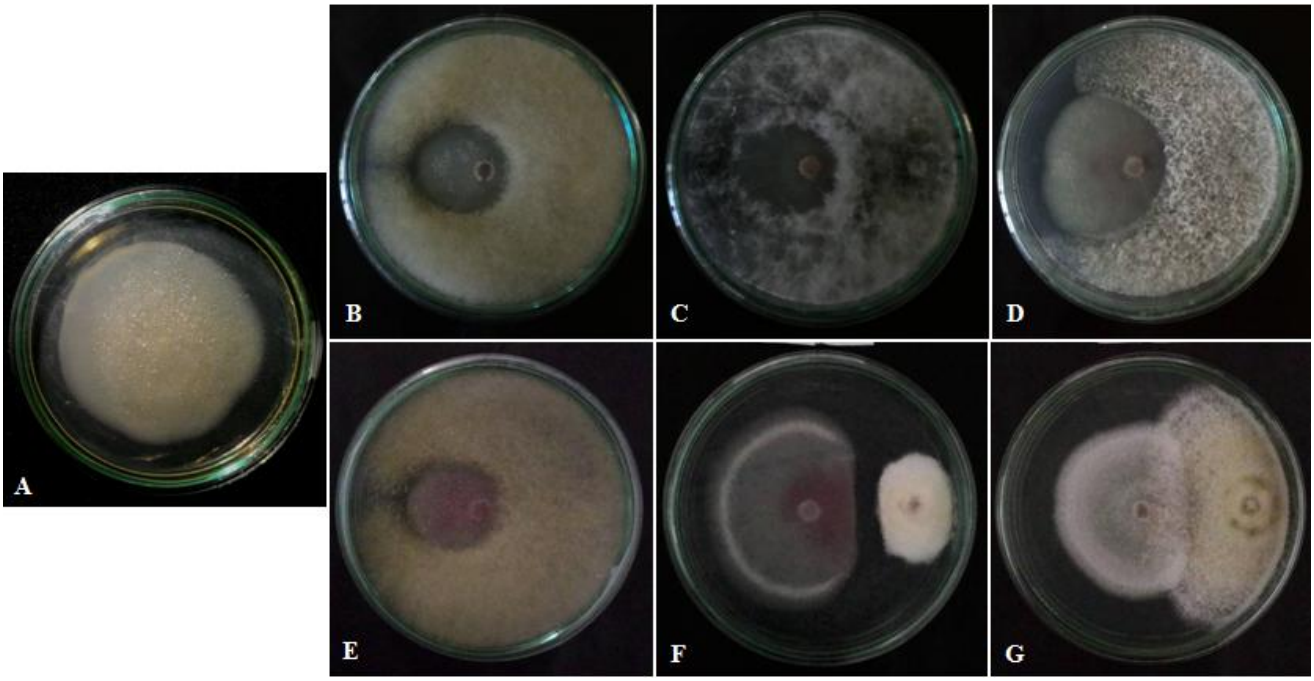


Figure 2. Inhibition of *Fusarium oxysporum* C2 by endophytic fungi isolated from *Solanum melongena*. (A) *F. oxysporum* control; (B-G) endophytic fungi tests - (B) FEB1, (C) FEB2, (D) FEB5, (E) FED1, (F) FED2, and (G) FED3. All cultures incubated for 7 days on PDA media.

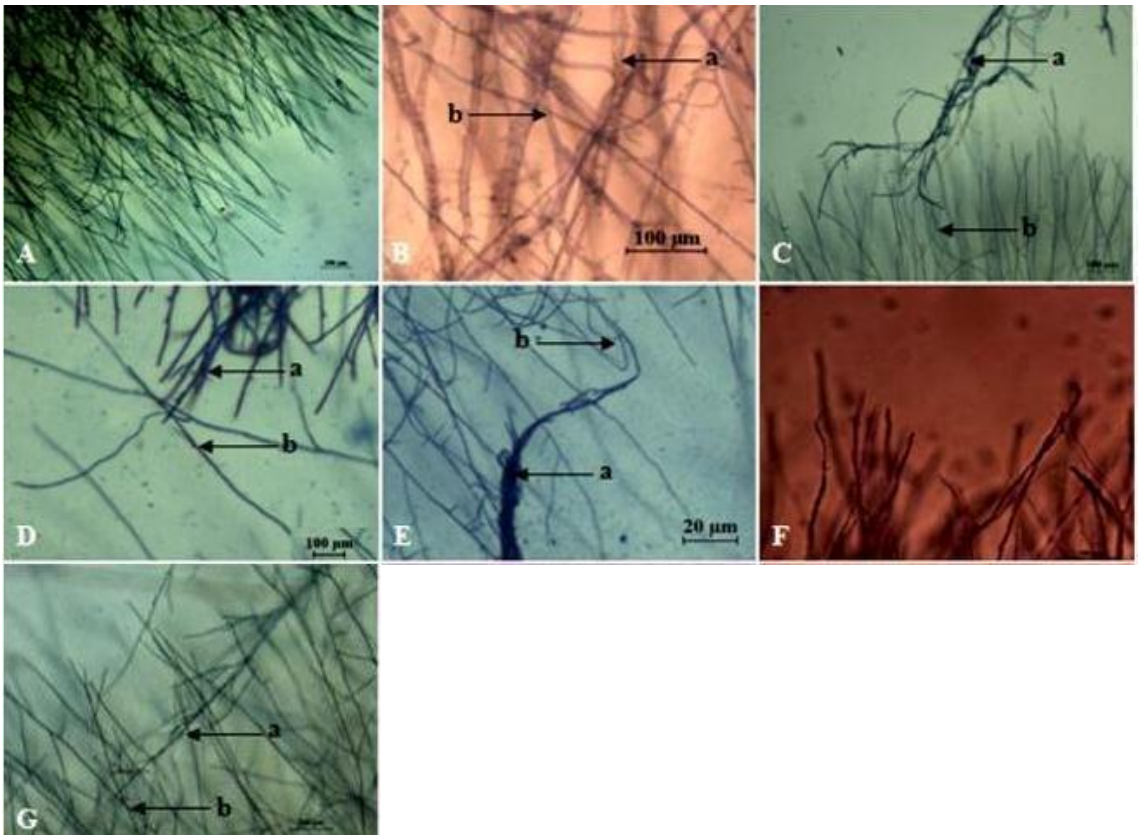


Figure 3. Interaction between endophytic fungi isolated from *Solanum melongena* and *Fusarium oxysporum* C2 (Fo); (A) normal Fo hyphae, (B) FEB1 and Fo hyphae, (C) FEB2 and Fo hyphae, (D) FEB5 and Fo hyphae, (E) FED1 and Fo hyphae, (F) Fo hypha becoming curly due to FED2 hyphae, (G) FED3 and Fo hyphae. (a) endophytic fungi (b) Fo

Morphological characteristics of endophytic microbes with potential as antagonistic agents

Characterization aimed to determine the macroscopic and microscopic characteristics of the endophytic microbes antagonistic to *F. oxysporum*. The observation of macroscopic features of the endophytic fungi showed that endophytic fungi have characteristics that can support their antagonistic activity; for example, exudate drops, aerial mycelia, and sclerotia. Exudate drops are the result of fungal metabolism that usually forms water droplets (Watanabe, 2002). The time when the exudate drops appear varies; there were some that appeared when the isolates were still at a young age (around 2-4 days after inoculation) or others at an old age (approximately 7 days and over). Isolates of the endophytic fungi appeared to produce antagonistically active compounds, except for FEB5 isolate.

A cluster of hyphae forms an intertwined mass called a mycelium that gets thicker through time and forms aerial hyphae or aerial mycelia (Gandjar et al. 2006). Endophytic fungal isolates that have a lot of aerial mycelia are highly benefited in terms of reproduction and dispersion. The fungal spores will quickly spread because the aerial mycelium can elongate and penetrate tissues inside the plant. In our study, isolates that had a lot of aerial hyphae were FEB1 and FED1 while the isolate that had only a few aerial hyphae was FED2.

Endophytic fungi form sclerotia at the relatively old age of about one month. Sclerotia are in the form of a hyphal mass that thickens and has a black color. According to Smith et al. (2014), the sclerotium is a structure that helps the fungi to survive against conditions such as freezing, desiccation, microbial attack, or the absence of the host. If the state of the environment is conducive, this resting cell will grow into hypha, mycelium, or stroma (Gandjar et al. 2006). Endophytic fungal isolates that had sclerotia in our study were FEB5 and FED3; they had a higher survival rate than other isolates.

The diameter of the endophytic fungi isolates was measured at 4 days after inoculation, at the time when one of the isolates had almost covered the surface of the PDA media in the Petri dishes. The diameters of the six antagonistic endophytic fungal isolates were larger (> 40 mm) than the diameter of the Fo (34.30 mm), except for FED2 isolate that inhibited the Fo by antibiosis. The rate of growth of the endophytic fungi isolates was higher (10.36-

27.18 mm/day) when compared to the Fo pathogenic fungi (8.85 mm/day), except for FED2 isolate (5.11 mm/day). Nevertheless, FED2 isolate was capable of producing active compounds to inhibit the growth of pathogenic fungi. With higher growth rates, the endophytic fungi can inhibit the growth of Fo. FEB1 was the isolate with the highest growth rate.

The colony shape of FEB1 isolate was circular. The color of the surface and reverse side of the colony color was yellow; it had a cottony texture and exudates drops. On the fourth day, the colony diameter was 73.8 mm. Hyphae of FEB1 were septate. Spores were semi-spherical, and they were arranged inside a sporangium supported by a sporangiophore. There was a root-like structure called a rhizoid (Figure 4).

The colony shape of isolate FEB2 was circular. The surface of the colony was white in color, while the reverse side was gray to black. The colony had a cottony texture, with exudates drops. On the fourth day, the colony diameter was 81.87 mm. Hyphae of FEB2 were septate. Its spore type was a helicospore supported by a conidiophore (Figure 5).

Isolate FEB5 was irregular in colony shape. The diameter of the colony was 60.25 mm on the fourth day. The color of the surface and reverse side of the colony both were white. The texture of the colony was cottony. Hyphae of FEB5 were septate and the spore type was a helicospore (Figure 6). This isolate formed sclerotia when the medium was running low (Figure 7).

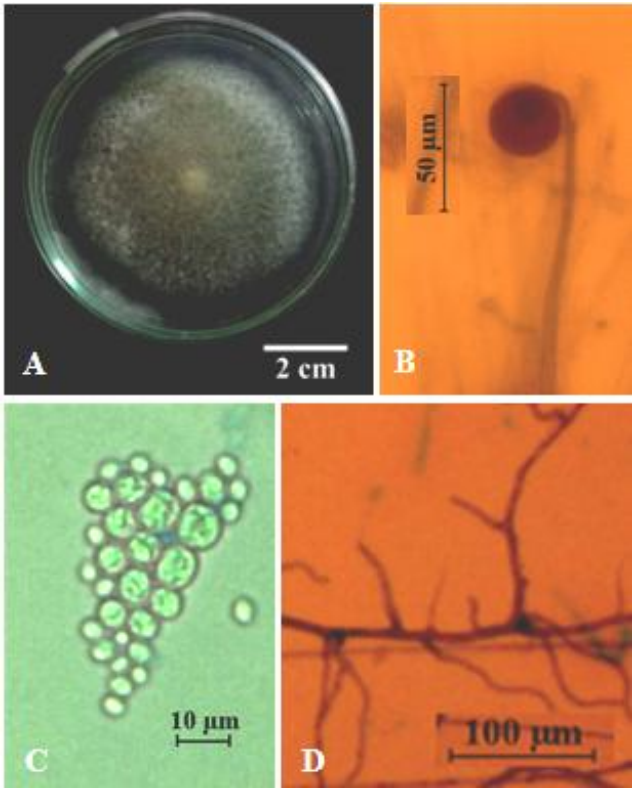


Figure 4. Isolate FEB1; (A) FEB1 colony on PDA medium after four days of incubation (B) sporangium supported by sporangiophore (C) spores (D) rhizoids.

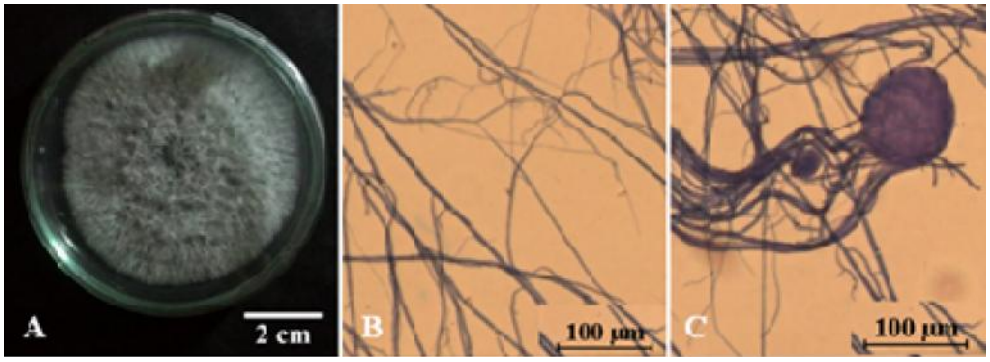


Figure 5. Isolate FEB2; (A) FEB2 colony on PDA medium after four days of incubation (B) hyphae (C) helicospore

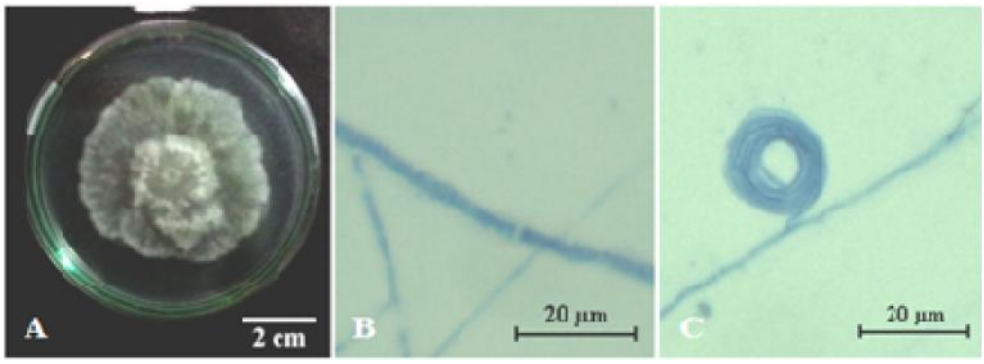


Figure 6. Isolate FEB5; (A) FEB5 colony on PDA medium after four days of incubation, (B) hypha, (C) helicospore.

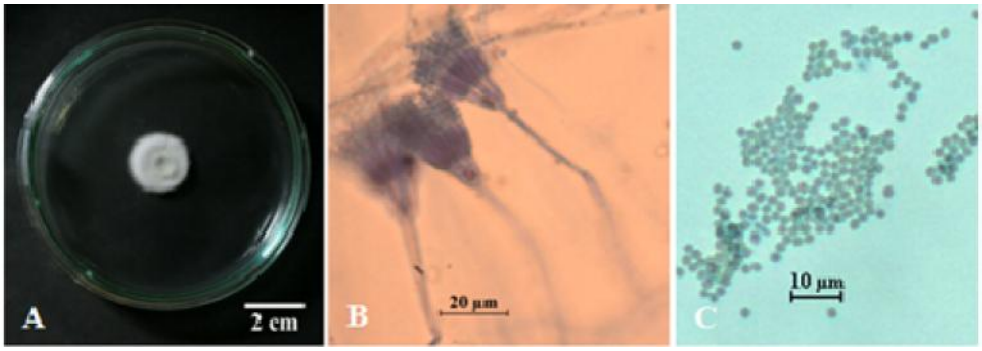


Figure 9. Isolate FED2; (A) FED2 colony on PDA medium after four days of incubation (B) conidiophore branching (C) conidiospores

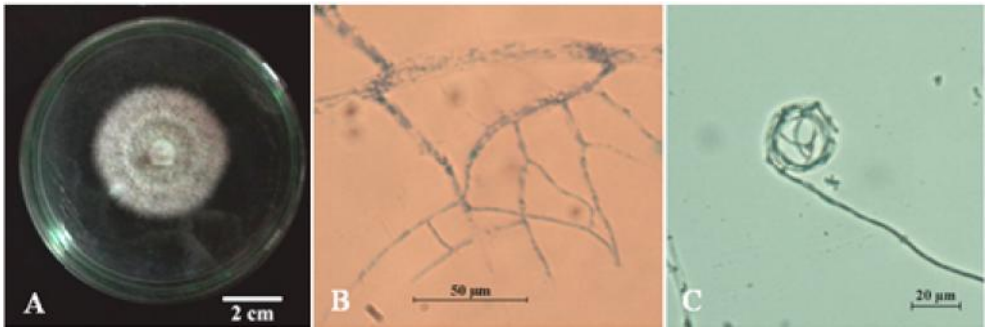


Figure 10. Isolate FED3; (A) FED3 colony on PDA media after four days of incubation, (B) hyphae, (C) helicospore

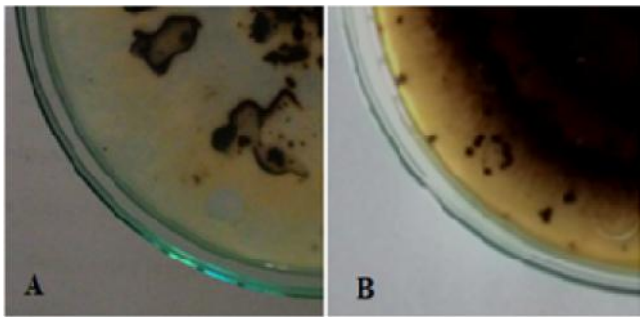


Figure 7. Sclerotia of FEB5 (A) and FED3(B)

Isolate FED1 had an irregular colony shape with a colony diameter of 73.95 mm on the fourth day. The color of the surface and reverse side of the colony both were yellow. The surface of the colony was cottony in texture, and there were exudate drops present. Hyphae were septate. Spores were produced in a sporangium. Spore shape was oblong (Figure 8). FED1 was similar to FEB1, except that FED1 did not have a rhizoid.

Isolate FED2 formed a circular colony with a velvety texture and exudates drops. Colony diameter was 19.67 mm on the fourth day. The color of the young colony was white and the mature colony was dark green. The reverse side of the young colony was pinky white in color, while the reverse of the mature colony was green white. Conidia were supported by mono-verticillate conidiophores. Conidiospores were round in shape (Figure 9).

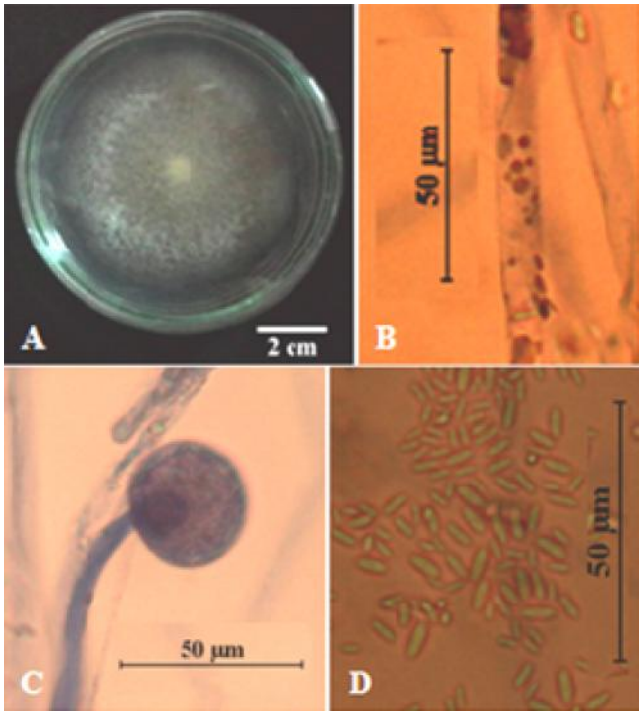


Figure 8. Isolate FED1; (A) FED1 colony on PDA medium after four days of incubation, (B) hypha (C) sporangium, (D) spores

Isolate FED3 formed a circular colony with cottony texture and exudates drops. The colony diameter was 48.1

mm on the fourth day. The color of the colony surface was white, and the color of the reverse side of the young colony was light brown while the mature colony reverse side was dark brown. Like FEB5, isolate FEB3 also formed sclerotia when the medium was running low. Its spore type was a helicospore (Figure 10).

From the characterization results, the genera of the endophytic fungi isolates could be determined, particularly considering the shapes of their asexual spores/conidia. Isolates FEB2, FEB5, and FED3 were identified as *Helicomyces* spp.*;* FEB1 was a *Rhizopus* sp.; FED1 a *Mucor* sp.*,* and FED2 a *Penicillium* sp.

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Carbon sequestration and growth of *Anthocephalus cadamba* plantation in North Kalimantan, Indonesia

AGUS SARJONO, ABUBAKAR M. LAHJIE♥, B.D.A.S. SIMARANGKIR, R. KRISTININGRUM, YOSEP RUSLIM♥♥

Faculty of Forestry, Mulawarman University. Jl. Ki Hajar Dewantara, PO Box 1013, Gunung Kelua, Samarinda Ulu, Samarinda-75116, East Kalimantan, Indonesia. Tel./Fax.: +62-541-735379. ♥email: lahjie@fahutan.unmul.ac.id, ♥♥yruslim@gmail.com

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Abstract. *Sarjono A, Lahjie AM, Simarangkir BDAS, Kristiningrum R, Ruslim Y. 2017. Carbon sequestration and growth of* Anthocephalus cadamba *plantation in North Kalimantan, Indonesia. Biodiversitas 18: 1385-1393.* Forest plantation of Jabon (*Anthocephalus cadamba*) plays many important roles for the primary source of timber production and also the carbon absorption for environmental amelioration. The growth of *A. cadamba* trees was measured using the formulas of MAI (Mean Annual Increment) and PAI (Periodic Annual Increment) as the basis of the increment measurement, while the analysis of biomass and carbon with simple linear regression (y = a + bx) was used, with the coefficient of R^2 = 0.90. The annual increment of *A. cadamba* trees reached its maximum point or the intersection between MAI and PAI at the age of 8 years, showing the maximum production of *A. cadamba* trees. Forest plantation of *A. cadamba* produced biomass and carbon sequestration of 81.90 ton ha^{-1} and 39.31 ton ha^{-1}, respectively at the age of 8 years. However, the amount of biomass and carbon in another plot of *A. cadamba* trees was 96.85 ton ha^{-1} and 46.49 ton ha^{-1}, respectively. While the values of the third plot were 116.84 ton ha^{-1} and 56.08 ton ha^{-1}, respectively. The correlation between the diameter and biomass production and the correlation between the age of stands and carbon production were very strong with R^2 value was higher than 0.90. This indicated that Jabon (*Anthocephalus cadamba*) plantation had important roles in carbon sequestration and biomass production.

Keywords: *Anthocephalus cadamba*, biomass, carbon, increment

INTRODUCTION

Tropical forests play an important role in global carbon sequestration. However, the increasing rate of deforestation and the impact of land-use changes need to be concerned prior to preventing the loss function of tropical forests. This work emphasizes the role of bulk density as the main driver of carbon (C) and nitrogen (N) stock in four land-use categories i.e natural forest, tree plantations, cropland and degraded soil (Tesfaye et al. 2016). In addition, the intense expansion of forest usage as the effect of human activities becomes a main focus to be solved especially the increased use of timber products (Hansen et al. 2013). Therefore, the development of more effective ways to reduce the illegal harvest activities should be done to protect the tropical forest diversity (Ruslim et al. 2016).

Birdsey and Pan (2015) had reviewed on how the function of forest has been changed in recent decades and summarized those implications for global carbon stocks. Although studies of specific management activities, including fertilization system, genotypes and species, site preparation and competition control have been applied in the industrial field trials for decades, the effect of these changes in the ecosystem level and in the interaction between land and atmosphere is still not been focussed, yet. Explicit comparisons of management effects using paired sites are rarely used (Herbst et al. 2015). More often extrapolations are based on the level of land use (Domec et al. 2015). The amount of this nutrient depletion depends on species characteristics, growth rate, tissue nutrient content, the period of harvesting rotation, the use of harvesting methods and nutrient reserves in the soil (Arias et al. 2011).

The mineralization of terrestrial detritus is primarily driven by soil microbes (Hobara et al. 2014; Hawke et al. 2015). The control of nutrient availability for plant uptake and soil microbes also drive C and N fluxes to the atmosphere and waterways. Multiple meta-analyses based on hundreds of studies have shown that the addition of N usually decreases the soil microbes that produce C (Lu et al. 2011). Conversely, P addition can either increase (Liu et al. 2012) or decrease (Groffman and Fisk 2011) forest soil microbial C regardless of the nutrient status of plants growing in the site (Turner and Wright 2014).

Increasing the rotation length would also increase the biomass carbon stock (Gonzalez-Benecke et al. 2015; Sharma et al. 2016; Panwar et al. 2017). The assessment of the whole potential of biomass carbon stock, and other potentials in order to change management activities is urgently needed for balancing the economic productivity with another ecosystem services such as carbon sequestration, the sustainability of soil health and water resources. This is important because the efforts to pursuit different management goals is very costly, but the established tools to evaluate the trade-offs is not ready, yet. (Birdsey and Pan 2015; Law and Waring 2015; Noormets et al. 2015),

A. Cadamba, one type of local Indonesian plants, is known as popular plants for forest plantation. *A. cadamba* trees can grow very rapidly with straight stands and its wood is qualified as one of the best raw materials in forestry industries such as plywood, furniture, pulp, paper, board and matches (Mansur and Tuheteru 2011). The nature of tree as carbon sinks is one of the most effective ways to capture greenhouse gas. The ability of fast-growing species to absorb carbon more rapidly compared with the slow-growing tree species is one of the reason for highly plantation of these trees in the private forest lands (Murdiyarso 2003; Chauhan et al. 2016a). In addition, forest plantation for wood production mostly provides environmental services such as water regulator and carbon absorber (Kanninen 2010; Chauhan et al. 2016b). These services can be sold to beneficiaries parties through Payment for Environmental Services (PES) (Wunder 2005). One of the largest existing environmental services markets is carbon markets through afforestation and reforestation activities in Clean Development Mechanism (CDM), which is integral component of the Kyoto Protocol Framework (Peters-Stanley et al. 2012). Some studies on optimal cycles in the forest plantation revealed that carbon for an environmental service has been included as an income source rather than wood in Europe and America (Van Kooten et al. 1995; Tasson et al. 2004; Galinato and Uchida 2010, 2011; Olschewski and Betitez 2010; Chauhan et al. 2015). Replacing one tree species with another one can considerably alter the functions of key forest ecosystem (Binkley and Menyailo 2005). Differences among species in growth rate, tissue nitrogen (N) concentrations, allocation to wood, foliage, and roots, litter chemistry, and mycorrhizal associations cause differences in net primary productivity (NPP), decomposition, soil carbon (C) storage, and N cycling (Crowley et al. 2016). However, the sustainability of fast-growing plantations may also be compromised. This occurs when the large amounts of nutrients removed biomass at the end of the rotations can not be compensated by nutrient inputs through fertilization, atmospheric deposition, or nitrogen biological fixation (Laclau et al. 2010). Therefore, this study aimed to analyze the increment of *A. cadamba* forest plant, and analyze the effect of forest plantation of *A. cadamba* on the production of biomass and carbon indicated by the value of coefficient of determination.

MATERIALS AND METHODS

Study site

The experiment was conducted from December 2015 to March 2016 in *A. cadamba* plantation area of Intracawood Manufacturing Company, North Kalimantan, Indonesia. The field experiments were conducted in four plots of *A. cadamba* plantation sites with the age of 8 years.

The area was located at a longitude of 116º, 47'21,0" East and at a latitude of 0º30'04,7" North and at an elevation of 40-50 m above sea level. The annual temperature ranged between 27-30ºC and annual rainfall was about 3,710.5 mm.

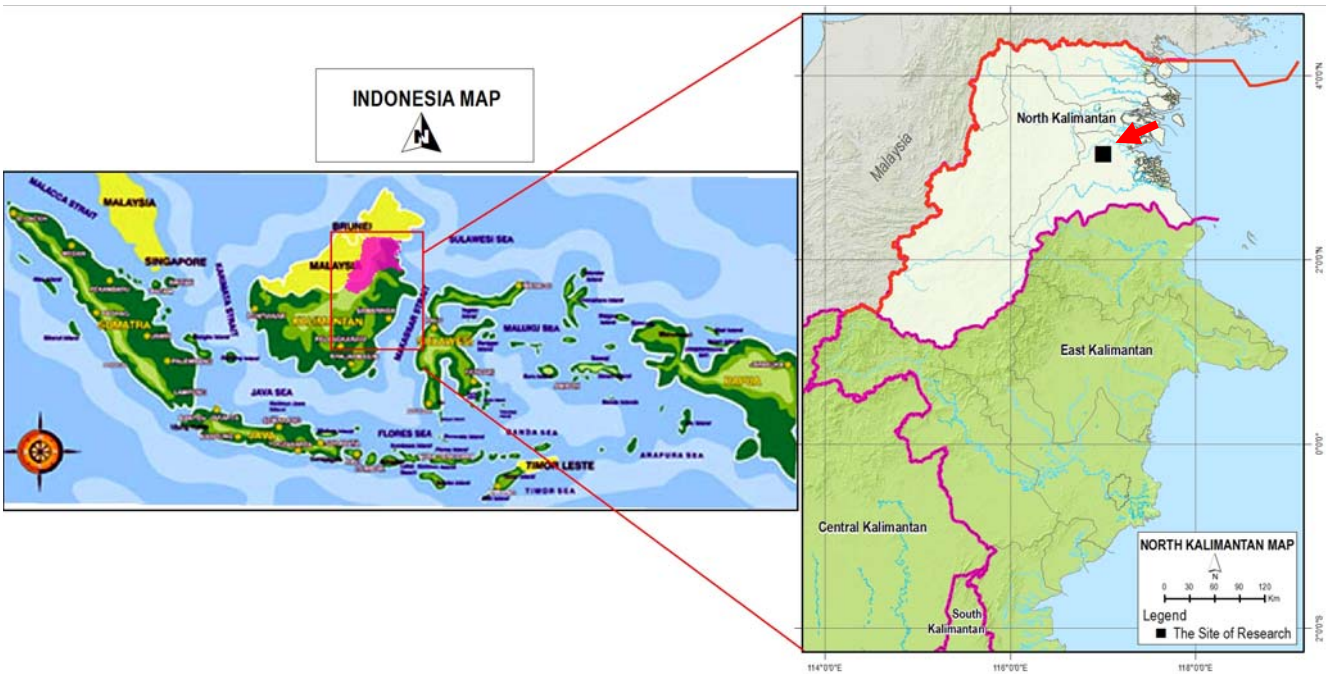


Figure 1. Location studies of Intraca manufacturing company (■) of North Kalimantan, Indonesia

Plot setting

Each plot had an area of 100m x 100m with a spacing of 4m x 4m. *A. cadamba* research plot consists of 3 (three) plots, ie. plot 1 as a control in the first year was given the manure fertilizer (bokashi) and NPK with the same dosage as the plot 2 and plot 3 with the value of fertilizer was 3-4 times from the price of biomass. For the advanced age, the plants were supplied with different fertilizer. Differences of fertilization doses in the second and subsequent years depended on the production of biomass (PAI). Differences in the economic value of fertilizer depended on Periodic Annual Increment (PAI) in that year. The dose of fertilizer was given to medium production ranged from 20-90% or the average total economic value of fertilizer 55% of biomass PAI (where young trees have the highest percentage). While at the maximum dose, the economic value ranged from 15 to 90% or the average total economic value of fertilizer 44% of biomass PAI where the price of manure IDR 1,000 kg^{-1} and NPK IDR 5,000 $kg^{-1.}$

Research procedure

Plot 1 was used to control the biomass and the growth, plot 2 and plot 3 were used to research and each stand age was used to evaluate the tree growth and to estimate its biomass production. The stem diameter at breast height (D; 1.3 m above the ground) and the tree height (H) of all *A. cadamba* trees were measured in the plots. The girth was measured using a tape. The diameter and the height was measured by Suunto clinometer.

The sample of the soil was firstly taken by determining the point of sample in the research plots. The soil composite samples were taken using a hoe or a shovel. The ground was dug up 30 cm deep, and then the soil sample was collected around 1 kg using a trowel.

Data analysis

Estimating MAI and PAI

According to Van Gardingen et al. (2003), to estimate the mean annual increment (MAI) and the periodic annual increment, the following mathematic formulas were used:

$$MAI = \frac{V_t}{t}$$

MAI = Mean annual increment
V_t = Total volume in ages t_0 - t (m^3)
t = Ages (years)

$$PAI - \frac{V_t - V_{t-1}}{T}$$

PAI = Periodic annual increment
V_t = Total volume in ages t_0 - t (m^3)
V_{t-1} = Previous total volume (m^3)
T = Second age t_0 - t, minus the first age (in year)

The estimation of tree biomass

The tree biomass can be expressed as the average of mass per unit area, or as the total of mass in the community. To formulate allometric equation for trees, about 10 different diameter-sized trees were cut down around the plots. This study was carried out through the destructive sampling technique of plant age on the level of *A. cadamba* started at 2 years old. To estimate the biomass of forest plants, the diameter and the height of the whole plants in an area (100 m x 100 m) were measured and the measurements were repeated three times. To build the allometric equation, this criterion was used: if the value of R^2 was high, then the variables had a strong correlation (Arezoo et al. 2014). Similarly, Chastain et al. 2006 used allometric modeling of the above ground biomass to quantify the importance of *Kalmia latifolia* and *Rhododendron maximum*, relative to stratum tree stands, in driving biogeochemical cycle in the Central Appalachian Mountains. Carbon sequestration and nitrogen and phosphorus storage potentials were investigated by running 50-year simulations of the ecosystem accounting model NuCSS for two situations: forests comprising the canopy over story layer with or without the evergreen understory layer (Darch et al. 2016). In addition to the quantity of the tree diameter and height, each of logs, branches, and twigs, leaves, and roots were also measured for its diameter and weight (Waring and Powers 2017). To find out the biomass of individual plant parts, about 250 grams of dried sample was taken and it was based on the ratio of wet weight and dry weight. Thus, the biomass per tree, parts of the stem, branches, and twigs, leaves and the total biomass can be estimated (Waring and Powers 2017). Biomass per plot is the sum of each individual tree biomass contained in the swaths of the designated study. Through the conversion of land coverage area, the accumulation of biomass or carbon content per hectare can be identified (Hardwick et al. 2015).

RESULTS AND DISCUSSIONS

Growth of *A. cadamba* stands

White Jabon (*A. cadamba*) can be cultivated in the different tread conditions (Haneda et al. 2012) and have significant exhibits on early growth of cultivation. However, the plants are threatened by the factor of excess water (Dunisch et al. 2003). Specifically, white jabon is very susceptible to water shortages, or not suitable for the rehabilitation of critical land.

The intensity of waterlogging and drought are predicted to increase in dry and rainy seasons due to climate change (Tong et al. 2016) and potentially affects on initial growth and successful forest and land rehabilitation activities. This condition can be used as reference in the development of seeds as well as a key climate change adaptation strategy (Millar et al. 2007). Jabon putih (*A. cadamba*) is a native forest species of South and Southeast Asia (Orwa et al. 2009). The characteristics of this species are quickly growth, straight growth, cylindrical boles, small diameter

branches and easily perform self-pruning. Jabon wood is very versatile as for plywood, light building materials, flooring, beams and rafters, boxes and wooden crate, wooden chests, packing boxes, coverings, ceiling boards, toys, wooden shoes, coils, shackles, carvings, chopsticks and pencils (Soerianegara and Lemmens 1993). In Indonesia, this species has been cultivated in West Java, East Java, South Kalimantan, East Kalimantan, Sumatra, Sulawesi, West Nusa Tenggara, and Papua (Martawijaya et al. 1989).

Detailed growth of A. cadamba from plot one plot two and plot three is presented in Table 1. According to Indrayana et al. (2013), *A. cadamba* trees cultivated in Java had the mean annual volume with the increment of 21 m^3 ha^{-1} $year^{-1}$, whereas, Krisnawati et al. (2011) stated that the mean annual volume of *A. cadamba* in small farms in South Kalimantan at the age of 9, reached 20 m^3 ha^{-1} $year^{-1}$. In this research, the maximum average increment at age 8 in plot one 20.47 m^3 ha^{-1} $year^{-1}$, in plot two 24.21 m^3 ha^{-1} $year^{-1}$, in plot 3 29.21 m^3 ha^{-1} $year^{-1}$. Can be seen in Table 1.

The average diameter of *A. cadamba* approximately 13 cm at age 5. While in this research, in plot one 22.7 cm, in plot two 23.4 cm and in plot three 26.2 cm. The mean of total height is 11 m, whereas in this research the clear bole height in the 5.8 m plot (total height 9 m), in the plot two clear bole height 6 m (total height of 9.6 m), in plot three clear bole height 7 m (total height 10.8 m). To estimate these difference factors, in South Kalimantan have rainfall 2200-2700 mm $year^{-1}$ while in the location of this study 3200-3700 mm $year^{-1}$. Another factor, the density of plants in one hectare more in South Kalimantan, therefore the total height of *A. cadamba* is higher.

What is interesting in the data table above is the Mean Annual Increment (MAI) precisely in year 8, where the MAI and PAI are equal in value even though the total of increment is different i.e. in plot one 163.79 m^3 ha^{-1} $year^{-1}$ in plot two 193.71 m^3 ha^{-1} $year^{-1}$ and on the plot three 233.68 m^3 ha^{-1} $years^{-1}$. This difference is known because of differences in fertility resulting from fertilization and silvicultural techniques by thinning. Seen on plot one number of tree 460 diameter 30 cm at age 8, in plot two number of tree 450 diameter 32.3 cm and in plot 3 number of tree 430 diameter 33.6 cm. According to Seo et al. (2015) the three plots are considered good growth.

The effect of fertilization on the increment growth of *A. cadamba*

Seo et al. (2015) has divided three categories of *A. cadamba* growth research in West Java into good growth in white jabon age 3.5 years diameter ranging from 15-17 cm, growth medium diameter ranged from 11-13 cm, poor growth 5-7 cm. In West Java, the most widely used fertilizers are NPK fertilizer, animal manure (bokashi), urea were widely applied and few TSP in some places. In general, the initial fertilization done at the time with the amount of fertilizer 15 grams per seedling. In South Kalimantan, urea and TSP are widely used at the time of growth of jabon age 2. Thus, at 3m x 3m spacing the amount of inorganic fertilizer reaches 15 kg ha^{-1}. In this research, initial fertilization was done on three plots, 56 g $seedling^{-1}$ NPK fertilizer and 1 kg animal manure $seedling^{-1}$. While the subsequent fertilization depends on the amount of increment and estimated price of stem biomass. In this study, it is not based on high increments and diameters but is based on increment volume stands or trees.

The increments of *A. cadamba* in each plot were different because of the different fertilizer applications in plot two and plot three. Plot one as the control plot was not fertilized, while plot two and plot three were fertilized to find out if there was any effect of fertilization on the growth of *A. cadamba*. The types of fertilizer applied were bokashi and NPK. The dosage and effect of the fertilizer can be seen in Table 2.

Table 1. The volume of *A. cadamba* in plot one (control plot), plot two and plot three

Ages	Plot 1							Plot 2							Plot 3						
	N	d	h	F	TV	MAI	PAI	N	d	h	F	TV	MAI	PAI	N	d	h	F	TV	MAI	PAI
2	590	13.7	4.0	0.78	27.12	13.56	-	580	14	4.3	0.78	29.93	14.97	-	580	14	4.8	0.77	32.98	16.49	-
3	570	17	4.8	0.76	47.17	15.72	20.05	560	17.4	4.7	0.77	50.07	16.69	21.00	570	16.7	5.7	0.77	54.77	18.26	21.79
4	550	19.6	5.5	0.75	68.42	17.10	21.24	540	20.2	5.5	0.75	72.67	18.17	22.60	550	20.0	6.3	0.77	83.78	20.94	29.01
5	530	22.7	5.8	0.74	92.01	18.40	24.29	520	23.4	6.0	0.74	99.24	19.85	26.57	520	24.0	7.0	0.76	125.09	25.02	41.31
6	500	26	6.1	0.73	118.15	19.69	26.14	490	27	6.6	0.73	135.10	22.52	35.86	510	26.2	8.0	0.75	164.89	27.48	39.80
7	480	28.3	6.6	0.72	143.40	20.49	25.25	470	30	7.0	0.73	169.68	24.24	34.58	480	30.0	8.2	0.74	205.78	29.40	40.89
8	460	30	7.0	0.72	163.79	20.47	20.39	450	32.3	7.3	0.72	193.71	24.21	24.03	430	33.6	8.4	0.73	233.68	29.21	27.90
9	455	30.6	7.2	0.71	170.97	19.00	7.17	440	33.1	7.4	0.72	201.93	22.44	8.22	400	35.4	8.6	0.72	243.65	27.07	9.97
10	440	31.1	7.5	0.70	174.47	17.45	3.50	430	34.1	7.5	0.71	205.97	20.60	4.05	380	36.5	8.8	0.71	248.30	24.83	4.65

Notes: N = Population of *A. cadamba* (tree ha^{-1}), d = Tree Diameter (cm), h = clear bole height (m), F = form factor, TV = Total Volume (m^3ha^{-1}), MAI = Mean Annual Increment (m^3 ha^{-1} $year^{-1}$), PAI = Periodic Annual Increment (m^3 ha^{-1} $year^{-1}$)

Table 2. The Effect of fertilization dosage on the growth of *A. cadamba* plot two and three

Age (year)	Plot 2						Plot 3					
	PAI 1	PAI 2	Difference PAI	Price	NPK	Bokashi	PAI 1	PAI 3	Difference PAI	Price	NPK	Bokashi
	(m^3 $ha^{-1}$$year^{-1}$)			(Rp.1,000 m^{-3})	(kg ha^{-1})		(m^3 $ha^{-1}$$year^{-1}$)			(Rp.1,000 m^{-3})	(kg ha^{-1})	
1	13.15	14.01	0.86	270	35	625	13.15	15.18	2.03	270	35	625
2	16.81	17.55	0.74	300	10	100	16.81	18.36	1.55	300	20	300
3	20.05	21.00	0.95	350	20	200	20.05	21.79	1.74	350	40	400
4	21.24	22.6	1.36	350	30	300	21.24	29.01	7.77	350	60	600
5	23.60	26.57	2.97	400	50	500	23.6	41.31	17.71	400	80	900
6	26.14	35.86	9.72	400	90	600	26.14	39.80	13.66	400	120	1100
7	25.25	34.58	9.33	500	60	800	25.25	40.89	15.64	500	80	900
8	20.39	24.03	3.64	500	40	300	20.39	27.90	7.51	500	50	600
9	7.17	8.01	0.84	550	30	100	7.17	9.97	2.80	550	30	300
10	3.50	4.20	0.70	600	20	90	3.50	4.65	1.15	600	20	200

Notes: PAI 1= Periodic annual increment of *A. cadamba* in plot 1, PAI 2 = Periodic annual increment of *A. cadamba* in plot 2 and PAI 3 = Periodic annual increment of *A. cadamba* in plot 3

Table 2 showed that the increment growth of *A. cadamba* in plot 2 was affected by the different dosages of NPK and bokhasi fertilization. This was indicated by the fact that the average periodic annual increment of *A. cadamba* in plot two was higher than that in plot one. The PAI of *A. cadamba* in plot two reached its maximum increment at the age of 6 years with the growth rate of 35.86 m^3 ha^{-1} $year^{-1}$. The different values of PAI which started from the age of 1 year to 10 years were 0.86; 0.74; 0.95; 1.36; 2.97; 9.72; 9.33; 3.64; 0.84 and 0.7 m^3 ha^{-1} $year^{-1}$ respectively. The dosages of NPK fertilizer were different for different ages. The fertilizer was applied to the trees aged 2 to 7 years with the increasing dosages of 10 kg; 20 kg; 30 kg, 50 kg; 90 kg; 60 kg in each hectare for each year. After the trees had reached the age of 7, the dosage of fertilizer was reduced to 40 kg; 30 kg and 20 kg. This followed the law of diminishing returns. The dosages of bokhasi fertilizer application were also different. At the age of 2 to 7 years the dosages were increased, and when the trees reached the age of 8 years, the dosages of bokhasi fertilizer were reduced. The amount of fertilizer applied was based on the total income derived from timber sales. The percentage of bokhasi fertilizer application was 60%, higher than the percentage of NPK fertilizer application which was only 40%.

Table 2 showed that the increment of *A. cadamba* in plot 3 was effected by the different dosages of NPK and bokhasi fertilizer application. This can be seen from the periodic annual increment of *A. cadamba* in plot three which was higher than that in plot one. The PAI of *A. cadamba* in plot three also reached its maximum increment at the age of 6 with the increment of 43.76 m^3 ha^{-1} $year^{-1}$. The different values of PAI reached at the age of 1 year to 10 years were 2.03; 1.55; 1.74; 7.77; 17.71; 13.60; 15.64; 7.51; 2.8; and 1.15 m^3 ha^{-1} $year^{-1}$ respectively. The dosages of NPK fertilizer application were different according to the tree ages, which began at the age of 2 years to 6 years with the increasing dosages of 20 kg; 40 kg; 60 kg, 80 kg and 120 kg, and after reaching the age 7 years, the dosages of NPK fertilizer application were reduced to 80 kg; 50 kg; 30 kg and 20 kg. This followed the law of diminishing returns. The application of bokashi fertilizer was also different. At the age of 2 to 6 years, the dosages were increased, and when the trees reached the age of 7 years, the dosages of bokhasi fertilizer were reduced. The amount of fertilizer applied to *A. cadamba* in plot three was the same as the amount of fertilizer applied to *A. cadamba* in plot two, namely it was based on the total income derived from timber sales. The percentage of bokhasi fertilizer application was 70%, higher than the percentage of NPK fertilizer application which was just 30%.

The addition of fertilizer in plot two, the result shows higher economic value (18.3%) compared to plot one. The addition of the fertilizer in plot three also shows higher economic value (24.4%) compared to plot one. However, the comparison of economic value between plot two and three is 6.1%. Futhermore, the result shows that the maximum of the amount of fertilizer is found in plot three.

Biomass in revegetation area

Biomass is closely related to the process of photosynthesis. Biomass increases because the plant absorbs CO_2 from the air and transforms it to organic compounds through the process of photosynthesis. The result of photosynthesis is used by plants to grow horizontally and vertically (Adinugroho and Sidiyasa 2009). Based on the growth volume of *A. cadamba* in all plots, it was found that there was a correlation between biomass and litter, between litter and age, and between litter and basal area. Therefore, the analysis of simple linear regression was needed. To measure the precision of the regression line which was used to identify the variability of data explained by the regression model, coefficient of determination was required, which was symbolized as R^2. The maximum value of R^2 was 100%, and the minimum value was 0%, with the following criteria: if the value of R^2 was high then there was a strong correlation between X and Y or if $R^2 = 0$, then there was no

any correlation between X and Y. If the value of R^2 was low, then the correlation between X and Y was weak (Handayani 2010). In addition, if the value of coefficient of determination (R^2) showed a precise and strong correlation between the independent and dependent variables, then according to this criterion, it could give greater confidence on the acceptance of model. The high value of R^2 means that there was a strong correlation between the variables (Grafen and Hails 2002; Arezoo et al. 2014).

Table 3 showed the data of the correlation of basal area, biomass, litter, and carbon of *A. cadamba* in plot one as a control plot. The older the age of one stand, the higher its value would be or it means that the value of a stand and its age were directly proportional. The values of biomass, litter, and carbon of *A. cadamba* in plot one at the age of 8 were 81.90 ton ha^{-1}; 2,500 kg ha^{-1} and 39.31 ton ha^{-1} respectively. The correlation can be seen in Figure 2.

Figure 2 showed that there was a very strong correlation and linear between carbon and litter, basal area and biomass of *A. cadamba* in plot one. The value of coefficient of determination (R^2) between carbon and biomass was 0.98.; the value of coefficient of determination (R^2) between carbon and basal area was 0.99, and the value of (R^2) between carbon and litter was 0.96. This indicated that there was a strong correlation between carbon and basal area also between biomass and litter because the value of coefficient of determination was higher than 0.90. The graph of each correlation formed a liner shape. This means that the older the age of an *A. cadamba* tree, the higher the content of its carbon, litter, biomass and basal area. The correlation of Basal Area, Biomass, Litter, and Carbon of *A. cadamba* in Plot two can be seen in Table 3.

Table 3 showed that there was a very strong correlation between carbon and litter, basal area and biomass of *A. cadamba* in plot two and the correlation form a linear shape. Graphically, the correlations can be seen in Figure 3, in which the value of R^2 for the correlation between carbon and biomass was 0.99; the value of R^2 for the correlation between carbon and basal area was 0.99, and the value of R^2 for the correlation between carbon and litter was 0.97. This indicated that there was a strong correlation between carbon and basal area, biomass and litter because the value of its coefficient of determination was higher than 0.90 and the graph of each correlation formed a linear shape. This means that the older the age of an *A. cadamba* tree, the higher the content of its carbon, litter, biomass and basal area. The amount of biomass, litter, and carbon of *A. cadamba* in plot two at the age of 8 were 96.85 ton ha^{-1}; 3,020 kg ha^{-1} and 46.49 ton ha^{-1} respectively.

The data concerning the correlation of basal area, biomass, litter, and carbon can be seen in Table 3. Table 3 showed the correlation of basal area, biomass, litter and carbon of *A. cadamba* in plot three, which had a linear correlation. This means that the older the age of one stand, the higher the content of its biomass, litter and carbon. This is supported by Figure 4 which shows that the correlation between carbon and litter, and between basal area and biomass of *A. cadamba* in plot three was very strong and linear. The value of R^2 for the correlation between carbon and biomass was 0.99; the value of R^2 for the correlation between carbon and basal area was 0.99, and the value of R^2 for the correlation between carbon and litter was 0.96. This means that there was a strong correlation between the content of carbon and basal area of *A. cadamba* trees in plot three and there was a strong correlation between biomass and litter because the values of their regression coefficients were higher than 0.90 and the graph of each correlation formed a linear shape. This means that the older the age of an *A. cadamba* tree, the higher the content of its carbon, litter, biomass, and basal area. The amount of biomass, litter, and carbon of *A. cadamba* in plot three at the age of 8 was 116.84 ton ha^{-1}; 3.650 kg ha^{-1} and 56.08 ton ha^{-1} respectively.

Based on Figure 2, 3 and 4, it can be concluded that the correlations between carbon and biomass, between litter and basal area in all research plots, were very strong and linear because the value of regression coefficient (R^2) was higher than 0.90.

Table 3. The correlation of basal area, biomass, litter, and carbon of *A. cadamba* in plot one, plot two and plot three

Ages (year)	Plot 1/control				Plot 2				Plot 3			
	Basal area (m^2)	Biomass (ton ha^{-1})	Litter (kg ha^{-1})	Carbon (ton ha^{-1})	Basal area (m^2)	Biomass (ton ha^{-1})	Litter (kg ha^{-1})	Carbon (ton ha^{-1})	Basal area (m^2)	Biomass (ton ha^{-1})	Litter (kg ha^{-1})	Carbon (ton ha^{-1})
2	8.69	12.75	1,080	5.74	8.92	14.07	1,184	6.33	8.92	15.50	1,300	6.98
3	12.93	22.41	1,250	10.20	14.02	23.78	1,300	10.82	12.52	25.71	1,350	11.70
4	16.59	32.84	1,480	15.11	17.62	34.88	1,540	16.05	17.27	40.21	1,800	18.50
5	21.44	44.63	1,800	20.75	22.35	48.13	1,920	22.38	21.25	58.75	2,350	27.32
6	26.53	57.89	2,000	27.21	28.04	66.20	2,380	31.11	27.48	80.80	2,900	37.97
7	30.18	70.98	2,300	33.72	33.21	83.99	2,800	39.90	33.69	101.18	3,360	48.06
8	32.50	81.90	2,500	39.31	36.85	96.85	3,020	46.49	38.11	116.84	3,650	56.08
9	33.44	86.34	2,450	41.87	37.90	101.21	2,950	49.08	39.35	123.04	3,500	59.68
10	33.62	89.45	2,350	43.83	38.68	102.88	2,850	50.41	39.40	125.94	3,450	61.71

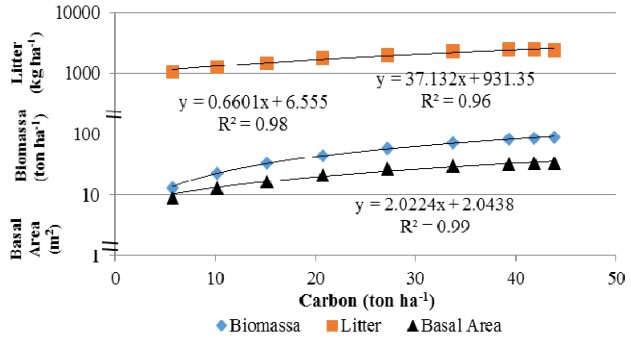


Figure 2. The Correlation between carbon and litter and basal area and biomass of *A. cadamba* in plot one

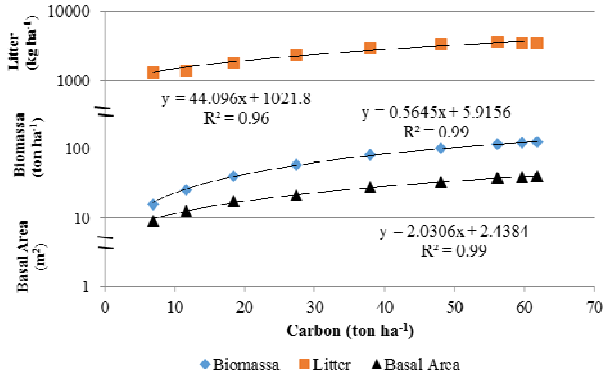


Figure 3. The correlation between carbon and litter, basal area and biomass of *A. cadamba* in plot two

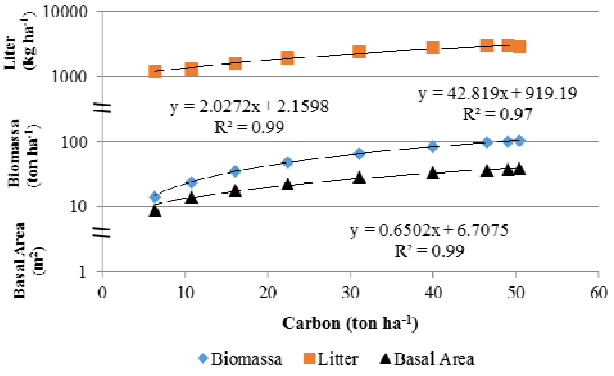


Figure 4. The correlation between carbon and litter, between basal area and biomass of *A. cadamba* in plot three

Carbon and biomass production

As the trees were getting older, their ability to absorb carbon was also high. Therefore, age was very influential on the production of carbon. The stand age, in relation to its influence on carbon sequestration, had a very strong and high correlation (R^2), reaching 0.90 (Figure 5). The tree phase was able to absorb more carbon compared with the pole phase, sapling phase, and seedling phase. The factor which influences the ability of a tree in storing carbon is the age of the tree (Mansur dan Tuheteru 2011).

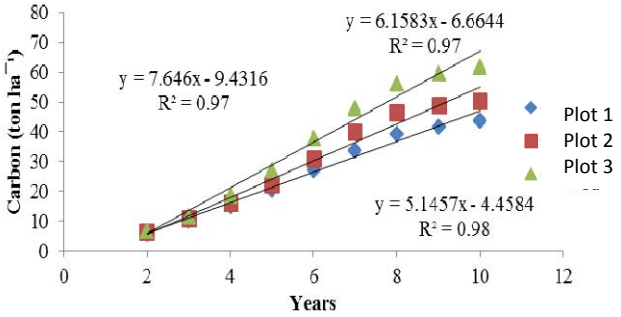


Figure 5. The correlation between the stand age of *A. cadamba* and the production of carbon

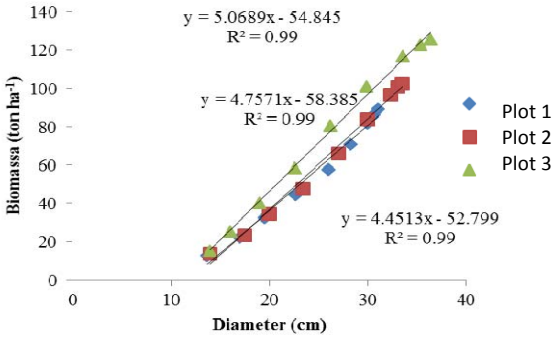


Figure 6. The correlation between diameter and biomass in the research plots

Tree age is closely related with tree stratification. The higher the stratification of a tree, the older the tree will be. The main differences of stratification level are the diameter and height of the tree. The older the age of a tree, the larger its diameter and the higher its trunk will be (Mansur and Tuheteru 2011). The same is applied to the diameter and biomass of a tree. The larger the diameter of one stand, the more biomass it will produce. Figure 6 showed a very significant correlation because the value of its R^2 reached 0.90.

Each plot produced different amount of biomass. Plot one as the control plot produced a very small amount of biomass compared with the production of biomass in plot two and plot three. This is indicated by the diameter which grew larger in plot two and plot three. Thus, forest plantation of *A. cadamba* plays important roles in absorbing carbon from the air and in producing biomass as the replacement of natural forests. *A. cadamba* is a tree that absorbs many nutrients, so it needs integration with cattle farms as a source of organic fertilizer. In 2.5 hectares *A. cadamba* trees required 1 cow to produce organic fertilizer. Organic fertilizer alone is not enough to accelerate the growth of *A. cadamba* trees, so it is necessary also an organic fertilizer as a decomposition stimulator. Economically, the total value of organic and an organic fertilizer is 30% from *A. cadamba* stem biomass margin growth value that can be utilized for timber industry. Fertilization is given to *A. cadamba* trees in a balanced manner. The comparison of organic economic value versus anorganic 7:3.

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Review: Progress and potential of DNA barcoding for species identification of fish species

AYESHA IMTIAZ[1,2], SITI AZIZAH MOHD NOR[1,3], DARLINA MD. NAIM[1,♥]

[1]School of Biological Sciences, Universiti Sains Malaysia (USM), 11800, Penang, Malaysia. Tel. +60-46534056, ♥email: darlinamdn@usm.my
[2]Government Degree College for Women 98NB, Punjab Education Department, Higher Education Wing, Sargodha, Pakistan
[3]Centre for Marine and Coastal Studies, Universiti Sains Malaysia, Penang, Malaysia

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Abstract. *Imtiaz A, Mohd Nor SA, Md. Naim D. 2017. Review: Progress and potential of DNA barcoding for species identification of fish species. Biodiversitas 18: 1394-1405.* DNA barcoding is a molecular technique to identify species by utilizing 600-800 base pairs genetic primer segments of mitochondrial gene cytochrome oxidase I. DNA barcoding has high potential to identify species into taxa, resolves ambiguousness in species identification, helps in accurate species identification, categorize species for conservation and also communize the information in the form of database system. The main challenge to this technique is regarding the use of barcoding information on 'biological species concept'. The extreme diversity of fish and their economic importance has made this group a major target of DNA barcoding. DNA barcoding can assign the status of known to unknown sample but it also has the ability to detect previously un-sampled species as distinct. In this review, we present an overview of DNA barcoding and introduce current advances and limitation of this promising technique.

Keywords: Cytochrome c oxidase I, barcode gap, DNA barcoding, fish diversity, NUMTS

INTRODUCTION

In modern society, the major environmental crisis is the loss of biodiversity and the decline is variable among different taxa. The issue has been highlighted at various levels. However, attempts to conserve biodiversity remained uncertain because the exact global baseline data is not available and the rate of loss is unknown, particularly in terms of background knowledge (Dirzo and Raven 2003). Although taxonomic history began since 250 years ago, there are still unknown numbers of species remain undiscovered and new species are waiting to be uncovered. Until now, there are only a few estimates about biodiversity have been published. Mora et al. (2011) reported in a study about globally unidentified biodiversity that 86% terrestrial species need their taxonomic identity whereas in marine biodiversity this number is extremely high (91% species). The standardized techniques and methods need to be developed for data acquisition of biodiversity in order to cope up the current biodiversity crisis. The importance of biodiversity in other fields of biology, i.e. ecology, evolutionary biology, molecular biology and biotechnology have been increased due to its crucial role in identifying specimens accurately (Simberloff et al. 2013). Being a most promising approach, 'DNA barcoding' is dominant among all identification techniques. Moreover, the advancement in biotechnology and taxonomic crisis helped in the creation of DNA barcoding technique.

DNA barcoding is helping taxonomists in terms of identification, discovery and genetically studying of specimens in order to achieve certain goals, i.e. knowledge about species diversity and level of variation among species. The challenge of DNA barcoding technique is not only trying to correctly identify species but also to make a standardized global reference library based on the identification of target specimen (Kerr et al. 2007). Several researchers recommended utilizing DNA barcoding techniques because it is cost effective, fast and authentic for species conservation (Li and Dao 2011). Using barcode data, stakeholders can collect conservation information, researchers can identify species without using the traditional method, taxonomists can resolve ambiguities in classification and policy makers can estimate the levels of conservation thus will depict proper management strategies for a species concerned (Francis et al. 2010).

The purpose of this review is to provide a critical overview of DNA barcoding and at what extent that this technique can realistically contribute to a practical identification of global biodiversity and biodiversity conservation efforts. We first address the scope of DNA barcoding as a standardized identification system, argue about the efficiency of DNA barcoding that depends on the use of integrative taxonomy, and assess the potential role of barcoding especially in the area of fisheries and most significantly in the conservation of known biodiversity.

OVERVIEW OF DNA BARCODING PROJECT

Short DNA sequences had been used for microbial species discrimination in early 80's (Kwok et al. 1984) and later had been tested on a variety of organisms (Chaves et

al. 2012). In 2003 it was the first time when Hebert et al. (2003) proposed that mitochondrial DNA cytochrome oxidase subunit I (COI) gene has the capability to serve as a barcode of all animals. DNA barcoding serves as a golden bullet for not only species identification, but also it delimits species boundaries. With this revolution in taxonomy, the Barcode of Life (BOL) project was launched to develop a globally accepted barcode system that is based on standard sequence of mitochondrial cytochrome oxidase I (COI) gene to identify eukaryotes and also enlist global biodiversity at a platform. Later in 2004, the Consortium for the Barcode of Life (CBOL) inaugurated this project. The aim of CBOL was to develop a standard protocol of DNA extraction, PCR and sequencing techniques, which aid the formation of a global DNA library. CBOL entered a new phase with the launch of iBOL (International Barcode of Life, http: //www.ibol.org) consisting of the collaboration of 26 countries that aimed to enlist eukaryotic biodiversity. In the initial few years, the iBOL concentrated mainly on a collection of maximum barcode sequences in a barcode library from all over the world to achieve the target of five million species barcodes. The iBOL also developed protocols, bioinformatics software with a huge data library. The purpose of these projects was to focus each animal group for making DNA barcode libraries. Some important project includes Fish-BOL (Fish barcode of life), Health BOL, Lepidoptera BOL, MarBOL (Marine Barcode of life), MBI (Mosquito Barcode of life), Mammalia Barcode of life campaign, Coral Reef Barcode of life, BeeBOL (Bee barcode of life) and CBOL fungal working group. Many countries have already collaborated to enlist their regional biodiversity and launched their own country's sub-projects i.e., Norway (NorBOL), Mexico (MexBOL), Japan (JBOLI) and Europe (ECBOL). Many regional based small projects on DNA barcoding are also under completion to identify unique biodiversity in small areas such as The Área de Conservación Guanacaste, a World Heritage Site in Costa Rica, which also combines barcoding project on insects (Hajibabaei et al. 2006; Janzen et al. 2009). Another example is the Moorea Bio Code Project, a comprehensive inventory of Moorea Island in French Polynesia that incorporates DNA barcoding (Check 2006). The DNA barcode from all projects was submitted to a database made by Canadian Center for DNA Barcoding (CCDB). BOLD is freely available to any researcher through the web page (http://www.boldsystems.og/index.php/Login/page?destination=MAS_Management_User Console).

DNA barcoding is the beginning of a revolution in species identification, in which can be accessed from the DNA libraries data record. BOLD provides information for basic identification by matching with the closely related individual, quality of sequences, as well as the quality of a core analysis by generating graphs of inter and intraspecific genetic distances, barcode gap, haplotype distributions and tree reconstructions (Ratnasingham and Hebert 2007).

AUTHENTICITY OF DNA BARCODING

DNA barcoding uses small regions of mitochondrial DNA that work as a barcode to amplify a gene. DNA sequencing and matching of unidentified sequence with the closely related individual in BOLD or NCBI libraries can be conducted within hours, so the response time depends greatly on available infrastructures, such as reference sequence or voucher specimen in NCBI and BOLD libraries. DNA barcoding is now well established; leads typically to accurate results and the DNA sequencing costs are low and constantly dropping. A major benefit of DNA-based analytical procedures is that they can be applied throughout the food supply chain, from whole specimens to trace samples (scales and fins), to highly processed and cooked fish products (Cawthorn et al. 2012). In addition, DNA analysis is use readily on not only fresh fish samples but also preserved historical material (bones and/or scales from museums).

The validity of the COI gene: For all or some species?

Mitochondrial Cytochrome c Oxidase subunit I (COI) is a mitochondrial DNA gene that codes a protein, which helps in cellular respiration. A 650 base pairs region of mitochondrial COI is considered as a universal barcode for animals because of its comparatively fast mutation rate over a relatively short time scale, has a high number of exons, high availability throughout cells and maternal inheritance (Steinke et al. 2009b). Due to these characteristic, the mitochondrial COI gene is a perfect universal barcode for species- level identification in sponges (Vargas et al. 2012), Platyhelminthes (Van Steenkiste et al. 2015), annelids (Achurra et al. 2013), molluscs (Layton et al. 2014), insects (Park et al. 2011), oysters (Zainal Abidin et al. 2016), echinoderms (Hemery et al. 2012), fish (Basheer et al. 2016), amphibians (Xia et al. 2012), reptiles (Hawlitschek et al. 2013), birds (Saitoh et al. 2015) and mammals (Alfonsi et al. 2013) (Table 1).

Although COI gene is considered as a universal barcode in animals, its potency is challenging in some protists, fungi, and plants (Schoch et al. 2012). In fungi, internal transcribed spacer (ITS) gene is more successful than COI gene to discriminate closely related taxa (Dentinger et al. 2010). Only a few published data are available on the successful of COI gene as a barcode in algae (Clarkston and Saunders 2010; Macaya and Zuccarello 2010). It has been reported that a universal plastid amplicon (UPA) works well as a barcode in algae instead of COI (Sherwood 2007). Furthermore, Saunder and Kucera (2010) reported that major advantage of UPA is its universality because this primer pair can reliably recover sequences from many groups of algae including green, red and brown marine macroalgae, diatoms, and also cyanobacteria (Sherwood and Presting 2007).

By the same token, the COI gene cannot be used as barcodes for some species of protists, which does not have mitochondrial DNA like anaerobic protists (Gómez 2014). Due to the ineffectiveness of the COI gene as a universal primer for protists, ribosomal subunits has been proposed to carry out that function well (Pawlowski et al. 2012). Moreover, low substitution rate, the high tendency of hybridization and polyploidy among closely related species of plants are the few factors that result in the failure of using mitochondrial COI gene as a universal primer in

plants (Kiewnick et al. 2014). Therefore, the combination of two alternative sequences in chloroplast DNA namely ribulose bisphosphate carboxylase and maturase K has been proposed as a standard barcode for the plant (Group et al. 2009).

DNA barcoding as an efficient tool for species identification

DNA barcoding is an advanced and efficient method for species identification. Various set of primers of mitochondrial gene for barcoding are available in published data. Researchers can also design primers by using different software, for example, PRiFi (Fredslund et al. 2005), BARCRAWL BARTAB (Frank 2009) and Primer-Blast (Ye et al. 2012). A good primer of a specific gene is basic key of success in PCR protocols. The translated sequences of unidentified samples after sequencing can be easily aligned by using various downloadable software or even online, such as ExPASy which is Sib information resource portal (Artimo et al. 2012) (www.expasy.org/genomics/sequence_alignment), MEGA version 6.06 (Tamura et al. 2013) (www.megasoftware.net) and MAFFT version 7 (Katoh and Sandley 2013) (http://mafft.cbrc.jp/alignment/software).

The identification of a voucher specimen that is barcoded can also be confirmed by traditional taxonomy. However, traditional taxonomy fails to discriminate some species due to several conditions, for example, species that having various external body colorations especially when specimens are not fresh (Sriwattanarothai et al. 2010), museum preserved species (Hebert et al. 2013) and samples showing phenotypic plasticity (Weigand et al. 2011). Unlike the traditional taxonomy, DNA barcoding gives information on cryptic (Lim et al. 2016; Thiriet et al. 2016) and sibling species (Blanco-Bercial et al. 2014; Shao'e-Sun et al. 2016). This identification technique fully supports the improvement of animal classification as well as helps to sort out any ambiguity at the species level. However, DNA barcoding with the support of traditional taxonomy has the capability to identify species complexes within populations (Martínez-Aquino et al. 2009; Iwatsuki et al. 2015).

Table 1. Universality of mitochondrial COI gene as barcode in various groups of animals

Taxa name	No of taxa	% identified	Sampling location	Mitochondrial COI Primers	References
Fish	203 species	> 98%	Canada	FishF1, FishR1	Hebert et al. (2003)
Birds	643 species	> 98%	North America	BirdF1, BirdR1, FalcoFA, Vertebrate R1, BirdR2	Hebert et al. (2004)
Neo tropical bats	87 species	> 97%	Guyana	CVF1di, CVR1di, CVF1LFT1, CVR1LRt1, LepF1t1, LepR1t1	Clare et al. (2007)
Fish	94 species	>98%	Canada	16S, COI1, COI2, COI 3, M13	Ivanova et al. (2007)
Chondrichthyes	200 species	>98%	North Atlantic and Australasia	FishF1, FishR1, HCO2198	Ward et al. (2008)
Ornamental fish	201 species	>98%	Canada	FishF1, FishR1	Steinke et al. (2009a)
Birds	756 species	>98%	Iran	BirdF1, BirdR1, BirdR2, 16SAL, 16SBH	Aliabadian et al. (2009)
Fish	115 species	>98%	India	FishF1, FishR1	Lakra et al. (2011)
Marine fish	158 species	>98%	Japan	FishF1, FishR1	Zhang and Hanner (2011)
Freshwater fish	105 species	100%	Brazil	CFishF1t1, CFishR1t1, VF1LFt1, VR1LRt1	de Carvalho et al. (2011)
Oriental Black (Diptera)	41 species	>96%	Thailand	LCO1490, HCO2198	Pramual and Adler (2014)
Amphibians	82 species	100%	China	dgLCO, LCO1490, Chmf4, COI-C02, COI-C01, LEPF1, dgHCO, HCO2198, Chmr4, COI-C04, COI-C03, LEP-R1	Che et al. (2012)
Reptiles	>250 species	100%	Madagascar	RepCOIF, RepCOIR	Nagy et al. (2012)
Reptiles	27 species	100%	Comoros Archipelago	LCO, HCO	Hawlitschek et al. (2013)
Amphibians	251 species	>94%	Madagascar	dgLCO1490, dgHCO2198	Perl et al. (2014)
Hymenoptera	4118 species	>90%	Europe	As used by Ivanova et al. 2006 and deWaard et al. 2008	Schmidt et al. (2015)
Cyprinid	35 species	100%	China	FishF1, FishR1, FishF2, FishR2	Shen et al. (2016)

STANDARD LABORATORY PROCEDURE (SLP) OF DNA BARCODING

Barcoding is a worldwide project where hundreds of laboratories have been involved in enlisting the global biodiversity. Accordingly, new methodologies of DNA extraction and Polymerase Chain Reaction (PCR) (Handy et al. 2016) has been increasingly developed in parallel with the development of barcoding primers. To standardize the handling of DNA barcoding samples, BOLD has already made standard laboratory procedure (SLP) (Keele et al. 2013). In SLP (Fig 1) preferably fresh few millimeter-sized tissue is placed in 1.5mL Eppendorf tube. Fish Barcoding Method (Handy et al. 2016) which is also available online at BOLD website (http: //www.dnabarcoding101.org/files/using-dna-barcodes.pdf) does DNA extraction. Species-specific primer or universal DNA barcoding primers (based on animals group i.e. invertebrate, plant, mammal, etc.) was used for PCR amplification. Positive control band (a sample that is known to give a positive band for the chosen primers) is included in PCR as a positive control. A negative control (a mixture containing no DNA template) is also loaded in PCR mixture to ensure that the master mix does not contain contaminating DNA. The PCR amplification is analyzed by loading PCR-amplified products on an agarose gel electrophoresis with a standard marker (DNA of known base pair size), such as 1-kilo base pairs (kbp) DNA ladder (i.e. First Base Laboratories, Sdn Bhd). If a bright band is present at the expected size, the PCR product will be purified. There were several purification kits are available in a market such as Mega quick spin of Intron Biotechnology Inc. After confirming a quality and quantity of the purified DNA, the PCR-amplified products were sent to a laboratory for sequencing.

Sequencing results can be analyzed by using National Centre of Biotechnology Information (NCBI) website (https: //www.ncbi.nlm.nih.gov) or BOLD website to determine the maximum identity of an organism with template sequences. BOLD compares unknown sequences with most closely resembling sequences available in the database. This database gives output in the form of resemblance percentage. The standard laboratory procedure is universally approved procedure but of course, for different organism modifications are required in order to obtain the best quality results. The DNA barcoding procedures totally fail if there is no barcode library to register barcoded samples (Ekrem et al. 2007). To develop barcode record, researchers required a species name, voucher specimen data (i.e. locality, date, repository of the specimen, photographs), a sequence data, PCR primers and trace files (sequencer's original outputs). It is estimated that sequencing can take place in less than 90 min at the cost of $2-5 per species, compared to several months of field work and a cost of at least $100 per specimen with morphological analyses (Stoeckle and Hebert 2008).

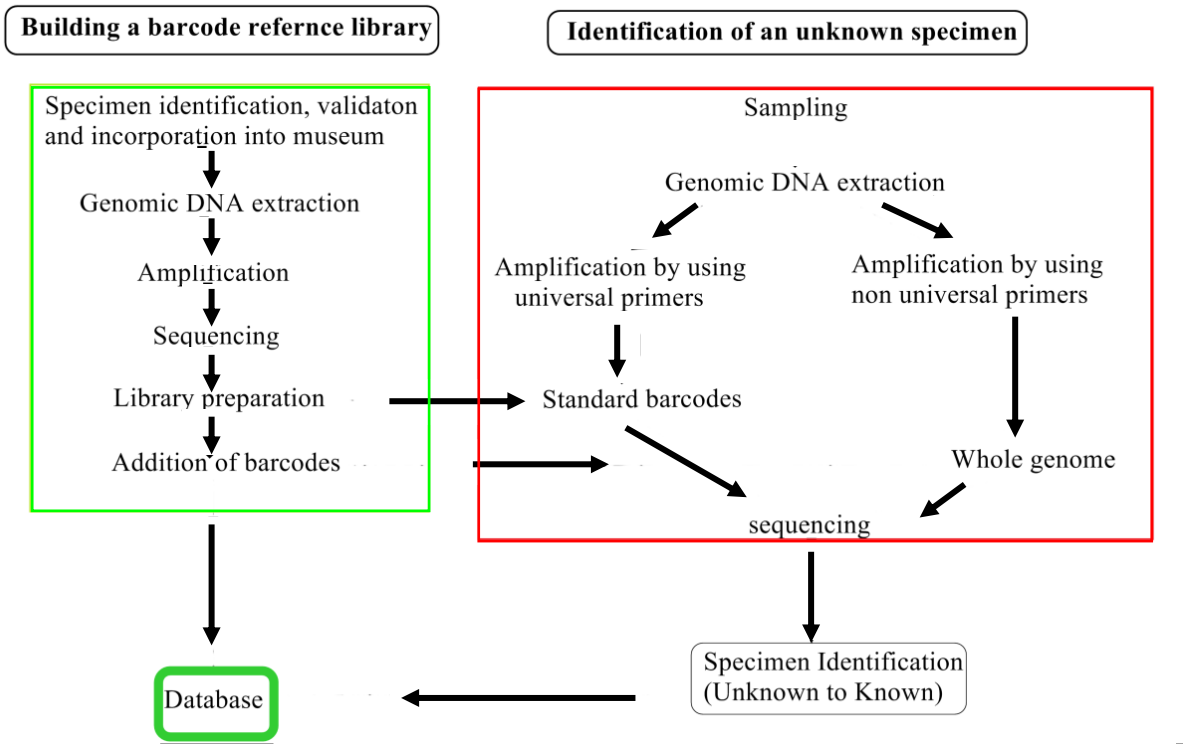


Figure 1. Overview of experimental procedures implemented for barcode libraries

USE OF LATEST TECHNIQUES IN DNA BARCODING

Real Time PCR

The real time PCR is the latest approach in DNA barcoding methods that is used to monitor the progress of PCR reaction in real time (Dias et al. 2016). At the same time, relatively small amount of PCR products cDNA or RNA can be quantified. One can start the PCR even with very small amount of nucleic acid and can quantify the end product quite accurately. Real time PCR technique is basically designed to maximize the amplification of barcode genes even in the presence of low-quality DNA (Doi et al. 2015). For this purpose a reporter molecule is used in PCR that binds to double stranded DNA that increases in number as PCR proceeds and produces fluorescence. The reporter molecules can be in the form of dye (i.e. SYBR Green) or sequence specific probes (Molecular Bacons or TaqMan Probes). The real time PCR is highly sensitive, easy to perform and cost effective, as no post PCR processing is needed that saves time and resources. Real time PCR is also called real time RT PCR that is used to convert DNA into RNA by an additional cycle of reverse transcription. Shokralla et al. (2015) used real time PCR based DNA barcode method as minibarcode system consisting127-314 base pair fragments of COI gene to identify all types of fish species used in fish products AND successfully identified 93.2% of samples upto species level. Feng et al. (2017) also recently identified four commercially important species of salmon and trout using real time PCR barcoding technique.

Microarrays

With the rise of next generation sequencing technologies and non-suitability of COI gene for design of oligonucleotide probes, it was believed that microarray technology is outdated. But developments in microarray technology (Microarray chips, mid-infrared chemical imaging) have refined the barcoding approach (Kochzius et al. 2010). Microarray chips are simple and can efficiently target specific group of species in a single assay. Mid-infrared chemical imaging (MICI) uses nanogold-silver augmented hybridized spots in microarrays and detects reflected substrate. These advanced methods of DNA barcoding have been applied in unknown species identification, point mutations, biodiversity assessment and food security (Trivedi et al. 2016). Handy et al. (2014) identified reported misidentification of seven catfish species in USA seafood market by using DNA microarrays with newly developed mid-infrared imaging detection method.

APPLICATIONS OF DNA BARCODING

With pits and falls of thirteen-year history, DNA barcoding project successfully not only identified a huge number of taxon but also addressed population and conservation issues in biodiversity. The unique features of DNA barcoding such as species identification, cryptic species and partial phylogenetic assessment is benefits to whole animal kingdom especially both basic and applied fisheries. Species identification methods using molecular data can help elucidate the relationships of morphologically variable individuals of the same species and individual's similarity in different developmental stages. In addition, in the features of typical non-barcode markers, the advantages of DNA barcoding include primer universality, the availability of information on a wide range of taxonomic groups, and its association with taxonomy.

DNA barcoding and species identification

Genetic identification of biodiversity is the necessity of time due to the presence of phenotypic similarities among neighboring species. Some organisms especially fish shows phenotypic plasticity with a change in its environment (Hutchings et al. 2007). DNA barcoding has the capability to identify not only in adult organisms but also at their early developmental stages. For example, Ko et al. (2013) used DNA barcoding technique to successfully identified 100 specimens of fish larvae with a success rate of > 65 percent at the species level. However, with an increase in taxonomic level the identity rate also increased up to > 85 Percent. Likewise, Naim et al. (2012) used COI gene to successfully identified approximately 60 individuals of mud crab into four species. Most recently, COI gene has been used to identify Ivory shell (Chiu et al. 2015), Atlantic goliath groupers (Damasceno et al. 2016) and yellowfin tuna (Higashi et al. 2016).

DNA barcoding and species delimiting boundaries

A great challenge associated with barcodes is to define species boundaries concepts. The literature on DNA barcoding studies suggests a different threshold approach in different taxa, as 2% difference in the DNA barcode of mammals implies that the samples are from different species (Cai et al. 2015). This value was considered as a divergence threshold and a standard cutoff value for species delimitation in fishes (Mabragaña et al. 2011). However, Ward et al. (2008) mentioned that 3% and 1% is the ideal threshold value for fish and birds respectively. These limitation values are based on the distribution of intra and inter-specific K2P genetic distance values in the approximately 172,000 species that have been barcoded (http: //www.boldsystems.org). It is important to set species delineation boundaries because, in homogenous populations, species identification is problematic. DNA barcoding can interpret a unique taxon as a separate species by recording intraspecific divergence which should be lower than interspecific threshold values.

DNA barcoding as a forensic tool

DNA samples can be used to check drug authenticity in forensic sciences (Carvalho et al. 2015). Forensically Informative Nucleotide Sequencing (FINS) was one of the earliest species diagnostic techniques for fish (Bartlett and Davidson 1992). In FINS the identity of an unidentified specimen is confirmed by comparing amplified sequence of

a mitochondrial COI gene fragment taken from the unknown sample to reference sequences in database library of known species. Such interrogative sequencing methodology used different types of genetic markers to identify a variety of meats (Pappalardo and Ferrito 2015). Barcoding provides a fast and reliable method to identify exotic species in a branch of biosecurity: one such example is the identification of the invasive North American bullfrog (*Rana catesbeiana*) found in water samples from ponds in France by using short mitochondrial fragments (Ficetola et al. 2008). Likewise, the Brazilian Forensic Government Programme reported that there is approximately 24 percent of seafood products has been mislabeled (Carvalho et al. 2015). Under those circumstances, biomedical research highlighted new aspects of DNA barcoding technique such as next-generation DNA sequencing, shotgun sequencing, transcriptomes and interactomes (Shendure and Ji 2008). All these techniques help in saving time and speeding up the process of collecting data that previously required a very extended period. DNA barcoding is not too difficult to handle as nonspecialized persons can participate in barcoding campaign by helping in samples collection from various geographical locations and uploading the pictures and other information on database system such as BOLD (http: //www.barcodinglife.org) for further taxonomic interpretation. This information will be used for forensic or biosecurity purposes as described above, henceforth, will encourage local participation in these matters. Such local involvement in barcoding project can lessen the field work of morphological taxonomists.

DNA barcoding and cryptic species

Fishes show significantly more divergence for COI gene at each hierarchical taxonomic level (Ward 2009). Barcodes have also complimented morphological studies in cases where taxa were easily misidentified due to the existence of cryptic species (Ward et al. 2008). If a sample from an unidentified specimen shows zero divergence from a previously identified specimen, or differs from it by only one or two base pairs, the probability is that it is the same species due to high similarity with the already identified specimen (>95%). If on the other hand, the unknown specimen is more than 2 percent divergent from the known specimen, it is very likely (probability greater than 95%) to be a different species. Khedkar et al. (2014) reported the possibility of sibling species of *Labeo dyocheilus* by analyzing inter and intraspecific divergence values. Hubert et al. (2012) also revealed cryptic fish diversity in coral reefs of Indo-Malay Philippines Archipelago. Hamaguchi et al. (2017) reported about cryptic diversity in flat oysters distributed in Japan.

DNA barcoding and population diversity

Mitochondrial DNA markers are not diploid because they are inherited from a single parent (maternal inheritance) so they are equally good for targeting population level studies (Avise 2004). Although the cytochrome oxidase I (COI) gene information from DNA barcoding is not sufficient to investigate population-level questions (Bazin et al. 2006), yet still it can be used to partially interpret the distribution of genomic diversity within taxa. Barcodes can give the status of species to an unknown specimen because they also consist information of genetic models (coalescent-model) based on population genetics (Abdo and Golding 2007). The COI gene provides information about genetic variation within a population of a single species and this information can help to deduce the phenomenon of migration as well as genetic drift in fish populations (Mohammad Geba et al. 2016). To demonstrate, Boonkusol et al. (2016) used mitochondrial COI gene sequences to access the genetic variability in snakehead fish of Thailand and deduced that genetic variation in the central river basin is due to fish dispersal by the flood. In the same fashion, the use of a combination of mitochondrial (COI) and nuclear genes (recombination activating gene-I (Rag I) and from nuclear alpha tropomyosin 'intron V') can fully address the question of population structure as done by Eytan and Helburg (2010) in Caribbean reef fish.

DNA barcoding and phylogenetic reconstruction

In barcoding, the information from an assemblage of species is in the form of genetic sequences, which is uploaded in a barcode library. However, the gene lengths from barcoding data are not sufficient to construct a deeper phylogenetic tree in resolving evolutionary relationship of organisms. Although the barcoding sequences have been used to construct Neighbour-Joining (NJ) tree, this barcode-based tree cannot be alternates of the phylogenetic tree. We strongly emphasize on the statement that DNA barcoding data can provide partial information about the phylogeny of species and can draw an outline for phylogeny that should be deeply analyzed by nuclear genes data. Faith and Williams (2005) argued that the most significant contribution of DNA barcoding to conservation efforts is its role in improving and speeding up phylogenetic diversity assessments. Lakra et al. (2011) successfully identified 115 marine fish species that clustered into 79 genera when NJ tree was constructed to see the phylogenetic relationship among collected samples. Ardura et al. (2010) identified 21 Amazonian species, with 100% bootstrap support was obtained at a specific level in the reconstructed NJ phylogenetic tree.

DNA barcoding and food safety

The global fish market is facing challenges of seafood authenticity and food safety, although identification keys and molecular approaches (DNA barcoding) have limitations in the identification of species. The market frauds regarding substitution of high priced fish with low-priced fish in fish products is very common. Wong and Hanner (2008) used barcoding technique in North American seafood to check barcoding aspects of traceability and their specimen data matched of >97 percent similarity with barcode library sequences. Twenty-five percent of the samples were potentially mislabeled, demonstrating that DNA barcodes are a powerful tool for identification of seafood to the species level. Likewise, Smith et al. (2008) detected ten families from smoked fish

products were mislabeled. Furthermore, Yan et al. (2016) identified *Alepes apercna*, which had surprisingly been labeled as *Rastrelliger brachysoma* by using COI gene in imported seafood products. Mislabeling in the fish market for economic benefits has become challenging. For this reason, the Food and Drug Administration Department in the United States has adopted DNA barcoding technique to reduce market frauds (Naaum and Hanner 2015).

DNA barcoding and biodiversity conservation

One of the main goals of DNA barcoding is a utilization of genetic data to boost conservation strategies, and indeed molecular tools were used in conservation biology at different levels of analysis (DeSalle and Amato 2004). Rapid biodiversity assessment is a research priority as species extinction/extirpation rate is high, and barcoding is contributing to biodiversity conservation in facilitating biodiversity assessment cheaply and quickly where financial resources are limited (Krishnamurthy and Francis 2012). The majority of described biodiversity is in developing countries, where resources for comprehensive biodiversity assessments are lacking. For conservation biology, genetic tools can aid in research as diverse as fine-scale management of coral reefs (Neigel et al. 2007) and regional management of fisheries (Swartz et al. 2008). DNA barcoding contributes to conservation policy in two important ways; 1) by speeding up local biodiversity assessments to prioritize conservation areas or evaluate the success of conservation actions, and 2) by providing information about evolutionary histories and phylogenetic diversity (Rubinoff 2006). Hubert et al. (2012) barcoded 2,276 specimens into 668 coral reef fish species and provided data to biodiversity conservationists for making conservation strategies as his findings remarkably suggested most of the coral reef diversity is accumulated in Indo-Malayan Archipelago. There have been several notable conservation successes using barcoding. For example, the rapid classification of 99% of 210 chondrichthyan species from 36 families from Australia (Ward et al. 2008) and the identification of larval and juvenile organisms for which morphological data is non-existent (Neigel et al. 2007), all of which have accelerated conservation priorities and policy response.

Barcoding prosecutes illegal trading of endangered species, even after organisms have been processed. For example, the illegal shark-fin trade is a significant threat to biodiversity in many coastal African countries. The barcoding can identify different varieties of shark, including those that were critically endangered and protected under international legislation (Swartz et al. 2008). Therefore, DNA barcoding in conjunction with a reference database potentially plays a key role in environmental law enforcement and conservation of species that are already studied (Williams et al. 2013).

LIMITATIONS IN DNA BARCODING

DNA barcoding has attracted significant attention in systematic studies. Yet, the project is controversial because many systematists feel that the conceptual foundation of DNA barcoding remains weak (Waterton et al. 2013). Here are few limitations of DNA barcoding that have been addressed in several types of research.

Barcode gap

The “barcode gap" refers to the differences between mean intra and interspecific genetic sequence variability of COI sequences within the same genus of animals (Meyer and Paulay 2005) (Fig 2). Large barcoding gaps are routinely used to predict DNA barcoding success for the taxon under study (Clare et al. 2007; Freshwater et al. 2010; Keskln and Atar 2013). The overlapping of genetic divergence values within and between species recorded in histograms shows that there is no gap between inter and intraspecific divergences in samples species (Mayer and Paulay 2005). In this regard, the inter-generic variation and overlapping of differences within and between species distances are ruled (Virgilio et al. 2010). We support the statement of Collins and Cruickshan (2013) that barcoding gaps are currently incorrectly computed and that the values reported in the barcoding literature are misleading. Various authors have pointed out that the use of smallest interspecific distances would be more appropriate (Cognato 2006; Roe and Sperling 2007).

Proper interspecific distances measurements are important for distance-based identification techniques. DNA barcodes with unusually large distances to conspecific sequences are often used to predict the possible existence of cryptic species (Clare et al. 2007). One cannot address the new species by considering barcoding gap only but the cryptic species can be predicted by using smallest intraspecific value (Will and Rubinoff 2004).

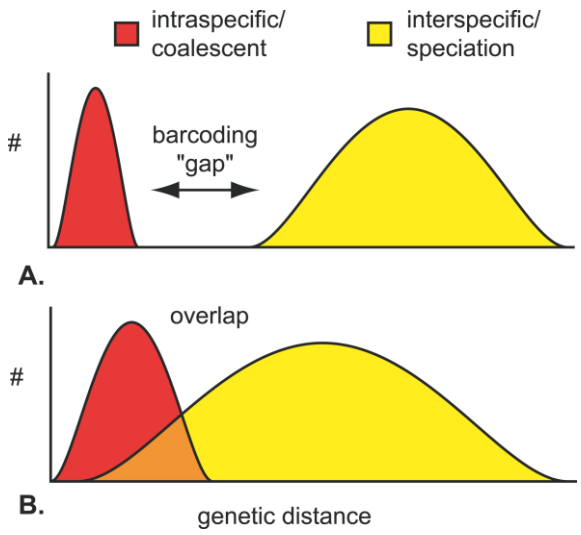


Figure 2. Barcode gap (Mayer and Paulay 2005). If there is no overlap between intraspecific and interspecific values as in A, it means the unknown sample has low threshed hold value all species are in their discrete distribution boundaries but if overlap is great as in B it means there are closely related taxa present and making this method problematic

Barcoding and phylogeny misinterpretation

Neighbor-Joining tree formation is easy to compute and is a part of standardized analytical procedure as used in various barcoding studies because NJ trees are fully resolved tree that is based on genetic distance matrix of similarity in sequences to create a clustering of taxon in the form of phenogram (Casiraghi et al. 2010). However, several authors have documented that phylogenetic approach to DNA barcoding is inappropriate (Goldstein and De Salle 2011). The NJ tree does not use cladistics sense as an identification system. NJ tree based identification can interpret wrong results about the identification of paraphyletic speciation and incomplete hierarchy (Lowenstein et al. 2010). Few species concepts require reciprocal monophyletic origin (Meier 2008) (Fig 3), and in any case, monophyly of mtDNA lineages can be an unrealistic scenario in many closely related groups (Zhang et al. 2012). It is important to note that a quantification of monophyly still remains a useful description of the data, and should be used but in conjunction with other methods. Problems mostly occur when NJ trees are the only analytical method presented (Little and Stevenson 2007). However, for the purpose of graphically summarizing the data, NJ trees can be considered appropriate.

For species identification, we suggest the mitochondrial plus nuclear gene combination with character based diagnostic methods should be used. Bayesian coalescent methods also promise statistical advantages, but may be too computationally inefficient in their current incarnations (Zhang et al. 2012).

NUMTS (Nuclear Mitochondrial Pseudo-genes)

Nuclear Mitochondrial Pseudo-genes (NUMTS) are non-active non-coding parts of mitochondrial DNA present in the nucleus of most eukaryotic organisms and can be easily amplified along with mitochondrial DNA even by using conserved universal primers (Song et al. 2008). The presence of co-amplification of NUMTS along with mitochondrial genes is problematic because DNA barcoding analysis can incorrectly overestimate the number of unique species. DNA barcoding is rapid and inexpensive technique of tagging species. However, the presence of NUMTS in COI amplified sequences can make DNA barcoding ambiguous (Buhay 2009). There are several techniques that can be used to control NUMTS such as (i) the use of muscle tissue as a source of DNA due to the presence of high concentration of mitochondrial DNA in those areas and (ii) amplification of longer fragments (750 bp) because barcode sequence is longer than NUMTS sequence (Pereira and Baker 2004). NUMTS is not only a major limitation of DNA barcoding, but also has significant taxonomic implications. Moulton et al. (2010) tried to identify Orthoptera species by using COI gene but the presence of NUMTS led to overestimation and misidentification of species. With attention to this matter, Song et al. (2008) suggested RT-PCR, long PCR, use of markers other than COI and choice of the mitochondrial rich region for extraction of DNA to avoid NUMTS. Similarly, BOLD also provides an opportunity to detect and delete NUMTS by analyzing sequences with Markov model during analysis.

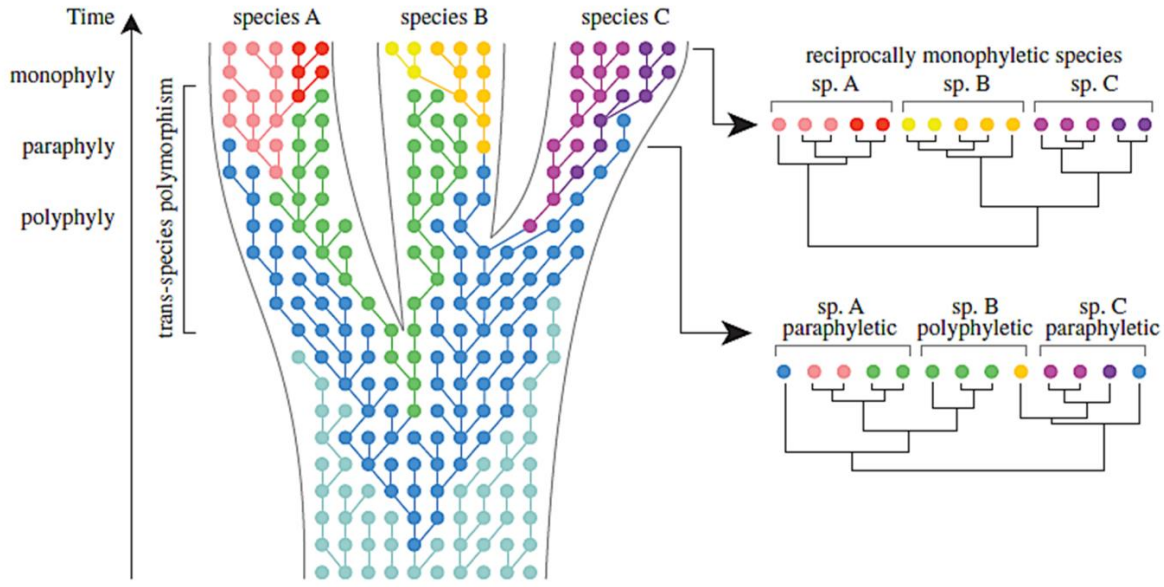


Figure 3. Differences among various types of phylogeny (Leliaert et al. 2014)

DISCUSSION

Although DNA barcoding is useful in classifying taxa, it is perhaps premature to suggest that DNA barcoding can offer a replacement for assessment of biodiversity and traditional taxonomy. Instead, we consider barcoding as an additional tool that links genetic data into existing studies. DNA barcodes have been particularly helpful in identifying cryptic diversity within species, especially when it is used in conjunction with other taxonomic methods such as morphology and ecology. DNA barcoding is worth capable of facilitating an integrative approach to species identification but with the involvement of traditional taxonomic studies. The role of DNA barcodes is positive to help in speeding up biodiversity assessments, however, integrative taxonomic methods are still required to address the 'taxonomic impediment' (Wilson 2000). There are three reasons for genetic barcoding should not be used alone for addressing the status of new species to a taxon as formerly described in this review; firstly, no standardized universal distance threshold has been identified for species delimitation, and thresholds vary between different taxa (Yassin et al. 2010). Secondly, discrimination of a closely related species is not an easy task by using a species definition based on only genetic distance data (Meier et al. 2006). Thirdly, for species identification, there is no universal identification technique, as species discovery requires a contribution from other branches of biology (Taylor and Harris 2012). DNA barcoding has the capability to discriminate species but it is quite unjustified to comment that DNA barcoding can replace traditional taxonomy. Additionally, barcoding research has limited success when identifying new species in unstudied groups (Meyer and Paulay 2005). For example, barcoding fails to identify recently isolated populations and reproductively isolated lineages of fish species that have undergone recent speciation (Zou et al. 2016). Even so, DNA barcoding has proved useful in enlisting global biodiversity by enhancing data availability for existing research problems (Rubinoff et al. 2006).

In summary, being taxonomist, we encourage DNA barcoding with a combination of traditional identification methods because it is an objective, quick, easy and replicable method to identify species. DNA barcoding has raised the profile of taxonomic research and contributed a valuable data for conservation of biodiversity. However, decisions for the conservation of a species concerned shall not be based only on DNA barcoding studies. The evidence-based approach to fundamental assessment about species, such as population size, species interaction with the environment and the possible effect of the ecosystem on species should be considered before jumped into conclusion. On the whole, DNA barcoding gives opportunity in various fields of biodiversity in a positive way and we think it will be regrettable to compromise on the efficiency of barcoding due to its presumed limitations.

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Short Communication: Lack of differentiation within the bigeye tuna population of Indonesia

NI PUTU DIAN PERTIWI[1], BUDI NUGRAHA[2], RIRIK KARTIKA SULISTYANINGSIH[2], IRWAN JATMIKO[2], ANDRIANUS SEMBIRING[1], ANGKA MAHARDINI[1], NI KADEK DITA CAHYANI[1], AJI WAHYU ANGGORO[1], HAWIS H. MADDUPPA[4], AMBARIYANTO AMBARIYANTO[5], PAUL H. BARBER[6], GUSTI NGURAH MAHARDIKA[1,7,♥]

[1]Indonesian Biodiversity Research Center. Jl Sesetan-Markisa 6, Denpasar 80226, Bali, Indonesia. Tel.: +62 361 8423062, Fax: +62 361 223 791, ♥email: gnmahardika@unud.ac.id
[2]Tuna Fisheries Research Center, The Ministry of Marine and Fisheries Affairs, Denpasar, Bali, Indonesia
[4]Department of Marine Science and Technology, Institut Pertanian Bogor. Jl. Agatis No 1, Darmaga, Bogor 16680, West Java, Indonesia
[5] Department of Marine, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Tembalang, Semarang City 50275, Central Java, Indonesia
[6]Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, CA 90095-7239, USA
[7] Laboratory of Animal Biomedical and Molecular Biology, Universitas Udayana. Jl Sesetan-Markisa 6, Denpasar, Bali, Indonesia

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Abstract. *Pertiwi NPD, Nugraha B, Kartika R, Sulistyaningsih RK, Jatmiko I, Sembiring A, Mahardini A, Cahyani NKD, Anggoro AW, Madduppa HH, Ambariyanto A, Barber PH, Mahardika GN. 2017. Short Communication: Lack of differentiation within the bigeye tuna population of Indonesia. Biodiversitas 18: 1406-1413.* All highly migratory tuna and tuna-like species have vast feeding grounds and spawning grounds. Indonesia's tuna catch is the largest in the world. However, genetic diversity in the population structure within particular tuna species in Indonesia is very limited. Here we provide genetic data for bigeye tuna *(Thunnus obesus)* covering fishing grounds and local fish markets throughout Indonesia. A fragment of mitochondrial DNA in the D-loop control region was amplified from samples collected across Indonesia in the biennium 2012-2013. The results showed high haplotype diversity and low nucleotide diversity in our samples. Little differentiation occured between the eleven diverse sampling locations, nor was any separation detected between general regions of Indonesia, nor between samples from fishing grounds and samples from fish markets.

Keywords: Bigeye tuna, mtDNA, Indonesia

INTRODUCTION

Having extensive archipelagic waters and an exclusive economic zone of 200 nautical miles, Indonesia should play a central role in maintaining the world's pelagic and coastal biomass. This country's seas are the migratory route, nursery and spawning ground for various species, including tuna. Indeed, tuna and their relatives are found in abundance here (Juan-Jorda et al. 2013), providing a significant source of income for Indonesia (FAO Fisheries 2013). Benefiting from this abundance, Indonesia is the biggest tuna-producing country in the world, contributing 15% to global tuna production in 2009 (Miyake et al. 2010; Sunoko and Huang 2014).

Genetic data should be incorporated in management models in order to develop more realistic conservation strategies. Genetic data for tuna fisheries is of particular interest. Many of the world's tuna populations have dwindled by up to 60% from 1950's levels, as the industry has increased. The long life-cycle of tuna has exacerbated this trend (Collette et al. 2011; Juan-Jorda et al. 2011). If current exploitation rates continue, it is estimated that the bigeye tuna population of the Central and Western Pacific will soon reach a stock level that will be incapable of producing maximum sustainable yields (Sibert et al. 2012).

To our knowledge, there is very limited population genetic data for tuna caught in Indonesia. While Indonesian-wide scientific coverage of the population genetics of other species, such as frigate tuna, kawakawa, skipjack tuna, Indian mackerel, and narrow-barred Spanish mackerel has been well documented (Jackson et al. 2014), genetic information is scarce for the principal market species of tuna – bluefin, yellowfin and bigeye. Therefore, in the research reported here, we examined genetic variability of bigeye tuna in the western, central, and eastern Indonesia waters using mitochondrial control region sequence data. This particular marker has been shown to be particularly sensitive in detecting the population genetic structure of marine migrating and predatory fish (Buonnacorsi et al. 2001).

MATERIALS AND METHODS

Sample collection

Fin clips of bigeye tuna samples were collected across Indonesia in 2012-2013, including in (1) Aceh, (2) Indian Ocean high sea, off-shore west of Sumatra (Bungus Island), (3) Pelabuhan Ratu (West Java), (4) Muara Baru, (5) Indian Ocean high sea, off-shore south of Java Island, (6) Lombok, (7) Bone (South Sulawesi), (8) Bitung (North

Sulawesi), (9) Ternate (North Maluku), (10) Ambon, and (11) Manokwari (West Papua). All locations are shown in Figure 1. The number of samples from each of the 11 locations, the general region of Indonesia represented, and the type of sample (originating either from a fishing ground or a fish market) are listed in Table 1.

Each fin clip was collected and preserved in ethanol 95%. Genomic DNA was extracted using a 10% Chelex solution (Walsh et al. 1991). The control region (CR) mtDNA fragment was amplified using polymerase chain reaction (PCR) with the published CRK-CRE primers (Lee et al. 1995). Following visualisation of PCR products on 1% agarose gels stained with ethidium bromide, the PCR products were sequenced using both PCR primers using the Big Dye Chain Termination protocol at the University of California Berkeley Sequencing Facility.

Population genetic analyses (haplotypic diversity (H), nucleotide diversity (π), index $\Phi_{ST)}$ were executed using Arlequin ver.3.5 (Excoffier and Lischer 2010) and DnaSP 5.1 (Librado and Rozas 2009). Samples from Aceh were not included in the population comparison due to a limited number of samples. Delineative statistics such as haplotypic diversity (H) (Nei et al. 1985) and nucleotide diversity (π) (Lynch and Crease 1990) were determined for each sampling location, for geographic regions, as well as for the type of sample (originating from fishing grounds or fish markets). The inter-haplotype levels of divergence were estimated using the index Φ_{ST} (Excoffier et al. 1992), which includes information on mitochondrial haplotype frequency (Weir and Hill 2002) and genetic distance (Tamura and Nei 1993). The analysis of molecular variance (AMOVA) (Excoffier et al. 2005) was used to examine the amount of genetic variability partitioned within and among populations. Permutation procedures (n = 20.000) were used to construct null distributions and to test the significance of variance components for each hierarchical comparison (Guo and Thompson 1992).

Phylogeny was reconstructed using the Neighbor-Joining method (Saitou and Nei 1987). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). The test of phylogeny was conducted using the bootstrap method with 1000 replicates. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). Included in the analysis were bigeye tuna (BET) reference accessions with the following accession numbers: AY640276-288 from East Atlantic and AY640299-301 from Western Pacific, South China Sea, and the Philippines (Chiang et al. 2006) as well as EF154397-417 from Indian Ocean off Seychelles, off-shore Southwestern and Southeastern India, as well as Cocos Islands (Chiang et al. 2008).

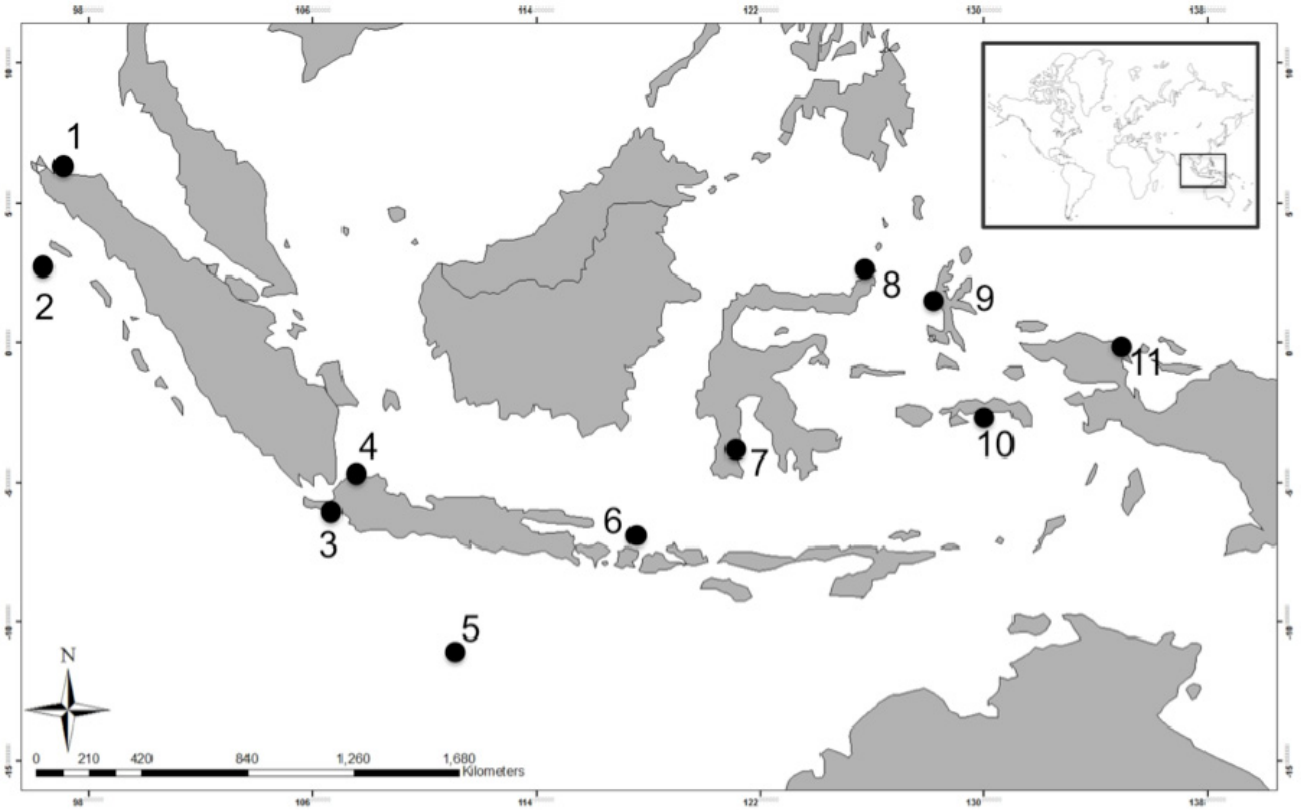


Figure 1. Map of Indonesia showing sampling locations for bigeye tuna (*Thunnus obesus*) coded by black circle. No 1 to 11 indicated each locations, (1) Aceh, (2) Indian Ocean high sea, off-shore west of Sumatra (Bungus Island), (3) Pelabuhan Ratu (West Java), (4) Muara Baru, (5) Indian Ocean high sea, off-shore south of Java Island, (6) Lombok, (7) Bone (South Sulawesi), (8) Bitung (North Sulawesi), (9) Ternate (North Maluku), (10) Ambon, (11) Manokwari (West Papua)

RESULTS AND DISCUSSION

Haplotype (H) and nucleotide diversity (π)

A total of 414 CR-mtDNA sequences for bigeye tuna were analysed in the study, based on a well-defined sequence of 447 bp. All sequence data has been deposited in GenBank with the following Accession Numbers: KR105968-KR106131 and KM871869-KM872030. The number of haplotypes (NH), the haplotype diversity (H_D) and the nucleotide diversity (π) estimated for each of the 11 sampling locations, for each region (West, Central or East) of Indonesia, and for the two type of sampling sites (fishing ground or fish market) are presented in Table 1. The results show that bigeye tuna possesses high haplotype and low nucleotide diversity throughout Indonesia. The lowest H_D was in Lombok (0.981), while the highest (1.00) was found in Aceh, Bungus, South Sulawesi, and Papua. The H_D's in west, center, and east Indonesia were 0.999, 0.998 and 0.997, respectively. Based on the type of sampling location, the H_D's in the fishing ground and fish market were 0.997 and 0.998. The overall value for nucleotide diversity (π) across locations was only 0.028±0.003.

Analysis of molecular variance (AMOVA)

The result of the analysis of molecular variance (AMOVA) of CR-mtDNA from various sampling locations is shown in Table 2. Population comparison between sampling area is shown in Table S1. Group comparisons based on the geographic area of Indonesia (west, center, and east) and the nature of sampling site (fishing ground and fish market) is summarized in Table 3. Overall fixation index (FST) was 0.00391. This index indicates there was no significant differentiation between bigeye sampling locations. Group comparisons resulted in no significant differentiation between geographic parts of Indonesia (west, center and east) and between the types of sampling locations (fishing ground and fish market). Population comparison between locations (Table S1) shows significant differentiations between Lombok and Ambon as well as between North Sulawesi's and North Maluku's bigeye samples.

Table 1. Origin, total number of readable sequences, number of haplotypes (NH), haplotype diversity (H) and nucleotide diversity (π) of bigeye and yellowfin tunas from various sampling locations, geographic part of Indonesia, as well as two types of sampling sites

Origin	Delineative statistics			
	No. Seq#	NH	H_D	*Π*
Aceh	3	3	1	0.026
Indian Ocean Bungus	19	19	1	0.028
West Java	67	65	0.999	0.029
Muara Baru	47	40	0.994	0.027
Indian Ocean Java	36	33	0.995	0.028
Lombok	15	13	0.981	0.031
South Sulawesi	25	25	1	0.028
North Sulawesi	126	103	0.996	0.029
Ambon	21	20	0.995	0.025
North Maluku	41	37	0.995	0.024
Papua	14	14	1	0.033
West Indonesia	89	86	0.999	0.029
Centre Indonesia	249	200	0.998	0.027
East Indonesia	76	68	0.997	0.026
Fishing Ground	55	51	0.997	0.028
Fish Market	359	279	0.998	0.026

Table 2. Analysis of molecular variance (AMOVA) of mtDNA control region of Bigeye tuna from various sampling locations

Source of variation	DF*	Sum of squares	Variance component	Percentage of variation
Among population	9	66.796	0.02535Va	0.39
Within-population	401	2587.214	6.45191Vb	99.61
Total	410	2654.01	6.47725	
Fixation Index (FST) =		0.00391		

Note: *: Aceh data was excluded from the analysis

Table 3. Group Comparison based on geographic area of Indonesia (west, center and east) and the nature of sampling sites (fishing ground and fish market) of bigeye tuna

Structure tested	Observed partition			
	Variance	% total	Φ statistics	*P*
Based on geographical regions (West, Center, and East Indonesia)				
Among regions	0.02278	0.35	0.00352	0.06648
Among locations within regions	0.00372	0.06	0.00058	0.37454
Within locations	6.44801	99.59	0.00409	0.1375
Based on type of sampling locations (fishing ground, and fish market)				
Among sample types	0.0135	0.21	0.00209	0.78459
Among locations within sample types	0.02256	0.35	0.00349	0.11178
Within locations	6.44801	99.86	0.00140	0.13975

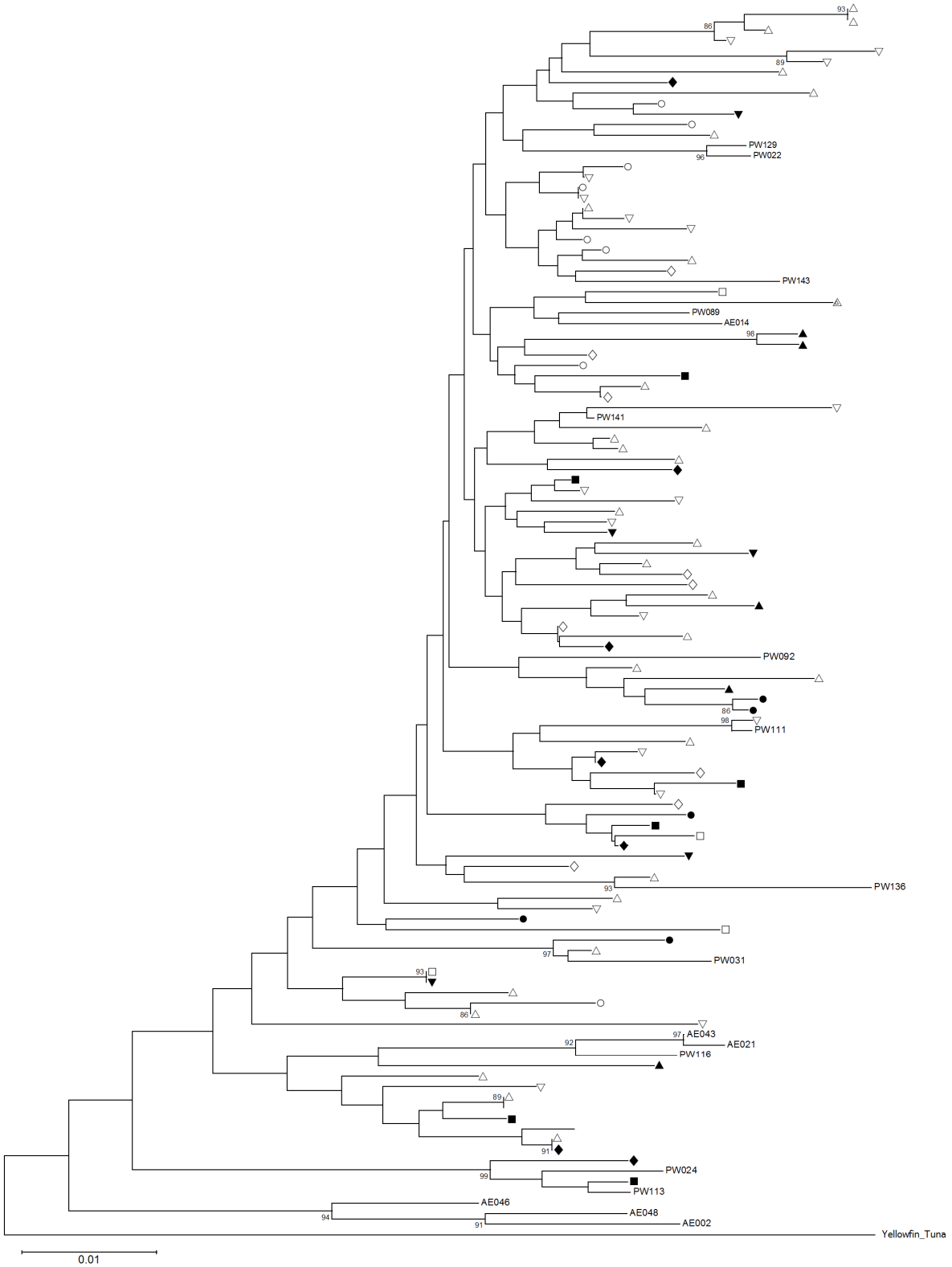


Figure 2. Neighbor-Joining tree of mtDNA control region fragment of bigeye tuna from various locations in Indonesia co-analyzed with secondary data available in GenBank from West Pacific, East Atlantic, and the Indian Ocean as previously published (Chiang et al., 2006, 2008). The taxon markers were filled inverted triangle for samples from Aceh, filled triangle from Bungus, unfilled inverted triangle from West Java, filled diamond from Muara Baru, unfilled circle from Java-Indian Ocean, filled circle from Lombok, filled square from South Sulawesi, unfilled triangle from North Sulawesi, unfilled square from Ambon, and unfilled diamond from North Maluku. The secondary data are indicated as PW and AE from the Indian Ocean, West Pacific, and East Atlantic. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Test of phylogeny was conducted using the bootstrap method with 1000 replicates. Bootstrap values of ≥80% are shown. A yellowfin tuna sequence (Acc. No. KP299042) was analysed as an outgroup

Evolutionary relationships

Evolutionary relationships for the mtDNA control region fragment of bigeye tuna from Indonesia were co-analyzed with data from Atlantic, Western Pacific and the Indian Ocean beyond Indonesia is shown in Figure 2. The phylogenetic tree shows that most of the Indonesian data belong to a single clade along with some Western Pacific and Indian Ocean East Atlantic data. A minor clade exists together with PW024 and PW113 secondary data (bootstrap value of 99%).

Discussion

We found that bigeye tuna in Indonesia seas possess high haplotype diversity and low nucleotide diversity based on analysis of a CR-mtDNA marker in populations sampled throughout the archipelago. Tuna is known as a highly migratory cosmopolitan marine fish (FAO 2000) and the origin of all species is thought to be polyphyletic (Kumar et al. 2014). Little migration occurs between Atlantic and Pacific-Indian Oceans for bigeye tuna (Chiang et al. 2006, 2008; Gonzalez et al. 2008). The fish are believed to possess large population sizes, that lead to the observed slow genetic drift with low-level or even no differentiation between Indo-Pacific and the Atlantic (Gonzalez et al. 2008).

Having vast inter-island waters, we expected some genetic differentiation among bigeye tuna. However, we found no differentiation between sampling locations, geographic regions (west, center, and east of Indonesia), and none between the types of sampling site (fishing ground and fish market). In the AMOVA between locations (Table S1), a significant difference was detected between Lombok and Ambon as well as between North Sulawesi and North Maluku. This small population differentiation might be due to the seasonal movement and age of tunas. The samples from those sites were mostly of young bigeye tuna collected from fish markets. This seems to be the harvest of traditional fishermen and artisanal fisheries. The artisanal tuna fisheries are most likely operating near shore and in inter-island waters or by using anchored fish aggregating devices (FAD) like 'payaos' in the Philippine (Dickson and Natividad 2000; Macusi et al. 2015). This traditional fishery might catch mostly baby tuna.

Many biological aspects of tuna are still unknown, especially regarding spawning and baby tuna. Adult bigeye tuna are known to be mesopelagic and mid-ocean species (Guojon and Majkowski 2000). Some tuna species, such as bluefin and yellowfin are known to exhibit homing behavior, returning to a specific place to spawn (Guojon and Majkowski 2000). Information for bigeye is lacking. This species might exhibit this behavior too. Further study is needed to explain the population differentiation found in some places, perhaps by simply analyzing samples of baby tuna.

The overall finding of our study is that there is a single population of bigeye tuna in Indonesia. This finding is in agreement with previous reports. Using CR-mtDNA, Chiang et al. (2008) confirmed the presence of various clades of bigeye, with the Atlantic clade limited in distribution to the Atlantic, and clades I and III dispersed in the Western Pacific, Indian Ocean, and Atlantic. No representatives of the Atlantic clade was identified in our study, as the reference accessions for the Atlantic clade (AE002, AE046 and AE048) formed a distinct cluster with bootstrap value of 94% (Figure 2).

Single dominant lineage has been identified in the South China Sea, Philippine Sea and Western Pacific Ocean, while minor clades are found in all waters (Chiang et al. 2006). A minor clade was also identified in this study from Indonesia (Figure 2). The demarcation of the specific Atlantic clade from the Pacific-Indian-Atlantic Oceans shared clade has been demonstrated using nucleotide sequencing from the same gene fragment used in our study (Martinez et al. 2006), as well as using random fragment length polymorphism (Chow et al. 2000). A barrier to gene flow at the Cape of a Good Hope, South Africa, has been proposed (Alvarado-Bremer et al. 1998; Chow et al. 2000). However, using data from nuclear microsatellite loci, Gonzalez et al. (2008) infered that bigeye tuna is a single worldwide panmictic unit The lack of congruence between mt and nDNA is thought to reflect past events of isolation and secondary contact (Gonzalez et al. 2008). The presence of representatives of the Pacific-Indian Ocean clade in the Atlantic is predicted based on hypothesized extensive migrations before the last glaciation. The discrepancy between mt and nDNA might also reflect behavioral differences between sexes such as female-biased philopatry and male-biased dispersal (Qiu et al. 2013).

This is the first spatial data covering Indonesia for bigeye tuna. The inference made in this study was based on a single mtDNA marker, the polymorphic control region, CR (Brown et al. 1986). This marker was selected in order to make a direct comparison with the global population data for tuna, as most other studies have targeted this fragment (Chiang et al. 2006, 2008). Using CR-mtDNA does have an advantage compared to other loci such as COI, as the coding regions are mostly under positive selection and accommodate viable mutations only. Therefore, greater variation occurs in CR compare to other coding regions. The weakness of CR-mtDNA is the lack of concordance with the results of using nuclear microsatellite loci (Gonzalez et al. 2008) in bigeye tuna. The discrepancy might reflect behavioral differences of male and female fishes (Qiu et al. 2013). Despite reservations about results obtained from different methods, the lack of differentiation we found amonst the bigeye tuna populations mirrors similar responses to evolutionary processes and biogeographic events observed in various comparable taxa (Carpenter et al. 2011).

The results of this study might reflect the high population size for bigeye tuna. Further study needs to be carried out concerning the temporal or seasonal population structure for this species. The techniques we have applied in this study have been made available within the country and will benefit government agencies in the responsible management of the tuna industry. Good quality fisheries data needs to be collected regularly - such as hook rates, voyaging distances, fishing grounds, fork lengths, maturity

levels and trends in cost per kilogram. This is required in order to provide a better understanding of the status of the tuna stocks and the strength of the fishery. This will allow decisons to be made on various management parameters such as maximum sustainable yields, and perhaps in the future total allowable catch levels.

In conclusion, the bigeye tunas catch in Indonesia has high haplotype diversity and low nucleotide diversity. Little population differentiation occurs between specific sampling locations, between geographic regions, and between types of samples taken to assess this diversity. Indonesian seas support a population of bigeye tuna assigned to the major Pacific and Indian Oceans clades for the species. Temporal differentiation needs to be examined in future studies, together with fisheries data, to generate a complete picture of the status of the tuna industry and to identify management actions needed to sustain the fishery for the benefit of peoples' livelihoods as well as the country's income.

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Table S1. The result of Fst test between locations of bigeye tuna samples from Indonesia

	Indian Ocean Java	**Lombok**	**Manokwari**	**South Sulawesi**	**North Sulawesi**	**Indian Ocean Bungus**	**West Java**	**Ambon**	**North Maluku**
Indian Ocean Java									
Lombok	-								
Manokwari	-	-							
South Sulawesi	-	-	-						
North Sulawesi	-	-	-	-					
Indian Ocean Bungus	-	-	-	-	-				
West Java	-	-	-	-	-	-			
Ambon	-	+	-	-	-	-	-		
North Maluku	-	-	-	-	+	-	-	-	
Muara Baru	-	-	-	-	-	-	-	-	-

Note: Positive (+) or negative (-) mean significant or not-significant differentiation.

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Identification and characterization of Talas banana, a superior local cultivar from East Kalimantan (Indonesia), based on morphological and agronomical characters

WIDI SUNARYO[1,♥], NURHASANAH[1], RAHMAN[2], ARIS SUGIARTO[1]

[1]Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman. Jl. Pasir Balengkong, Kampus Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia. ♥email: widi_sunaryo@yahoo.com

[2]UPTD Pengawasan dan Sertifikasi Benih Tanaman Pangan dan Hortikultura, Dinas Pertanian Tanaman Pangan, Propinsi Kalimantan Timur. Jl. PM Noor, Samarinda 75119, East Kalimantan, Indonesia.

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Abstract. *Sunaryo W, Nurhasanah, Rahman, Sugiarto A. 2017. Identification and characterization of Talas banana, a superior local cultivar from East Kalimantan (Indonesia), based on morphological and agronomical characters. Biodiversitas 18: 1414-1423.* Talas banana is a local cultivar grown in the certain area of East Kalimantan that is not explored and identified yet as other popular bananas such as Ambon, Raja, Kepok, Maulin, Susu and Cavendish. Based on the pre-observation, *Talas* Banana had a superior and unique taste compared to other commercial dessert and plantain bananas. The morphological and agronomical performance observation were carried out to investigate the possibility of Talas Banana developed as commercial and national excellent variety. *Talas* banana samples were collected and explored from four districts in East Kalimantan i.e. Samarinda, Kutai Kertanegara, Paser and East Kutai. The on spot morphological observation indicated that *Talas* banana is a combination of dessert and plantain suggesting a hybrid banana (*Musa acuminate* vs *Musa balbisiana*).Talas banana has characters possessed by *Musa Acuminata* as shown by the bract shape and color, male flower shape and color, and bud color, margin of the petiole canal,blotches color, and scars prominence. On the other hand, some other characters especially in fruit performance showed properties of plantain banana (*Musa balbisiana*) such as pedicle length, fruit apex shape and length, fruit shape, sugar and carbohydrate/starch content. Although based on the agronomical performance, Talas banana had low number of hands (7-11) and small fruit size resulting in lower production per hectar, it suggested to have important characters to meet commercial products such as long self-life, delicious taste, and high edible portion compared to other local commercial bananas. In conclusion, Talas banana is a potential cultivar to be developed as a superior and commercial variety.

Keywords: Talas banana, superior cultivar, *Musa acuminata, Musa balbisiana*, East Kalimantan

INTRODUCTION

Bananas are the most important fruit and serve major staple food for million people in the World (De et al. 2009). Most commercial and edible bananas are originated from two species of *Musa* spp. i.e. *Musa acuminata* (A-genome, 2n = 2x = 22), *Musa balbisiana* (B-genome, 2n = 2x = 22) and their interspecific triploid hybridscan be referred to as *Musa x paradisiaca* (Espino et al. 1992). Most triploid hybrids are plantains (AAB genotype) and other cooking bananas (ABB genotype). Almost all of commercial bananas are selected from the triploid hybrid plant for the seedless, vigorous, taste, shelf-life, fruit performance, and nutritional properties.

Indonesia is one of countries surrounded by the largest tropical rainforest in the world after Brazil (Seymor 2014). The humid-tropical rainforest covers part of Sumatra and almost all of Kalimantan and Papua Islands. Such forest contains a very high biological diversity of plants, animals, and other organisms including bananas. The biodiversity of banana in Kalimantan Island especially in East Kalimantan is still unknown, therefore efforts of exploration are very important for the banana breeding program to develop superior and commercial cultivars.

Bananas are cultivated and consumed by nearly 100 tropical and subtropical countries and has very important social and economic values in the world. In developing countries, bananas are the fourth important crop after rice, wheat and maize (INIBAP 2000). There are about 1,000 known cultivars of bananas (Heslop-Harrison and Schwarzacher 2007) and nearly 200 cultivars/clones are cultivated throughout the Indonesian archipelago (Valmayor et al. 2000). Bananas are very popular and widely consumed not only because of its delicious taste but also due to the high content of vitamins and minerals, especially vitamin A and C (Wall 2006).

The improvement of local fruit commodities as commercial products is one of the efforts to increase the competitiveness of such fruits in national and global markets. The local fruit has benefits in terms of the diversity of taste, aroma, color, and aesthetic aspects. Moreover, the local fruit commodities have a broad range adaptation on the growing regions that do not require high-input technology. Therefore, the development of the Indonesian local fruit commodities based on the development of adapted-local fruit centers is a promising strategy for the development of Indonesian agriculture,

especially in horticulture sectors that are recently still undeveloped optimally.

Talas Banana is a banana variant growing endemically on the Kalimantan Island which is not widely known as other commercial bananas such as Ambon, Kepok, Raja, Emas, Tanduk, Mauli or Barangan. Based on preliminary research, Talas banana has a good taste possessing excellent characters such as soft texture, sweet, not astringent or sour, low water content, and long shelf-life. In addition to good taste, Talas banana has also a unique and interesting physical characters such as yellow-orange/red pulpcolor and long-curved fruit apex. In local market, Talas banana shows a higher economic value represented by the higher price compared to other local bananas. In this paper, we report the results of the exploration and identification of Talas banana from East Kalimantan based on the morphological and agronomical characters as a preliminary effort in improving Talas banana as a national excellent and commercial variety.

MATERIALS AND METHODS

Site Visit

This study was conducted by field visit to the centers of Talas banana cultivation in East Kalimantan. The information of the cultivation centers was collected from several sources such as farmers, banana sellers, and from correlated agricultural institution. Based on the information, the site visit was focused to 4districts in East Kalimantan i.e. Samarinda, Kutai Kertanegara, Paser, and East Kutai (Figure 1). The observation, identification and observationwere conducted on spot centers of cultivation areasspread out in each district.

Morphological and agronomical identification

Several morphological and agronomical characters were observed for plant identification. For the characterization, 12 sample plants were identified for each research spot. Other important information such as number, type, origin of plant, local name, and location were also collected.

The whole plant and specific organ pictures such as leaves, pseudostem, male bud, suckers,bunch and fruit were taken using a Nikon Camera. The fruit characterization was conducted to investigate the fruit performance such as fruit color, fruit shape, fruit size, fruit productivity, peel and pulp thickness, percentage of edible part, nutritional content, and shelf-life of fruit at room temperature. The criteria of plant and fruit morphological characters used in this research were based on the ”Guideline book to make a description and to test the correctness of horticulture plant varieties, Ministry of Agriculture, Republic of Indonesia, number: 700/Kpts/OT.320/D/12/2011 (in Indonesian) and ”Descriptor for banana (*Musa* spp.)” (INIBAB, 1996).

Data analysis

The quantitative data such as plant height, pseudostem girth, leaf lamina length etc were calculated for mean values and standard deviation. The qualitative data were analyzed and characterized by comparing the similarity of samples with the descriptor.

RESULTS AND DISCUSSION

Plant distribution

Talas banana is cultivated in many ditricts in East Kalimantan including Samarinda, Kutai Kertanegara, Paser and East Kutai (Table 1), but only few of them were successfully explored and identified during the field visit. From 4 districtsexplored, 13 spots ofcultivation areaswere foundcomprising different number of clumps ranked from 332 to 153 clumps. The largest area of Talas banana cultivation was found in Paser districts, especially at the Muara Komam regencycomposed of 332 clumps from 3 spots of cultivated area. In total, it was observed 90 clumps from 13 spots of Talas banana cultivation in East Kalimantan (Table 1).

The distribution of Talas banana cultivation in east Kalimantan is influenced by the preference of the farmers, the economic value and the resistance to banana diseases mainly Bacterial and/or Fusarium wilt (Personal communication to the farmers). The economic value of banana is determined and indicated by the price in local market. Compared to the other local commercial cultivars, *Talas* banana is more preferred by local farmers due to the high price. Moreover, Talas banana is preferred by consumers because of its very good/delicious and unique taste (unpublished data). The most important factors influencing consumer preferences toward banana is quality (Dhamotharan and Selvaraj 2013). Fruit performance factors such as fruit taste, size and number of fruit per hand/fingers are considered the most important parameters influencing the consumer purchase in urban area, while appearances like color and shelf-life are considered less important (Ayinde et al. 2015).

Plant morphology

Most edible and modern bananas were derived from *Musa* spp. especially from the crosses of *Musa acuminata* (Genome AA) and *Musa balbisiana* (Genome BB) producing cultivated sweet and plantain bananas (Simmonds and Sheperd 1955). *M. acuminate* and *M. balbisiana* show significant differences of plant morphology. The characters of the ancestor/parents often appear in the modern cultivated bananas resulted from the crosses and could be detected using morphological observation. *M. Acuminata* has specific remarkable properties that could be recognized easily. *M. acuminata* is marked by brown or black blotches in pseudostem, erect or spreading margin canal petiole, downy or hairy peduncle, short pedicels, the roll back bract after opening, lanceolate/ovate bract shape, acute bract apex, prominent bract scars, and creamy white of male flower color(Simmonds and Sheperd 1955; D'Hont et al. 2012).On the other hand, *M. Balbisiana* possesses morphological properties such as no or few blotches on the pseudostem, inclosed margin of the petiole canal, not winged but clasping pseudostem, glabrous peduncle, long pedicels,

four irregular rows of ovules in each loculus, width/length ratio of bract shoulder usually low (more than 0.30), bracts do not reflex,broadley ovate bract shape, obtuse bract apex, scarcely prominent bract scars, a distinctive brownish-purple bract coloroutside, bright crimson inside, and male flower color variably flushed with pink (Simmonds and Sheperd 1955; Davey et al. 2013). These characters are remarkable at both species and easily observed to distinguish and identify the species.

Table 1. Talas banana cultivation in four districts of East Kalimantan, Indonesia

Explored districts	Number of observed spots	Number of clumps per spot	Total clumps observed
Samarinda	3	153	459
Kutai Kertanegara	5	202	1010
Paser	3	332	996
East Kutai	2	154	308
Total	13	841	2773

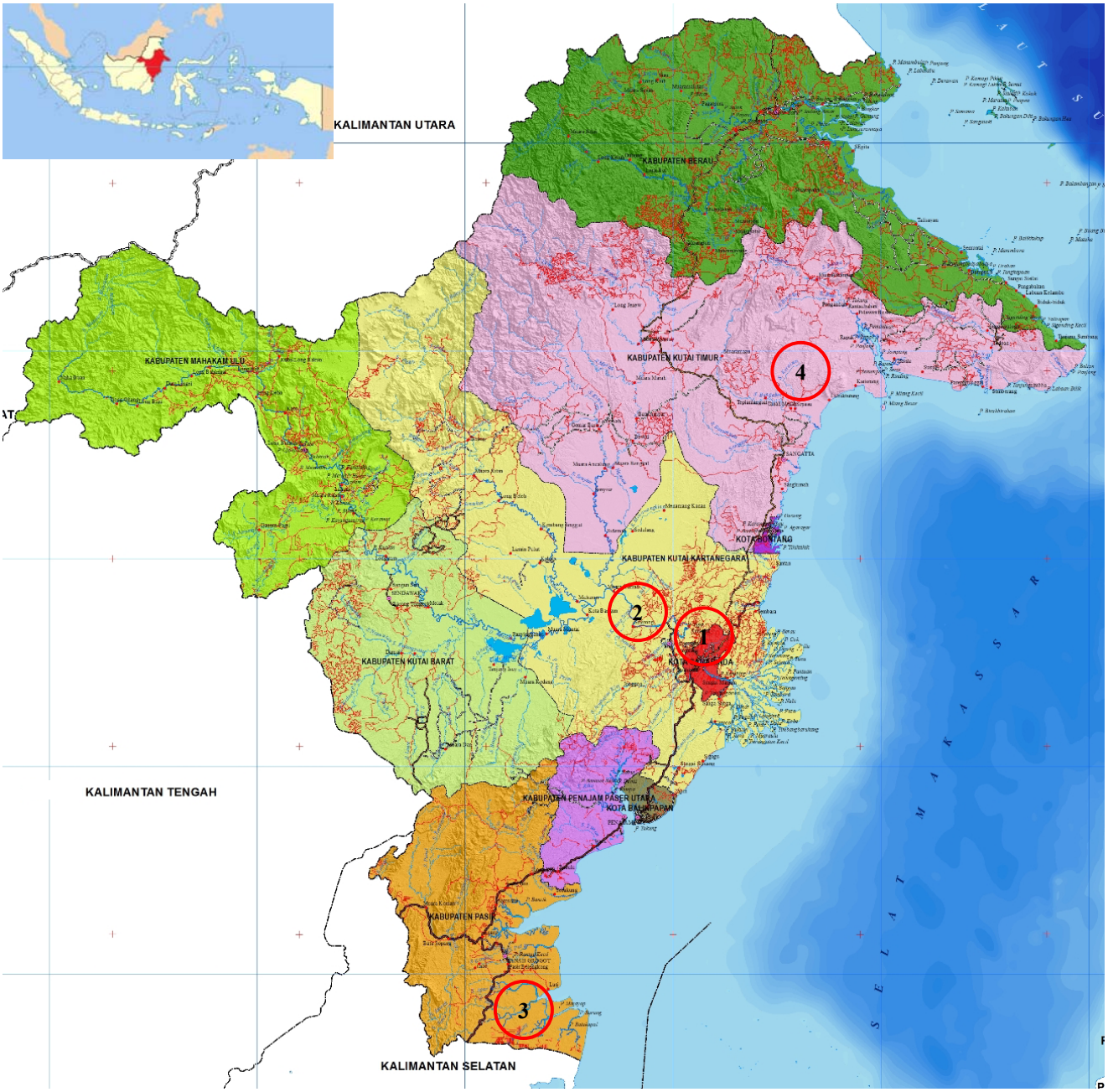


Figure 1. Research exploration sites of Talas banana in East Kalimantan Province, Indonesia. 1. Samarinda, 2. Kutai Kertanegara, 3. Paser, and 4. East Kutai

Morphological observation during site visit showed that Talas banana is very much closed to *Musa paradisiaca* (Simmonds and Sheperd 1955) derived from the crossing between *M. acuminata* and *M. balbisiana*. With plant height (pseudostem height) about 2.82 m as almost similar as to other local commercial bananas such as Ambon, Susu, or Raja bananas (Table 2, Figure 1A).*Talas* banana is grouped as a normal banana if it is classified based on the plant height and dwarfism. The height of cultivated banana is in the range of 2-9 m (INIBAB 2000). The pseudostem girth of Talas banana is slimy (53.13 cm) with dark-purple blotches color (Table 2, Figure 1B). The pseudostem blotches dominated by brown or black color is a remarkable character derived from *M. acuminata*. Among banana cultivars, the pseudostem morphology of banana possessed high variation in length and coloration (Pillay and Tripathi 2007).

The leaves emergence and growth of *Talas* banana is in intermediate shape (Not erect/not drop) with the leaf ratio (length:width) more than 3 : 1. *Talas* banana contains about 10 leaves which has 257.7 cm length and 72.3 cm width in average (table 2). The leaves has the green and not shiny color in upper and green-yellow on lower lamina (figure 3 A and B). The lamina base is *obtusus* (both sides pointed) which forms an *oblonge* type of leaf shape (figure 3 D) with a ratio of length and width 3.56 : 1. According to Nugroho (2003), the length and width ratio of leaves in *M. acuminata* is between 2.5-5 : 1 which accordingly will be categorized as an oblong type. Interestingly, the margin of leaf petiole shows a straight with erect margins indicating a *M. acuminata*-derived character (Table 2, Figure 3A). The cross section of petiole shows a U-shaped and a semi-hollow structure (figure 4A). This structure was as reported by Ennos et al. (2000) in *Musa textillis* and found in many other bananas (Sumardi and Wulandari 2010). The base of petioles forms a strong overlapping structure with a grey, green and dark purple color combination (Figure 3C).

The male bud is in a medium-slimy size and ovate/lanceolate shape with curly (rolling) *brachtea* after opening and before falling (figure 2D) emerging from the erect leave petioles(Figure 2C).The young bracts are greatly overlap to form a compact imbrication of male bud. These properties are *M. acuminata* belonging. On the other hand the bract internal facecolor is orange-red showing a *M. balbisiana*-derived property (Simmonds and Sheperd 1955).

The bunch is hanging slightly angled on the pseudostem and has green color with a spiral and dense (compact) fruit (Fable 2, Figure 2E). However, it was found that the fruit density and size were different between the initial harvest pant in the new opened cultivation land (Figure 4A) and those of in the continuous cropping land (figure 4B). The initial bunch usually contains fewer number of hands, but has bigger fruit size. It might be caused by their differences on the level of land fertility resulting in the different fruit productivity. As reported by Zhong et al. (2014) that banana continuous cropping results in the decline of the yield due to the decrease of soil quality as showed by increase of soil acidity, decrease of total organic C, accumulation of N, P, K, Ca and Cu, and deficiency of Mg, S, Fe, Mn, and Zn.

The peduncle is slightly hairy, dark green, with the length less than 30 cm. The rachis is falling vertically and the present flower and male bud may be degenerated or persistent with very prominent bract scars on rachis (table 2, figure 2C, D). These properties is also in line with the *M. acuminata* characters and in contrast to *M. balbisiana* posing a glabrous peduncle and scarcely prominent bract scars (Simmonds and Sheperd 1955; D'Hont et al. 2012).

The fruit is emerging and developing from the yellow-white flower and stand curved upward (obliquely, at a 45°angle upward) on the rachis. It is not like a sweet banana in general (*M. acuminata*-derived characters). Talas banana has fruit shape and properties like a plantain with a curved fruit shape, short pedicle and lengthily pointed long fruit apex (table 2, figure5A, B, C).These properties indicate a *M. balbisiana*-derived fruit shape. The pedicles are not fused and can be separated easily. The fruit size is classified in a medium size with 17.00 cm fruit length and 11.07 fruit girth. The interesting character of Talas banana is the persistentremains of flower relicts at fruit apex and the fruit does not peel easily (Table 2, Figure 5A, B, C), therefore the fruits have a longer shelf-life (20-24 days, table 3).

The immature fruit peel has light-green color(table 2, figure 2E) and when mature, the color is bright-yellow and waxy (table 2, figure 5A). The peel is classified as a thin peel with the thickness less than 2 mm. The color of fruit pulp is yellow bright with fourirregular rows of ovules in each loculus (Figure 5E, F). The mature fruits stand strongly on the pedicle without falling from hands (persistent). This property is much in contrast with other local cultivars such as Ambon, Mauli or Susu that can fall from the hands easily after certain time of maturity. The fruit flesh/aril shows seedless, soft texture, sweet and tasty properties. As a dessert and plantain, Talas banana can be cookedand also freshly eaten. These could be due to the starch (carbohydrates) content in the flesh is high enough as starch content as in common plantain cultivars. Compared to other local commercial bananas, Talas banana was preferred by consumers living in the surrounded cultivation areas in East Kalimantan because of the sweet, tasty and delicious taste (Personal communication to consumers). The seedless character indicates that Talas banana hasthe possible triploid structure of chromosomes resulted from the natural genetic modification such as crossing, and mutation/deletions orinsertions (Dolezel et al. 1994).

Table 2. Morphological characters of *Talas* banana identified based on the banana descriptor (INIBAB 1996).

Morphological characters	Descriptor	Note
Leaf habit	Intermediate	Not erect/not drop
Dwarfism	Normal	leaves not overlapped and leaf Ratio inferior to 2.5
Pesudostem height	2.1-2.9 m	2.82 ± 0.41 m
Pesudostem aspect	Slender	-
Pseudostem color	Green-yellow	-
Peudostem appearance	Dull (not shiny)	-
Pseodostem predominantcolor	Brown-purple	-
Sap color	Watery	-
Development of suckers	Between 1/4 and 3/4 of the height of the parent plant	-
Sucker Position	Close to parent (vertical growth)	-
Blotches at the petiole base	Small blotches	-
Blotches color	Brown-purple	-
Petiole canal leaf	Straight with erect margins	-
Petiole margins	Not winged and not clasping the pseudostem	-
Petiole margin color	Pink-purple to red	-
Leaf blade length [cm]	221 to 260 cm	257.70 ± 27.66 cm
Leaf blade width [cm]	≤70 cm	70.23 ± 5.83
Leaf ratio	≥3	3.68
Petiole length	51 to 70 cm	52.21 ± 22.67 cm
Color of leaf upper surface	Green	-
Appearance of leaf upper surface	Dull (not shiny)	-
Color of leaf lower surface	Green-yellow	-
Appearance of leaf lower surface	Dull (not shiny)	-
Wax on leaves	Few wax	-
Insertion point of leaf blades on petiole	Asymmetric	-
Shape of leaf blade base	Both sides pointed	-
Leaf corrugation	Even, smooth	-
Color of midrib dorsal surface	Green-yellow	-
Color of midrib ventral surface	White-green	-
Color of cigar leaf dorsal surface	Green	-
Peduncle length [cm]	≤30 cm	22.44 ± 12.43 cm
Peduncle width [cm]	≤6 cm	5.89 ± 1.32 cm
Peduncle color	Dark green	-
Peduncle hairiness	Slightly hairy	-
Bunch position	Hanging Slightly angled	-
Bunch shape	Spiral	-
Bunch appearance	Compact	-
Flowers that form fruits	Female (absence of pollens)	-
Male flower color	White-yellow	
Male bud type	Present	-
Male bud shape	Intermediate (Ovate)	-
Male bud size	≤20 cm	Small
Positioning of fruits on the crown	Uniseriate	-
Rachis type	Present and male bud may be degenerated or persistent	-
Rachis position	Falling vertically	-
Bract base shape	Small Shoulder	-
Bract apex shape	Slightly pointed	-
Bract imbrication	Young bracts greatly overlap	Like *Musa acuminata*
Color of the bract external face	Purple	-
Color of the bract internal face	Orange-red	-
Color on the bract apex	Color is uniform until apex	-
Color stripes on bract	Without discolored lines (not ridges) on the external face	-
Bract scars on rachis	Very prominent	-
Bract behaviour before falling	Revolute (rolling)	-
Wax on the bract	Moderately waxy	-
Presence of grooves on the bract	Not grooved	-

Fruit position	Curved upward (obliquely, at a 45°angle upward)	-
Number of fruits	13-16	16.00 ± 1.97
Fruit length [cm]	16-20	17.00 ± 0.44
Fruit shape	Curved	-
Transverse section of fruit	Rounded	-
Fruit apex	Lengthily pointed	Like plantain
Remains of flower relicts at fruit apex	Persistent style	-
Fruit pedicel length [mm]	≥21 mm	25,3 ± 0.35
Fruit pedicel width [mm]	5 to 10 mm	8.19 ± 0.23
Pedicel surface	Hairless	-
Fusion of pedicels	No visible sign of fusion	-
Immature fruit peel color	Light green	-
Mature fruit peel color	Bright yellow	-
Fruit peel thickness [mm]	≤ 2 mm	-
Adherence of the fruit peel	Fruit does not peel easily	-
Cracks in fruit peel	Without cracks	-
Pulp in fruit	With pulp	-
Pulp color before maturity	White	-
Pulp color at maturity	Yellow Goldish	-
Fruits fall from hands	Persistent	-
Flesh texture	Soft	-
Predominant taste	Sweet (*like Cavendish*) and tasty	-
Presence of seed with source of polle	Seedless	-



Figure 2. A. Talas banana plant, B. Pseudostem, C-D.. Male bud, D. Flower, and E. Bunch of *Talas* banana

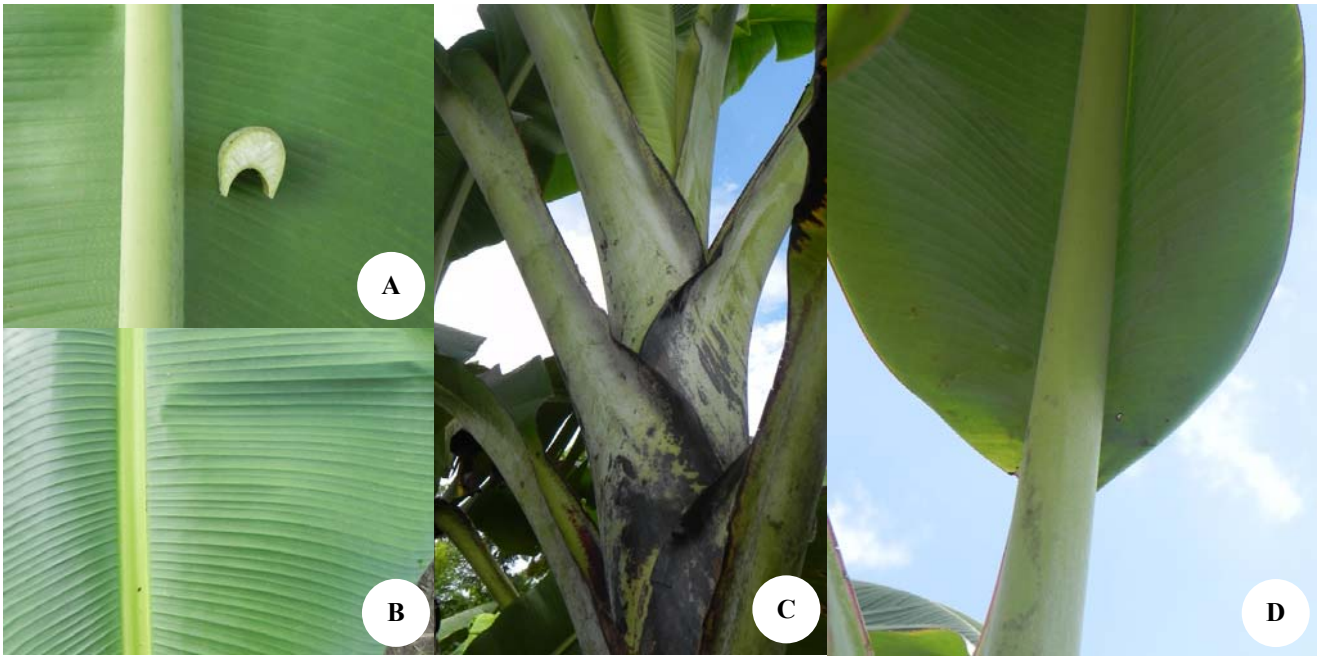


Figure 3. Leaves and petioles of Talas banana. A. Lower surface and petiole canal, B. Upper surface, C. Petiole arrangement in pseudostem and D. leaf base



Figure 4. Two different types of Talas banana bunches: A. banana bunch produced in a new opened lands plenty in soil nutrition, and B. Banana bunch grown in the continuous cropping lands

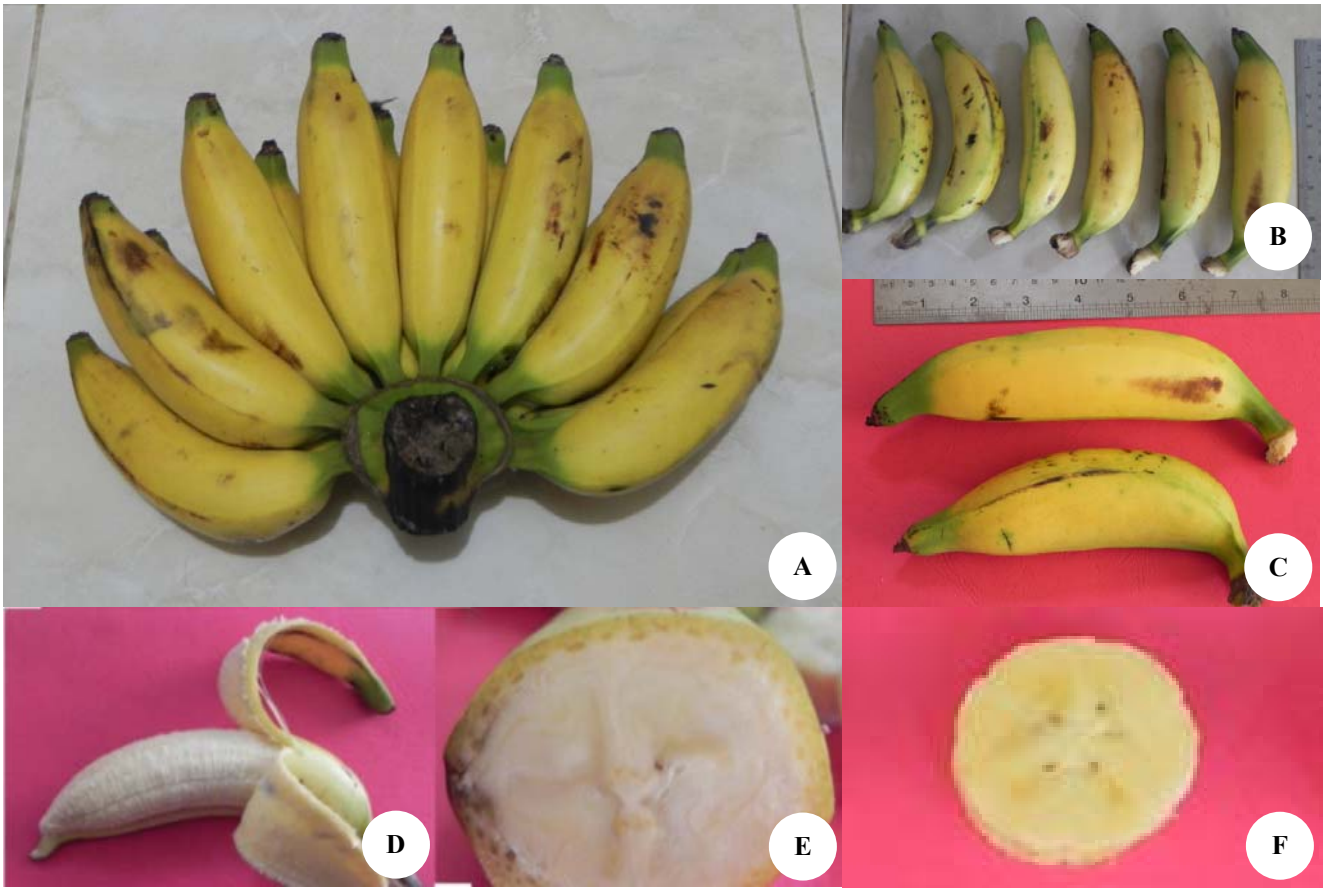


Figure 5. A. Talas banana hand, B-C. Fruits, D. Peeled fruit, E. Cross sectioned, F. Fruit

Plant agronomical performances

The observation of fruit productivity showed that with the traditional system of cultivation, *Talas* banana produced yield per hectare approximately 1500-2000 bunches or 16-23 t ha^{-1} per year indicating 1-2 bunches per clump per year with plant space 2 m x 3 m (table 3) with the population around 1660 clumps. In an intensive cultivation, banana cultivation in Southern Mindanau (Philipines) can produce 34 t ha^{-1}, but in average, banana in Philippines only produced 11 t ha^{-1}. On a poor nutrient management, banana production in Guangxi, China was 18 to 20 t ha^{-1} (Hongwei et al. 2004). Production banana is varied among the cultivation system, technology input system, and banana cultivars. The yield of banana is influenced by many factors such as environmental conditions, agronomic practices, the cultivars and ratooning management (Morton, 1987). If the innovation of cultivation technology especially the balanced land fertilization is applied, the yield could increase significantly. The application of N, P, and K fertilizer can increase the banana yield and reached 39 t ha^{-1} especially by producing more hands and increasing the single fruit weight (Hongwei et al. 2004).

Even though the number of hands per bunch of *Talas* banana was low (8-9 hands) but the number of single fruit per hand was relatively high approximately 16 fruit per hand (table 3). With the yield 1-2 bunches per clump per year, the production of *Talas* banana is categorized as low. The good and intensive cultivation of banana usually will result in 2-3 bunches per clump per year. The productivity of bunches of banana is closely correlated with the nutrient supply and management. The Giant Cavendish produced20-60 kg per bunch (in average 23 kg per bunch) or 1.5-2 carton and 2100 carton per hectare and 7-14 fingers (Broadley et al. 2014). Lady finger, a relative small banana, produced 10-30 kg per bunch (in average 13 kg per bunch) or 1 carton and 750 carton per hectare and 7-10 fingers (Broadley et al. 2014). The low yield of the observed plant production could be due to the application of low even zero input to the technology cultivation system especially the nutrient balanced technology. The uncontrolled sucker (ratoon) management was also the other practice contributing the low yield Talas banana. In the commercial Cavendish banana cultivation, the ratoon management was conducted by desuckering and remaining only 2 ratoons per clump besides the main plant (Broadley et al. 2004). Most farmers observed in this research were using a traditional system and do not apply the ratoon management technology for the Talas banana cultivation and maintained almost 4-5 suckers per clump (Unpublished data). Therefore, the resource (light, water, and soil nutrient) competition resulted in the low yield. The use of

intensive system such as balanced fertilization, weed control, soil tillage, and pest and disease control would also increase the yield significantly.

Beside morphological, nutritional, and agronomical characters, the shelf-life plays an important role in concerning to improve agricultural products like fruits as commercial commodities. Shelf-life determines the length/time of the product can be harvested, stored, transported, and used by consumers. Therefore, it also determines the economic value of such fruit product. Talas banana showed shelf-life about 20-24 days after harvesting (Table 3). It is also longer time compared to other local commercial cultivars such as Ambon, Susu, Raja, and Mauli (Unpublished data). Moreover, due to the thin fruit peel, Talas banana has very high edible portion around 72.45% (Table 3). These positive properties are preferable by consumers and contributing the high economic values.

Plant taxonomy

Most modern commercial bananas are derived from the two banana ancestor i.e. *Musa acuminata* and *Musa balbisiana* (McKey et al. 2010; Simmonds and Sheperd 1955; Heslop-Harrison and Schwarzager 2007). The natural crossing of both species (referred as *Musa x paradisiaca*; Simmonds and Sheperd 1955) and the genetic modification produces plenty variants of modern and edible bananas (called as domesticated bananas; Heslop-Harrison and Schwarzager 2007) possessing good characters such as sweet and seedless banana. Both of *M. acuminata* and *M. balbisiana* are diploids with genome AA (descending sweet/dessert bananas) and BB (descending plantain/ cooking bananas). Most domesticated bananas are triploids containing A and/or B genomes and classified into six variants/groupsAA, AAA, AB, AAB, ABB and ABBB (Simmonds and Sheperd 1955).

Table 3. The agronomical charactersof Talas banana based on 12 sample plants observed.

Agronomic characters	Mean ± SD*)
Plant height (m)	2.82 ± 0.41
Pseudostem girth (cm)	53.13 ± 8.60
Leaf number	10.00 ± 1.58
Leaf length (cm)	257.70 ± 27.66
Leaf wide (cm)	70.23 ± 5.83
Number of suckers (anakan)	10.03 ± 0.84
Fruit number per hand	16.00 ± 1.97
Number of hands	8.13 ± 1.88
Fruit length (cm)	17.00 ± 0.44
Fruit girth (cm)	11.07 ± 0.21
Tip length (cm)	2.40 ± 0.10
Petiole length (cm)	2.53 ± 0.35
Bunch weight (kg)	11.93 ± 3.34
Hand weight (g)	1466.60 ± 53.24
Fruit weight (g)	79.03 ± 13.56
Fruit aril weight (g)	57.26 ± 4.51
Edible portion (%)	72.45 ± 6.72
Production per hectar (bunches per year)	1500-2000
Production per hectar (ton ha^{-1})	16-23
Shelflife from the fisrst day harvesting (days)	20-24

Note: *) The value was calculated based on 12 sample plants observed. SD: Standard Deviation

The taxonomy status of Talas banana is still unclear and unidentified yet. But based on the morphology observation (Table 2 and Figure 2,3,4,5), most likely Talas banana is originated from the crossing of two species i.e.*M. acuminata* (AA genome) and *M. balbisiana* (BB genome) and possibly has triploids chromosome sets since the fruit pulp/flesh is sweet and has no seeds (seedless) (table 3). To investigate the genetic constitution of *Talas* banana needs further genomic analysis such as chromosome (flow cytometry for chromosome calculation) or molecular marker analysis.

In conclusion, morphological observation indicated that *Talas* banana is a combination of dessert and plantain suggesting a hybrid banana (*Musa acuminate* vs *Musa balbisiana*). *Talas* banana has characters possessed by *Musa acuminata*as shown by the bract shape and color, male flower and bud shape, male flower color, margin of the petiole canal, blotches color, and scars prominence. On the other hand, some other characters especially in fruit performance showed properties of plantain banana (derived from *Musa balbisina*) such as pedicle length, fruit apex shape and length, fruit shape, sugar and carbohydrate/starch content. Although, based on the agronomical performance, *Talas* banana had low number of hands (7-11) and small fruit size resulting in lower production per hectar, it suggested to have important characters to meet commercial products such as long self-life, delicious taste, and high edible portion compared to other local commercial bananas. In conclusion, Talas banana is a potential cultivar to be developed as a superior and commercial variety.

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Short Communication: The potential of various indigenous *Trichoderma* spp. to suppress *Plasmodiophora brassicae* the pathogen of clubroot disease on cabbage

I KETUT SUADA
Program of Agroecotechnology, Faculty of Agriculture, Universitas Udayana. Jl. P. B. Sudirman, Denpasar 80225, Bali, Indonesia. Tel./fax.: +62-361-702801, email: ketutsuada@unud.ac.id

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Abstract. *Suada IK. 2017. Short Communication: The potential of various indigenous* Trichoderma *spp. to suppress* Plasmodiophora brassicae*, the pathogen of clubroot disease on cabbage. Biodiversitas 18: 1424-1429.* On the island of Bali, cabbage (*Brassica oleracea* L.) is a vegetable that has high economic value especially for farmers who cultivate it as a cash crop. However, the clubroot disease caused by the microbial pathogen *Plasmodiophora brassicae* decreases its productivity. Much effort has been expended in attempts to decrease the pathogen attack by use of synthetic fungicides, however, this has not had much success; more over such pesticide applications generate environmental pollution. Therefore, biological control using *Trichoderma* or other organisms antagonistic to the pathogen is an attractive alternative. The purpose of this study was to find, isolate and identify *Trichoderma* spp. able to decrease the disease incidence and increase plant growth. Twelve indigenous *Trichoderma* isolates were tested on cabbage plants grown in polybags containing local soil media culture treated with the *Trichoderma* inocula at a concentration of $1.5x10^6$ CFU per polybag. The results showed that three *Trichoderma* isolates, i.e. *T. hamatum*-1*, T. harzianum*-1, and *T. harzianum*-2, were able to decrease the clubroot disease and increase cabbage growth as well.

Keywords: Biocontrol, cabbage, clubroot, *Trichoderma*

INTRODUCTION

Cabbage (*Brassica oleracea* L.) is a popular vegetable beneficial to health because it contains vitamins A, B1, C, and minerals, and is therefore in high demand on the island of Bali, Indonesia. However, the production of local cabbages in Bali has been in steady decline since 2010: in that year, the production of cabbages in Bali amounted to 47,077 tons, in 2011 it was 42,926 tons, in 2012 40,167 tons, but in 2013 it only reached 35,781 tons (Central Bureau of Statistics of Bali Province 2014). This decline in production was closely related to the observed high incidence of clubroot disease caused by the microbial pathogen *Plasmodiophora brassicae* Woronin, responsible not only for the disease in cabbage plants, but also for attacking other members of the family Brassicaceae.

According to local farmers, clubroot disease is an important issue in every crop season and occurs in almost every cabbage-growing area in Bali. Disease control using pesticides is often not effective against soil borne pathogens such as the clubroot pathogen. Moreover, excessive use of fungicides is polluting the environment. As a result, public attention has been increasingly drawn to environmental safety concerns, leading to limitations being placed on pesticide application.

A potential alternative to the use of chemical pesticides in the control of clubroot disease of cabbages is an environmentally friendly control system based on biological agents such as fungi or bacteria antagonistic to the clubroot pathogen. One such antagonist with potential to be used as a biocontrol agent is the fungal genus *Trichoderma* Persoon. Besides being able to protect the cabbage against clubroot-disease caused by *Plasmodiophora brassicae*, *Trichoderma* also can control various other diseases caused by fungal pathogens (Cheah and Page 1997; Navi and Bandyopadhyay 2002). Fungal pathogens that have been reported as being controlled to some degree by *Trichoderma* spp. Include *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotium,* and *Verticillium* (Nederhoff 2001; Agrios 2005; Arya and Perello 2010). The use of introduced *Trichoderma* spp. has been reported to reduce pathogen attacks by about 25%, while the use of fermented livestock manure combined with *Trichoderma* spp. in cabbage reduced pathogen attack by 51% (Legowo 2010). In addition, to acting as antagonists against pathogens, *Trichoderma* can also act as a plant growth promoter, because the interaction between *Trichoderma* and plants has been reported to stimulate formation of plant growth hormones(Harman et al. 1991).

In the research literature, there are documented cases of obstacles encountered in testing for the effectiveness of various antagonist agents against pathogens. The major factors that influence the effectiveness of antagonist agents are environmental extremes include temperature fluctuations, ultraviolet light, pH, availability of water and nutrients, as well as competition from indigenous microflora (Agrios 2005; Renwick and Poole 1989; Zhu et al. 2016). As a result, there are problems in the adaptation of some introduced biological agents to the environment of their application site.

Microbial control agents introduced to help protect crops against pathogens that cause root disease must adapt to the environment of the application site and to the rhizosphere of the crop plants if they are to function properly as antagonist agents. Moreover, an antagonist agent is usually propagated in an artificial media so becoming accustomed to the optimum conditions of that medium. This tends to reduce its completive abilities when introduced to the open field conditions of a growing crop. Furthermore, the genetics of the pathogenic microbe and also of the antagonistic agent generally has a capacity to change quickly, and so the effectiveness of the antagonist against the pathogen can change rapidly as well. At the site of introduction, biological agents can be inhibited by the new environmental conditions resulting in lower than expected effectiveness under field conditions.

Weller (1988), in his wheat research, has clearly revealed that although introduced microbes may dominate in a new location for the few first weeks, by the end of the growing season they can decline in vigor and their population drop to less than 2% of the original population. Even genetically modified agents can experience the same thing, such that their population shrinks drastically and they no longer remain effective as biological agents.

The research reported in this paper was undertaken to obtain and identify local *Trichoderma* spp. isolates able to be used as biocontrol agents in cabbage crops grown in Bali. The purpose of the study was to culture these *Trichoderma* isolates and apply them at populations suitable for controlling clubroot disease and enhancing growth of cabbage plants under local conditions.

MATERIALS AND METHODS

Soil sampling

Soils were sampled in farmers' vegetable gardens, from the rhizosphere of various type of cabbages infected by clubroot disease in the area of Bedugul, Tabanan, Bali. Each sample of 200 g was placed in a plastic bag. The samples were packaged in a box that had been filled with ice to protect them from sun damage and overheating, and then transported to the Biotechnology Laboratory of the Faculty of Agriculture, University of Udayana, Denpasar, Bali, Indonesia.

Isolation and identification of *Trichoderma* spp.

In the Biotechnology Laboratory, isolation of *Trichoderma* spp. from the soil samples was carried out by culturing on PDA (Potato Dextrose Agar) media containing100 ppm Streptomycin as an antibiotic for bacteria.

Colonies of growing fungi were subcultured and then identified by observation under a microscope; matching the fruiting bodies of the fungal isolates to reference images documented in Alexopoulos and Mims (1979), Barnett and Hunter (1972), CMI (1988), Webster and Weber (2007), Domsch et al. (1980), and Watanabe (2002), and also by using the taxonomic key of Samuels et al. (2006). The right *Trichoderma* inocula were subcultured using a single spore isolation technique. A serial dilution method was used to obtain dilutions of 10^{-1}-10^{-7}. One milliliter of suspension of dilution rate of 10^{-3}-10^{-7} was cultured in sterile PDA medium containing 100 ppm Streptomycin to prevent bacterial contaminants growth.

Design of the experiment to test *Trichoderma* isolates on cabbage plants

The experiment to test isolates was performed in a greenhouse of the Faculty of Agriculture. Polybags (diameter 12.5 cm x height 20 cm) received 2.5 kg of a mixed medium (local soil:compost, at a ratio of 3:1). Each polybag was planted with one three-week-old cabbage seedlings. For every isolate to be tested, 9 polybags were assigned. Altogether, twelve *Trichoderma* isolates were tested in the experiment, and their impacts on cabbage plant growth was compared with a control treatment that consisted of cabbage plants with no *Trichoderma* application. Thus, the experiment consisted of a total of thirteen treatments with three replications per treatment (each with three sample plants per polybag), arranged in a completely randomized design. Each of the twelve *Trichoderma* treatments received $1.5x10^6$ spores of the particular assigned *Trichoderma* isolate in 200 ml water, applied as a drench to the soil around the base of the cabbage plants.

Maintenance of the cabbage plants

The polybags were fertilized twice; i.e. at one and three weeks after planting. Fertilizer was given at a dose of 1.3 g/plant ZA (*Zwavelzure Ammoniak*, 20.8% Nitrogen), 1.8 g/plants TSP (Triple Super Phosphate, 45% P_2O_5), and 0,7 g/plant KCl (Potassium chloride, 60% K_2O). Plants were watered until the soil moisture was stable at field capacity. Plants of all treatments were sprayed with foliar fertilizer DI Grow (liquid organic fertilizer contained macro, micro elements, and growth regulator) at a concentration of 2 cc/L at a dose equivalent to 400 L/ha.

Observation variables

The observed variables were: plant height, measured from the ground to the tip of the leaf held erect; total number of leaves; number of galls (i.e. number of large and/or small galls per plant), counted after the plants were upended at the end of the experiment; percentage of plants attacked (%), calculated from the number of plants that had a gall after the plant was upended; leaf chlorophyll content, measured by a SPAD Chlorophyll Meter; leaf area, measured by a Leaf Area Meter. All these variables were observed at7 weeks after planting, i.e. prior to the plants forming cabbage hearts.

Data analysis

Data were analyzed by ANOVA appropriate to a completely randomized design (CRD), followed by a Duncan's Multiple Range Test at a 1% and 5% significance level, to differentiate the effects of isolates.

RESULTS AND DISCUSSION

Antagonistic *Trichoderma* spp.

From the soil obtained in the rhizosphere of various plants sampled at the study site, twelve specimens of *Trichoderma* spp. were isolated in the laboratory. The characteristics of the twelve isolates are described in Table 1 and Figure 1.

Based on observation by microscope, the isolates could be assigned to four different groups with the characteristics shown in Table 2.

As listed in Table 2, the twelve isolates were identified as belonging to four *Trichoderma* spp., namely *T. hamantum, T. harzianum, T. polysporum,* and *T. koningii*. Generally, the *Trichoderma* fungal colonies were found to be white-green in color after three days growth on PDA but they gradually turned bluish toward the seventh days. Within 6 to 7 days, colonies had reached a diameter of 9 cm, which resulted in them reaching the outer edge of the media in the petri dishes.

Performance of the cabbage plants in the experiment evaluating the *Trichoderma* isolates

Observations carried out on the cabbage plants evaluated for the effects of the *Trichoderma* isolates comprised measurements of plant height, leaf number, leaf area, and leaf chlorophyll at seven weeks after planting, just before the plants began to form cabbage hearts.

Plant height and total leaf number did not show a significant difference between the isolate treatments (based on Duncan Multiple Range test at 5% level). However, there was a significant difference between isolate treatments in leaf area and leaf chlorophyll. The largest leaf area was found in isolate treatment Tri-1 (*T. hamatum*-1) with a total leaf area of 95.24 cm^2 leaf area. Its chlorophyll estimate was also the highest, namely 3596.26 SPAD units of chlorophyll (see Wood et al. 1992). These results indicated a beneficial response of the cabbage plant to the fungus *T. hamatum*-1, an interaction that promoted plant growth (Table 3). According to Suwahyono and Wahyudi (2004), *Trichoderma* can act not only as a biological control agent, but the fungus can also act as a driver of growth, improving plant performance: *Trichoderma* can stimulate the formation of growth hormones that enhance plant growth. *Trichoderma* species that have been reported to act in this way are *T. harzianum, T. polysporum*, and *T. viridae*. In this current study, the most probable isolate with the described property was *T. hamatum*-1. Compared to other isolates, *T. hamatum* in this study was better in its effects on cabbage plant performance than *T. harzianum* and *T. polysporum.*

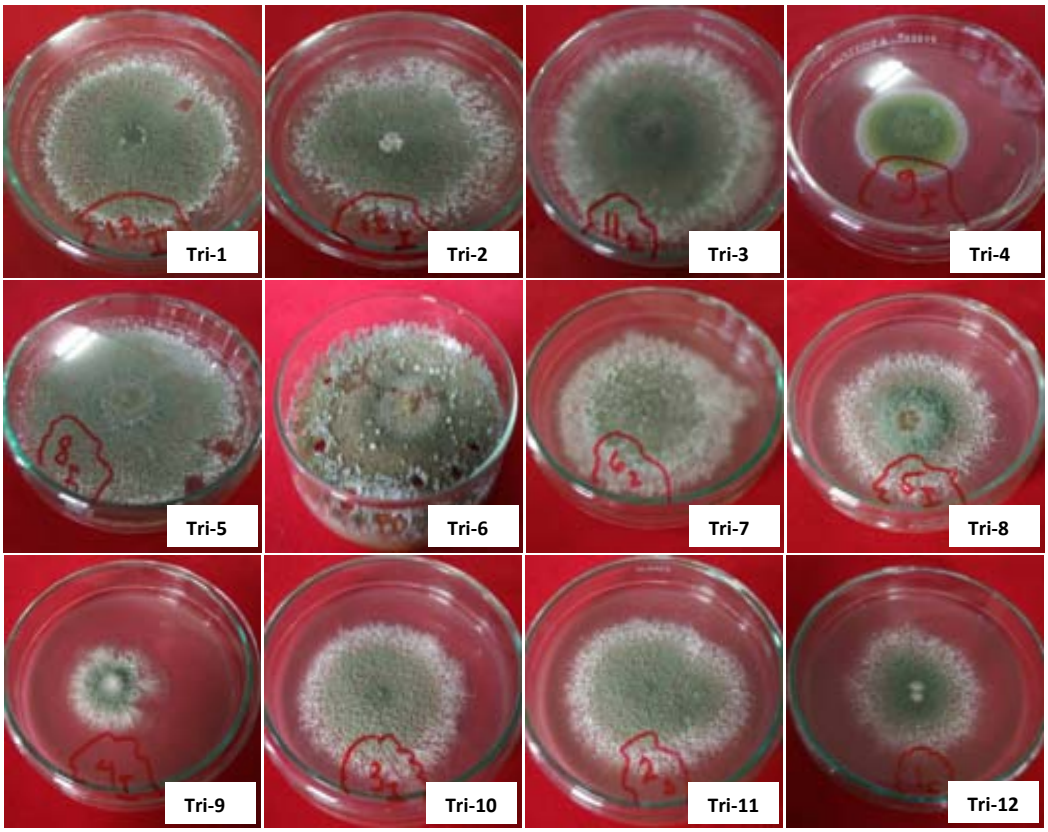


Figure 1. Various types of *Trichoderma* spp. colonies isolated from the rhizosphere of healthy plants in farmers' fields where clubroot disease was prevalent

Table 1. Characteristics of the colony development of twelve *Trichoderma* spp. isolates cultured on PDA medium

Isolate	Color at colony age (day 1 to 7)							Colony form
	1	2	3	4	5	6	7	
Tri-1	White	Greenish white	Greenish white	Greenish white old	Light green	Green	Dark green	Round
Tri-2	White	Greenish white	Light green	Greenish white old	Light green	Green	Green	Round
Tri-3	White	White	Greenish white	Greenish white old	Light green	Green	Dark green	Round
Tri-4	White	White	Green	Dark green	Light green	Dark green	Dark green	Round
Tri-5	White	Greenish white	Greenish white	Greenish white old	Light green	Green	Dark green	Round
Tri-6	White	Greenish white	Greenish white	Greenish white old	Light green	Green	Dark green	Round
Tri-7	White	Greenish white	Light green	Greenish white old	Light green	Green	Dark green	Round
Tri-8	White	Greenish white	Light green	Greenish white old	Light green	Green	Dark green	Round
Tri-9	White	Greenish white	Light green	Greenish white old	Light green	Green	Dark green	Round
Tri-10	White	White	Greenish white	Greenish white old	Light green	Green	Dark green	Round
Tri-11	White	Greenish white	Greenish white	Greenish white old	Light green	Green	Dark green	Round
Tri-12	White	Greenish white	Light green	Greenish white old	Light green	Green	Dark green	Round

Note: Tri=*Trichoderma* spp. Numbers that follow Tri is an identifying numbers for isolates obtained from various healthy plants in farmers' fields where clubroot disease was endemic

Table 2. Characteristics of *Trichoderma* spp. isolated from the rhizosphere of various healthy plants

Isolate	Microscopic characteristics			Species
	Conidiophore	Phialides	Conidia	
Tri-1	Erect, branched	Short, thick	Oval	*T. hamantum*-1
Tri-2	Erect, branched	Short, thick	Oval	*T. harzianum*-1
Tri-3	Branched	Long, very thick	Oval	*T. polysporum*-1
Tri-4	Erect, branched	Short, thicker	Oval	*T. harzianum*-2
Tri-5	Erect, branched	Short, thick	Oval	*T. harzianum*-3
Tri-6	Erect, branched	Short, thick	Oval	*T. hamantum*-2
Tri-7	Erect, branched	Short, thick	Oval	*T. hamantum*-3
Tri-8	Erect, branched	Small, taper	Oval and round	*T. koningii*-1
Tri-9	Erect, branched	Short, thick	Oval	*T. hamantum*-4
Tri-10	Branched	Long, very thick	Oval	*T. polysporum*-2
Tri-11	Branched	Long, very thick	Oval	*T. polysporum*-3
Tri-12	Erect, branched	Small, taper	Oval and round	*T. koningii*-2

Note: Tri=*Trichoderma* spp. Numbers that follow Tri are identifying numbers for the isolates obtained from various healthy plants in the field areas where clubroot disease was prevalent.

Table 3. Effect of isolates of *Trichoderma* spp. on several cabbage plant variables at seven weeks after planting

Treatment	Variables of plant performance			
	Height (cm)	Total leaf number	Leaf area (cm^2)	Leaf chlorophyll (SPAD)
Control (no Tri)	14.45±3.23 a	9.33±0.89 a	28.43±43 b	1007.84±2.56 d
Tri-1	17.79±2.44 a	11.00±5.14 a	95.24±6.75 a	3596.26±3.66 a
Tri-2	16.45±4.35 a	10.00±2.11 a	64.36±3.76 ab	2413.50±7.54 b
Tri-3	14.88±1.57 a	9.66±3.55 a	43.42±5.22 b	1577.44±6.77 c
Tri-4	16.08±2.65 a	9.66±2.45 a	64.01±6.32 ab	2349.16±12.69 b
Tri-5	14.56±3.56 a	9.33±1.79 a	31.66±4.33 b	1122.34±11.54 d
Tri-6	14.45±3.77 a	10.00±2.19 a	35.48±3.22 b	1321.98±32.56 d
Tri-7	14.54±1.33 a	9.33±2.17 a	49.09±2.11 ab	1819.27±18.23 c
Tri-8	17.06±2.35 a	10.33±1.35 a	58.40±3.24 ab	2219.93±10.23 b
Tri-9	16.54±2.33 a	9.66±3.44 a	61.45±3.44 ab	2284.09±9.34 b
Tri-10	15.54±1.14 a	10.00±1.35 a	62.25±4.68 ab	2311.34±16.88 b
Tri-11	15.63±4.12 a	9.66±2.33 a	41.38±3.56 b	1596.02±24.15 c
Tri-12	14.94±3.66 a	10.00±2.56 a	26.09±2.77 b	965.33±13.24 d

Note: Tri=*Trichoderma* spp. Numbers that follow Tri are numbers identifying particular isolates obtained from various healthy plants in a field area where clubroot disease was prevalent. Data are expressed as means±SDs. Values followed by the same letters in the same column do not differ significantly at 5% probability level according to Duncan Multiple Range test



Figure 2. The appearance of the plant roots at seven weeks after planting. Asymptomatic roots (*above*) and galled roots (*below*)

Table 4. Incidence of clubroot disease observed in cabbage plants treated with different *Trichoderma* spp. isolates

Treatment	Observation variables	
	Gall number (pieces/plant)	Disease incidence (%)
Control (no Tri)	8.94±3.03 a	100.00 a
Tri-1	0.00 d	0.00 d
Tri-2	0.00 d	0.00 d
Tri-3	8.22±2.00 a	100.00 a
Tri-4	2.33±0.21 c	11.11±0.25 c
Tri-5	7.89±2.13 a	55.55±05.00 b
Tri-6	7.45±2.22 a	55.55±1.00 b
Tri-7	7.35±3.02 a	33.33±3.95 b
Tri-8	4.33±2.41 b	11.11±2.13 c
Tri-9	2.78±0.02 c	11.11±2.16 c
Tri-10	7.35±1.80 a	55.55±05.00 b
Tri-11	7.78±2.02 a	44.44±3.55 b

Note: Tri=*Trichoderma* spp. Numbers that follow Tri are numbers identifying particular isolates obtained from various healthy plant in a field area where clubroot disease was prevalent. Data are means±SDs. Values followed by the same letters within the same column are not significantly different at a 5% level of significance, based on Duncan Multiple Range test. Percentage data were analyzed after arc-sin $\sqrt{(x+1/2)}$ conversion.

Incidence of clubroot on cabbage plants

The effect of the *Trichoderma* isolates on incidence of clubroot in the cabbage was rated based on two variables i.e. the number of clubroot galls and the percentage of infected plants (Figure 2). It proved not possible to effectively categorize all galls according to size into large or small, and so all galls irrespective of size were counted as single galls. The percentage of infected plants was obtained by dividing the number of plants with gall(s) by the total number of plants observed, expressed as a percentage.

Tri-1, that is *T. hamatum*-1, completely suppressed the formation of galls, so that the incidence of clubroot in this treatment was zero, this suggests that Tri-1 may be antagonistic to the growth of the pathogen (*P. brassicae*). Furthermore, Tri-2 (*T. harzianum*-1) also demonstrated the same total suppression of gall formation, while Tri-4, Tri-8 and Tri-9 showed a relatively low disease incidence (11.11%) (Table 4). The isolates Tri-1 and Tri-2 were effective in disease-suppression and also appeared to promote the health of the cabbage plants as indicated by high total chlorophyll estimates of 3596.26 and 2413.50 SPAD respectively. That means that the two *Trichoderma* isolates may act not only as biological control agents, but may also enhance plant growth. Plants with a high leaf chlorophyll content would be expected to have enhanced photosynthesis and hence enhanced ability to form carbohydrates. Many studies have reported that increased chlorophyll concentrations in plant leaves, achieved by improved nutrition, are correlated with higher yields in agricultural and horticultural crops (Wood et al. 1992; Kararurt et al. 2009; Fan et al. 2014).

It is possible that a beneficial *Trichoderma*-crop association results in growth enhancement of the plants via promotion of photosynthesis as indicated by the high chlorophyll content and leaf area observed in Tri-1 and Tr-2 treatments. A conclusion that follows from this research is that *Trichoderma* isolates capable of suppressing clubroot disease in cabbage plants also improved some parameters of cabbage plant growth. The best isolates were *Trichoderma hamatum*-1, followed by *Trichoderma harzianum*-1, and *Trichoderma harzianum*-2. Further research is needed to determine the most effective formulations that can be applied in the field.

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Evaluation of expression analysis of putrescine n-methyltransferase gene during different stages of growth in the medicinal plant *Physalis divaricata* (Solanaceae)

ELHAM MOALLEM[1], ABDOLLAH GHASEMIPIRBALOUTI[2], TAHER NEJADSATTARI[1], ALIREZA IRANBAKHSH[1], IRAJ MEHREGAN[1,♥]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran, Tel: +98-2144865327, ♥email: iraj@daad-alumni.de, imehregan@srbiau.ac.ir

[2]Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

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Abstract. *Moallem E, Ghasemipirbalouti A, Nejadsattari T, Iranbakhsh A, Mehregan I. 2017. Evaluation of expression analysis of putrescine n-methyltransferase gene during different stages of growth in the medicinal plant* Physalis divaricata *(Solanaceae). Biodiversitas 18: 1430-1437. Physalis divaricata* (Solanaceae) is one of the most prevalent weeds in summer crops. Putrescine N-methyltransferase (PMT) is a key enzyme in the biosynthesis of nicotine, tropane alkaloids atropine, scopolamine, cocaine, and calystegines. The present study set to compare *PMT* gene expression during different growth stages of *Ph. divaricata* using RT-qPCR assay. RNA extraction was performed from root and leaf samples of a total number of 40 individuals *Ph. divaricata* at different growth stages (late vegetative and fruiting stages) collected from southwestern Iran. RT-qPCR of cDNA reversely synthesized from RNA was carried out using SYBR®Premix Ex Taq™ II kit. *PMT* gene expression levels were analyzed using ΔΔCT method. The results showed that expression level of *PMT* in late vegetative stage samples was significantly higher compared to fruiting stage samples. The expression level of *PMT* similarly changed in root and leaf samples. Direct visualization of alkaloids in different tissues using Wagner histochemical tests showed more concentrations of alkaloids in leaf idioblasts and root stele.

Keyword: Histochemical, *Physalis divaricata*, PMT, RT-qPCR, Wagner test

INTRODUCTION

Of the estimated about 500,000 plant species, only a small group has been investigated phytochemically, and even a smaller group has been studied in terms of their pharmacological properties (Hamburger and Hostettmann 1991).

Physalis divaricata D. Don (Solanaceae) is an annual ground cherry, has recently become a most serious damaging weed in many minor crops and summer annual crops. The generic name *Physalis* is originated from the Greek name phusa; the fruit is attached and in bladder shape of flower bowl with five clefts, which increases in size after falling off the corolla. It bears smooth, dark-green ovate, lanceolate- or rhomboid-ovate, acuminate leaves, and 4-5 mm long yellowish-white flowers. Fruits are yellow, spherical and ca. 10 mm in diameter, containing numerous reniform-lenticular, reticulate-rugose seeds. *Ph. divaricata* is distributed in India, Pakistan, and Afghanistan, and recently introduced in Iran (Schönbeck-Temesy 1972; Rates 2001).

Physalis divaricata is an invasive weed with a length of 15-60 cm. Its yellow color flowers are cup-shaped and solitary, which appear from May to July. The number of seeds produced can range from 126 to 16,300 per plant; also, it produces 4 to 70 seeds per berry. This weed prefers rich nutrient soils. According to the preliminary observations, the freshly harvested seeds indicate high levels of dormancy. This species has become destructive to annual summer crops, such as potato, tomato, maize, sugar beet, and some minor crops in Iran. The sticky materials released from Ph. divaricata berries cause harvest problems and crop quality reduction. One of the most important stages in the life cycle of *Ph. divaricata* is seed germination. *Physalis divaricata* seeds apparently have no special tools to disperse for long and short distances (Stanton and Lemerle 2012). Different factors can highly affect seed germination and seedling emergence. These include light (Huebner 2011), osmotic potential (Boyd and Van Acker 2004), seed burial depth (Wilson et al. 2006), and temperature (Foley 2008). Interactions of those factors with internal conditions of seeds would also have impact on seed germination. Chemical control of *Ph. divaricata* is difficult due to its proximity to other *Solanaceae* crops, especially when it grows in the fields of such crops (Gorski and Wertz 1987). Development of effective control measures, prediction of invasion potential, and weed management would be easier with understanding of the factors affecting seed germination and seedling emergence (Ghersa and Holt 1995).

Due to its alkaloids. *Ph. divaricata*, an invasive weed to many summer fodder fields, is toxic to domestic animals. It is important to investigate the amount of alkaloid production during different developmental stages. *Putrescine N-methyltransferase* (*PMT*) is one of the most important enzymes of plant secondary metabolism at the

beginning of the specific nicotine biosynthesis, calystegines, and tropane alkaloids that are glycosidase inhibitors with nortropane structure. *PMT* is fictitious to have developed from spermidine synthases (SPDS), participating in ubiquitous polyamine metabolism (Teuber et al. 2007). *PMT* in the nicotine biosynthesis is the first pathway-specific enzyme of tropane alkaloids atropine, cocaine, scopolamine, and calystegines. Calystegines are polyhydroxy alkaloids with strong glycosidase inhibitory activity. They occur fictitiously more among the plant kingdom before the distribution of tropane alkaloids (Griffin and Lin 2000); Brassicaceae and Moraceae contain calystegines (Brock et al. 2006). The first *PMT* cDNA sequence was obtained by subtractive hybridization on cDNA banks exploiting different transcript levels between tobacco cultivars rich and poor in nicotine (Hibi et al. 1994). Furthermore, *PMT* cDNA sequences from *Nicotiana* species were found screening genomic DNA libraries by 5c-terminal *PMT*-fragments as probes that contain 33 base pairs repeated two to nine times (Hashimoto et al. 1998).

Northern blot analysis is usually used for determination of gene expression levels. Large quantities of RNA are required for northern blot technique (Dean et al. 2002). RT-qPCR is widely used for clinical diagnostic, the gene expression analyses, and for plant studies (Gachon et al. 2004). Many reference genes required for the normalization of expression signals are well characterized (Sturzenbaum and Kille 2001). Actin, glyceraldehyde-3-phosphate dehydrogenase, ribosomal genes, cyclophilin, 18s rRNA, and elongation factor 1-a (ef1a) are amongst those well-known genes (Volkov et al. 2003). This list might also include adenine phosphoribosyltransferase (APRT) and tubulin. The vast majority of the studies dealing with housekeeping gene expression has mainly focused on human tissues, bacteria, and viruses. This figure is much fewer in case of plants, and usually limited to concerned plants such as barley, rice, poplar, *Arabidopsis thaliana*, and tobacco (Langer et al. 2002). Consequently, it is very important to choose an appropriate internal control for gene expression quantification (Nicot et al. 2005). Using 18s rRNA could be a valuable alternative for quantification of genes of interest (Kadam 1999).

Some studies have shown that *PMT* gene expression in Solanaceae is restricted to root. It is also shown that at least two members of *PMT* gene family are also involved in the regulation synthesis of alkaloid (Laue et al. 2000).

The *PMT* protein expression in nicotine was reported to be confined to roots and tropane alkaloid producing plants; the only exceptions were young potato tuber sprouts that synthesize calystegines and wounded tobacco leaves (Sachan and Falcone 2002). *PMT* transcript localization in roots was confirmed for all plants in this study and considered as typical for non-wounded plants. Calystegines transport or preceding metabolites in aerial organs must be inferred from the *PMT* expression usually restricted to roots (Korolev 2002). Other key alkaloids like physalins are usually diagnosed at the development and all stages of growth. Physalin concentration increases double during the developmental changes such as during flowering, twice the germination stage (El-Tayeb et al. 1997).

The present study aimed to evaluate expression analysis of *PMT* gene during different stages of growth (late vegetative and fruiting stages) in the medicinal plant *Ph. divaricata,* using RT-qPCR assay.

Histochemistry is dealing with the identification of chemical components of cells and tissues (Dhale 2011). Alkaloids are degradation of the protein, investigated using two methods, namely, Mayer's reagent and Wagner's reagent (Dhale 2011). The present study aimed to investigate storage alkaloid that occurs in the tissues and cells of root and leaf of *physalis divaricata* at late vegetative and fruiting stages.

MATERIALS AND METHODS

Plant material

To determine the *PMT* gene, 20 samples at the late vegetative stage (Figure 1.A) and 20 samples at fruiting stage (Figure 1.B) were randomly selected. Root and leaf samples were obtained from *Ph. divaricata* plants carefully. All samples were stored in RNAlater immediately after resection until use. The protocols of samples preparation referred to the Studies Committee at the Islamic Azad University of Shahrekord, Iran with grant number 17621105.

RNA extraction and cDNA synthesis

RNX^{TM}-Plus solution (SinaClon, Iran) was used for extraction of total RNA from root and leaf samples. DNA was destroyed by adding an extended 1 h treatment with DNase I. Quality of RNA was checked on a Thermo Scientific NanoDrop™ 1000 spectrophotometer. Presence and integrity of RNA were initially checked on 2% agarose gel. Synthesis of complementary DNA (cDNA) was performed by taking 1 µ and using random hexamer priming and PrimeScript^{TM}-RT reagent kit (TaKaRa, Japan). The concentration of synthesized cDNA was checked spectrophotometrically.



Figure 1. *Physalisdivaricata*late vegetative stage (A), and at fruiting stage (B)

Quantitative Real-Time PCR

Assessment of all samples was carried out on a rotor gene 6000 Corbett detection system. qPCR became possible using SYBR®Premix Ex Taq™ II kit (TaKaRa, Japan) after manufacturer's instructions. PCR conditions included an initial activation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 65°C for 1 min. Each run also included a "no template control" (NTC) consisting of H2O. Amplification of the target genes was performed by using following forward and reverse primer pairs: PMT-f: 5′-TTC TGT CAA CTA CGC TTG GA-3′; PMT-r: 5′-CTA ATT CGG ACT TGA TAT GAG C-3′; 18S-f: 5′-GGG CAT TCG TAT TTC ATA GTC AGA G-3′; 18S-r: 5′-CGG TTC TTG ATT AAT GAA AAC ATC CT-3′. Specificity of PCR products verification was performed using melting curve analysis. In order to verify product sizes and specificity, PCR products were checked on 2% agarose gel. All samples were normalized to 18s rRNA. We used the mean value in each triplicate to calculate relative *PMT* concentrations (ΔCt = Ct mean PMT-Ct mean 18s rRNA). $2^{-\Delta\Delta Ct}$ methods were used to calculate the expression fold changes. The qPCR assays were carried out with three replicates for each sample, and the data were presented as the mean ± standard error of the mean (SEM).

Histochemical tests

In order to find the cells and tissues where alkaloids being stored, we tried to investigate the fresh tissues using the method first described by Furr and Mahlberg (1981). This method is specially designed to show the actual places of alkaloid storage. The histochemical tests were performed with fresh tissue samples, sectioned by hand from median region of the leaves, and roots cross-sections were taken from different regions. Looking for alkaloid components, we screened all plant parts at different growth stages. They are not recognizable morphologically.Sections which store alkaloid compounds are determined using the Wagner reagent. Wagner reagents and Dittmar reagent were introduced by Furr and Mahlberg (1981). The sections were installed in 50% glycerin on a slide with coverslip according to the semi-permanent slide preparation methodology described previously (Johansen 1940). An optical microscope (Olympus AX70) coupled with a digital camera was used to obtain photomicrographs.

Statistical analysis

In order to determine the significance, a pairwise fixed reallocation randomization test with 2000 iterations was performed using the REST 2009 software package. The software package SPSS v.20 (SPSS Inc., Chicago, IL) was used for statistical analysis of demographic and clinical data. Testing the relationship between categorical variables was made possible using Chi-square and independent t-tests ($P<0.05$).

RESULTS AND DISCUSSION

Results

To explore the role of *PMT* in *Ph. divaricata* plants, the relative expression of mentioned gene was examined in late vegetative stage samples and the fruiting stage samples with RT-qPCR technique. The relative expression of *PMT* is shown in Figure 2.The relative expression level of *PMT* in samples at late vegetative stage was significantly higher(2 times) compared to the samples at the fruiting stage. On the other hand, the expression level of *PMT* had a similar change in both root and leaf samples (2 times).

The analysis by REST 2009 confirms the results. Overexpression of *PMT* in samples at a late vegetative stage in root samples is shown in Figure 3. As seen in Figure 3, 50% of the relative expression has an expression distribution between 8% and 15%.

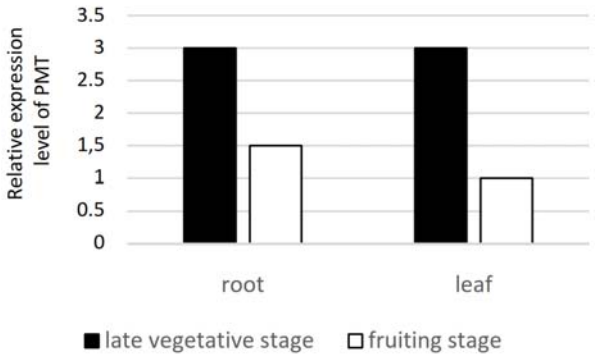


Figure 2. The relative expression level of PMT gene in two groups of samples

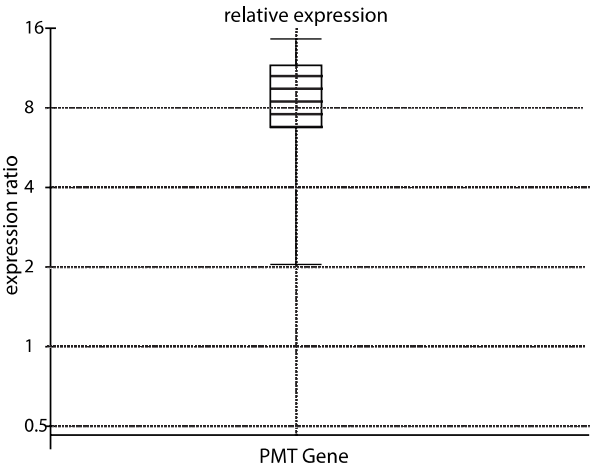


Figure 3. The relative expression level of PMT in root samples by REST 2009

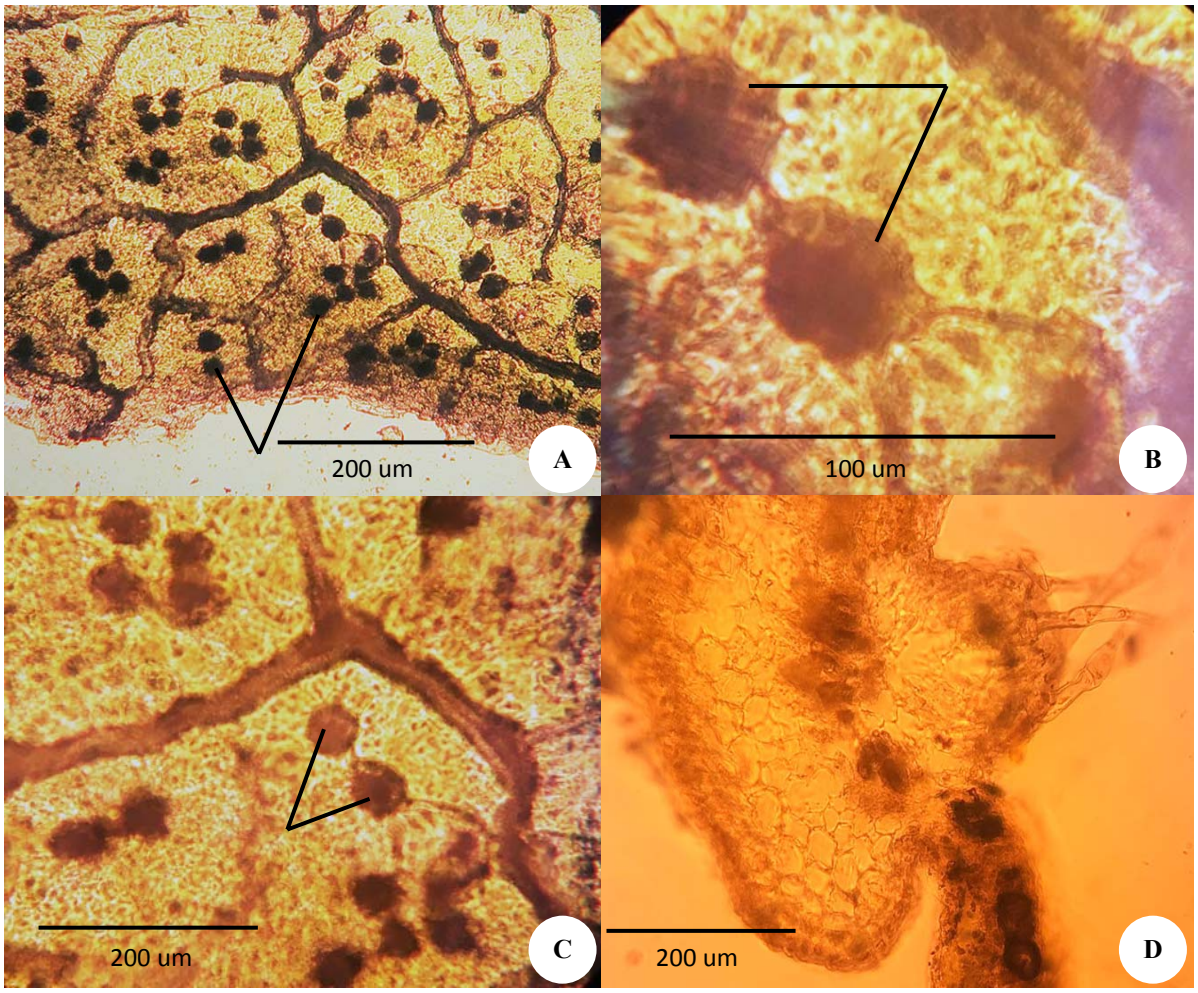


Figure 4. Sample spots treated with Wagner reagent; *Physalis divaricata* leaves at late vegetative stage detail are shown by cross arrows, under light microscopy. A. Leaf blade section stained with Wagner reagent. B. Detail of the mesophyll and several idioblasts in the parenchyma. C. Detail of the same parenchyma cells with a few idioblasts. D. Detail of the main vein.

In Mayer's reagent, alkaloids were observed in the scattered cells of mesophyll of leaves. In Wagner's reagent, alkaloids were found in the cells of mesophyll and cells of cortex parenchyma and pith parenchyma of the stem of *Adhatoda zeylanica*, *Ruta graveiolence Vitex negundo* (Dhale 2011).

Spots of Wagner reagents are seen in separate mesophilic cells or idioblasts cells of leaves at the end of the vegetative stage. (Figure 4.A, B, C), and at the fruiting stage, the spots have a density of less than the late vegetative stage (Figure 5.D), but more trimmed (Figure 5.A, B, C). Of course, they are not found in the parenchyma around the main vein (Figure 4.D). Wagner reaction reveals in the secondary phloem, primary phloem, epiderm, the pericycle bounded, under the epiderm, and medullary rays of root at late vegetative (Figure 4.A, B, C, D, E, F) and fruiting stages that spots are only the pith and rey parenchyma cells with less density than the late vegetative stage (Figure 5A, B) of *Ph. divaricata*.

Alkaloids generally accumulate in specific cell types owing to their cytotoxicity and probable role in plant defense responses. For example, alkaloids are sequestered to isolated idioblasts and laticifers in *Catharanthus roseus* (St-Pierre et al. 1999), root endodermis, and stem cortex/pith in *Thalictrum flavum* (Samanani et al. 2005), and laticifers in opium poppy (Bird et al. 2003). Thus, in this research, alkaloids were evidenced in leaf idioblasts cells, root cortex/endodermis/stele by Wagner histochemical tests.

Discussion

Our results showed that Wagner reagent is a suitable tool to spot accumulation of alkaloids in different tissues and organs. Both aerial and underground parts of *Ph. divaricata* showed to accumulate alkaloids. Accumulation of alkaloids in vegetative parts of *Ph. divaricata* increases with growth of those organs. The maximum amount of alkaloid accumulation was observed in the late vegetative

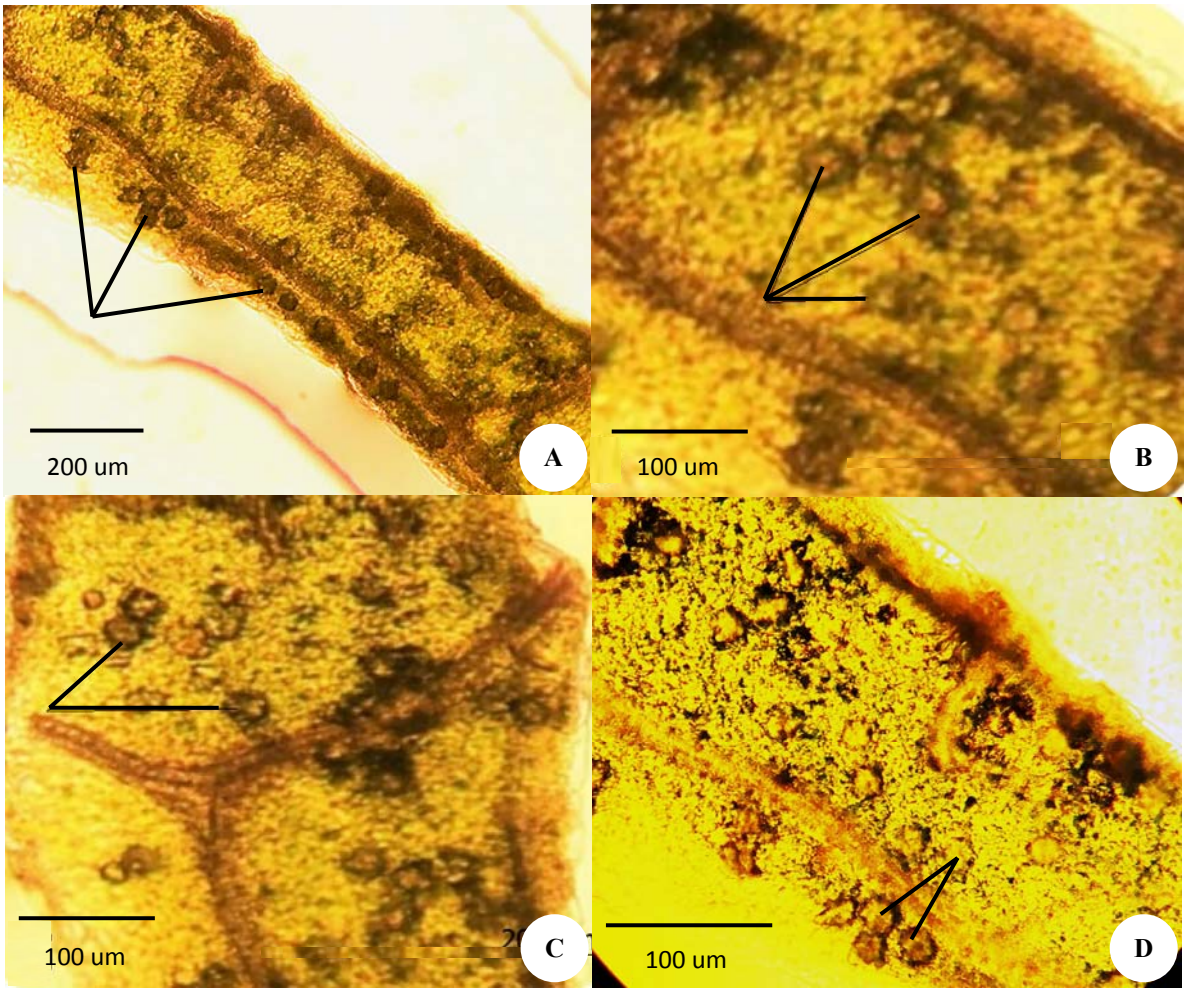


Figure 5. Sample spots treated with Wagner reagent; the section in *Physalis divaricata* leaves at fruiting stage detail is shown by cross arrows, under light microscopy. A. Leaf blade section stained with Wagner reagent, B. Detail of the mesophyll and several idioblasts in the parenchyma, C, D. Detail of other parts of the mesophyll and several idioblasts in the parenchyma

stage. Accumulation of alkaloids in the vegetative parts start to decline with plant enters to the reproductive phase demonstrated by flowering and then fruiting. These results were firmly supported by investigation of *PMT* expression. Expression ratio of *PMT* in the vegetative parts showed a peak at end of vegetative stage.

Alkaloids generally collect in specific cell types owing to their cytotoxicity and probable role in plant defense responses. They biosynthetic pathways are associated with different cell types (Ziegler and Facchini 2008). Tropane alkaloids are one of the main classes of plant-derived anticholinergic compounds, such as scopolamine and hyoscyamine, which occur in several *Hyoscyamus*, *Datura*, and *Atropa* species. Calystegines that function as selective glucosidase inhibitors are more widely spread than scopolamine and hyoscyamine in the plant kingdom and occur mainly in *Solanaceae* (Drager 2004).

Five *PMT*-encoding genes have been known in *N. tabacum* species: *NtPMT1*, *NtPMT2*, NtPMT3, *NtPMT4*, and *NtPMT1a*. In many studies, the rapid induction of *PMT* encoding genes has been documented by wounding, herbivore attack, or exogenous applications of JA or MeJA in roots (Hara et al. 2000). *H6H* and *PMT*, catalyzing the last and first steps, respectively, in the tropane alkaloid scopolamine biosynthesis, localize to the pericycle in the roots of *A. belladonna* and *Hyoscyamus muticus* (Suzuki et al. 1999). This gene also catalyzes the first step in biosynthesis of nicotine and localizes to the outer cortex, endodermis, and xylem in *Nicotiana sylvestris* (Shoji et al. 2002).

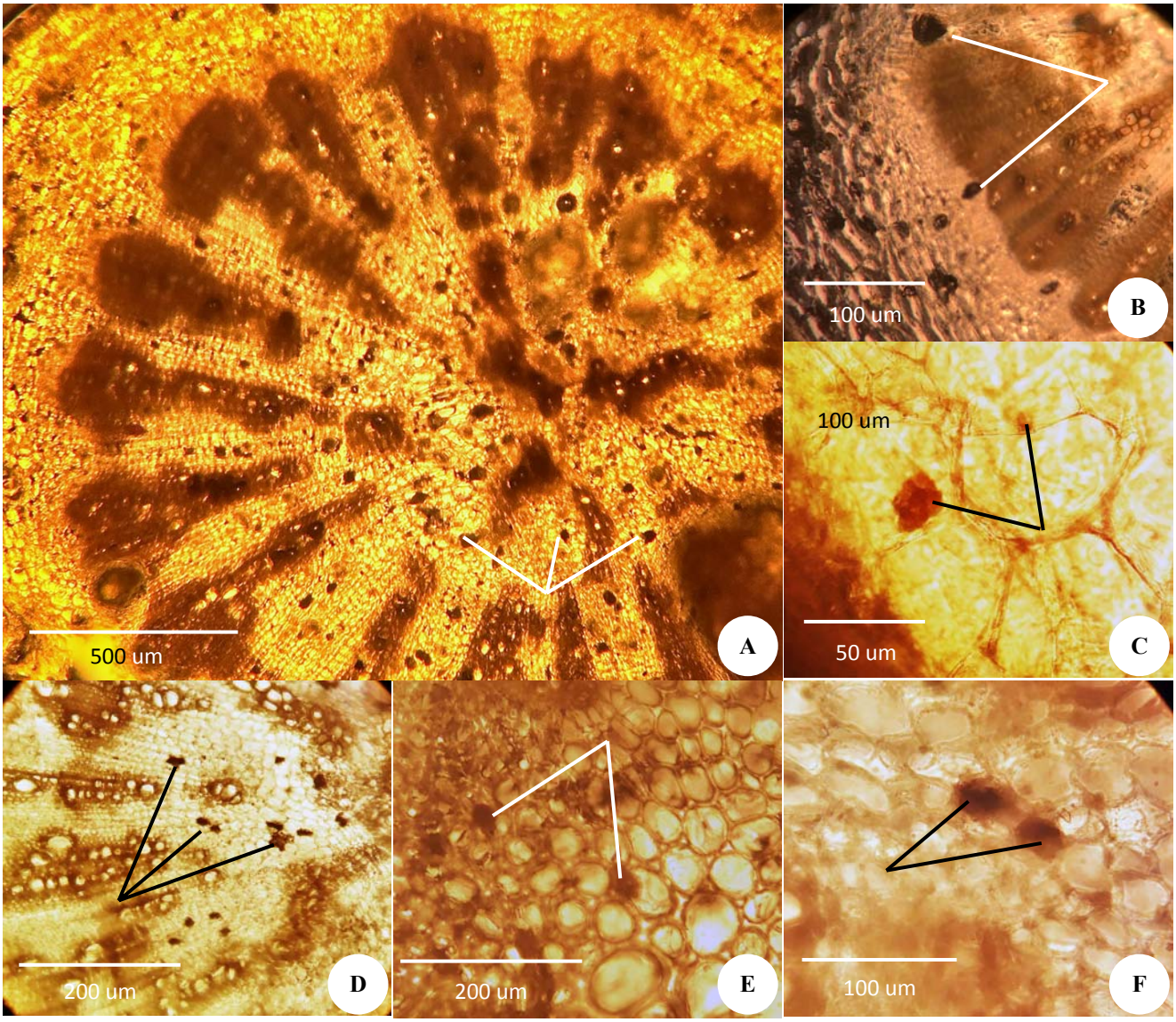


Figure 6. Sample spots treated with Wagner reagent; the section in *Physalis divaricata* roots at the late vegetative stage is shown by cross arrows, under light microscopy. A. Root section stained with Wagner reagent, B. Detail of the cortical parenchyma, C. Detail of the cortical parenchyma tissue and one parenchyma cell, D. Detail of the pith and rey parenchyma cells. E) Detail of the pith parenchyma tissue and several parenchyma cells. F) Detail of the pith parenchyma tissue and two parenchyma cells

In a similar study, Sachan and Falcone (2002) indicated that during the course of studying the regulation of alkaloid biosynthesis in *Nicotiana*, evidence was obtained for low *PMT* expression levels in leaves, which are abruptly upregulated over wounding. This upregulated expression was found to be highly localized and short-lived. On the other hand, expression was observed in leaves when they were wounded mechanically. Little evidence of long-distance signaling could be found as the expression was highly localized around the wound site. The results of Sachan and Falcone (2002) confirm the present findings. The relative *PMT* expression level in samples at late vegetative stage had a significant increase compared to the samples at the fruiting stage. However, *PMT* expression level in both root and leaf samples had similar change.

Facchini et al. (1996) reported the effect of the alkaloids morphine concentration, confirmed in poppy cultivation. Their study showed that adding botrytis pathogenic stimuli to the poppy medium causes accumulation of sanguinarine (Facchini and Park 2003).

The spots created by Wagner reagent on roots' section at the fruiting stage are observed only in a few cells with less density than the late vegetative stage (Figure 7.A, B) and in the scattered mesophyll cells of leaves; at the fruiting stage, the density of spots is less (Figure 5.D) or the spots are seen less trimmed (Figures 5.A, B, C). Of course, the results are in line with the results of PMT gene expression.

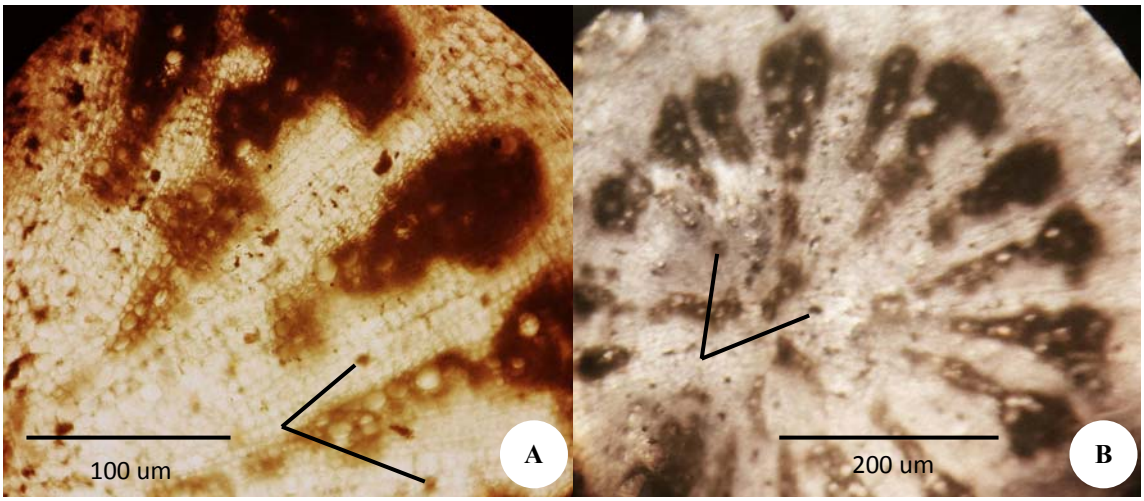


Figure 7. Sample spots treated with Wagner reagent; the section in *Physalis divaricata* roots at fruiting stage is shown by cross arrows, under light microscopy. A. Root section stained with Wagner reagent, B. Detail of the pith and rey parenchyma cells.

In conclusion and according to tropane alkaloid biosynthesis pathway and *PMT* gene expression, it seems that *PMT* gene in the root of *Ph. divaricata* at late vegetative is extremely active. For further confirmation of tropane alkaloid biosynthesis pathway, it is required to determine the amounts of other tropinones in root and leaves at these two stages and compare these data.

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Short Communication: New record of a primitive brachiopod, *Lingula* sp. in Lubuk Damar, Indonesia

ANANINGTYAS S. DARMARINI[1,♥], YUSLI WARDIATNO[2,♥♥], TRI PRARTONO[3], KADARWAN SOEWARDI[2]

[1]Doctoral Program in Aquatic Resources Management, Graduate School, Institut Pertanian Bogor. Jl. Raya Darmaga, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. ♥email: ananingtyas.s@gmail.com

[2]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Institut Pertanian Bogor. Jl. Raya Darmaga, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8622932, ♥♥email: yusli@ipb.ac.id

[3]Department of Marine Sciences and Technology, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jalan Raya Darmaga, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia.

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Abstract. *Darmarini AS, Wardiatno Y, Prartono T, Soewardi K. 2017. Short Communication: New record of primitive brachiopod,* Lingula *sp. in mangrove ecosystem of Lubuk Damar, Aceh Tamiang, Indonesia. Biodiversitas 18: 1438-1444.*. This study reports the occurrence of the primitive brachiopod, *Lingula* in mangrove ecosystem as a new record in Indonesia. Specimens were collected from the mangrove sediment at a depth of between the sediment surface and 20 cm beneath. . *Lingula* has a glossy shell and oval-ovate shell shape. The color of shell is brownish or greenish. In posterior a point where pedicle occurs is found. The pedicle is a prolongation of the body like an anchor, with varying length, rough, hard and striped texture. Pedicle has soft transparent layer and the edges are covered by the substrate. The inside body of *Lingula*, there is a lophophore, which is the characteristic of this species The total length of the brachiopod ranges from 3-122 mm. The texture of habitat consists of 39.67% sand, 50.95% silt, and 11.45% clay.

Keywords: Benthos, living fossil, Malacca Strait, marine biodiversity, soft sediment

INTRODUCTION

As an ecosystem, mangrove has been revealed to play ecological function as the center of marine biodiversity by supplying habitat for various organisms (Robertsen and Alongi 1992; Field et al. 1998; Wei-dong et al. 2003; Lee et al. 2014). For Indonesia, recent studies related to marine biodiversity have been focused mainly on the occurrence of edible marine invertebrate species, such as lobsters (e.g. Wardiatno et al. 2016a,b,c; Wahyudin et al. 2016; 2017a,b), hippoid crabs (e.g. Wardiatno 2015a,b; Mashar et al. 2014, 2015; Ardika et al. 2015). Information on the occurrence and distribution of non-edible marine organisms such as brachiopods are limited.

Brachiopoda are components significant of the early Cambrian marine faunas (Carlson 2016) and was initially included in the class of mollusk, which was then at the end of the 19 century was removed from the mollusk group (Emig 2008). Hutchins et al. (2003) mention grouping this fauna an exclusively marine group of lophophorate animals. They are called “inarticulated” because their shells lack articulation. Nonarticulate lampshells are also known as inarticulated brachiopods.

Brachiopoda is one phylum of Lophophorates (Pechenik, 2010) and the lophophore, an essential organ of the Brachiopoda (Zhang et al. 2003). Grouping Lophophorates be based animals take possession of a lophophore (Carlson 2016) and as it related to a way of eating that is using lophophore (Emig 1992; Samanta et al. 2014a, b; Carlson 2016), but is morphologically similar to the mollusk with two shells (Sundaram and Deshmukh 2011; Samanta et al. 2014). Aditionally, the brachiopoda are differentiated from the mollusk because their shells have anteroposterior symmetry. One common genus of brachiopods is *Lingula* and received appellation as a living fossil (Mudjiono and Suparman 1992; Emig 2003). According to Emig (1997), various types of inarticulate brachiopods spread over coastal waters at bathyal zone until abyssal and are found many in the intertidal zone.

The area of study in Lubuk Damar Aceh Tamiang is located along the coast of Malacca Strait with about ±3 km beach length. The dominant vegetation is real mangrove vegetation consisting of several species such as *Sonneratia* sp., *Bruguiera sexangula*, *Scyphiphora hydrophyllaceae*, *Avicennia alba*, and *Rizophora stylosa*. The study was aimed to report the occurrence of *Lingula* sp. in a mangrove ecosystem in Province Aceh as a new record of Indonesia.

MATERIALS AND METHODS

Lingula sp. specimens were collected from mangrove ecosystem in Lubuk Damar, Aceh Tamiang, Province Aceh, Indonesia (Figure 1) on January 2017 during low tide at three stations. Sampling was carried out by using core with the area 0.0133 m^2 at 300 sampling points and 74 individuals were obtained from 0-20 cm sediment depth.

Collected specimens were stored in 70% ethanol after being fixed in 10% formaldehyde and sorting. Laboratory works were conducted in Biomicro Laboratory, Department of Aquatic Resources Management, Faculty of Fisheries and Marine, Bogor Agricultural University, West Java, Indonesia. Substrate analysis was conducted in Soil Laboratory, Faculty of Agriculture, University of Syiah Kuala, Banda Aceh, Indonesia. Morphological picture of the specimens was taken by the dinolite Pro 2 and macro camera of Canon Power Shot D30. Description of the specimens was based on morphology descriptions by Emig (2003), Lee and Campbell (1987), and Yugan et al. (1982).

RESULTS AND DISCUSSION

Systematics

Phylum Brachiopoda Duméril 1806; subphylum linguliformea Williams, Carlson, Brunton, Holmer & Popov, 1996; Class Lingulata Gorjansky and Popov 1985; Order Lingulida Waagen 1885; Superfamily Linguladea Menke 1828; Family Lingulidae Menke 1828; Genus *Lingula* Bruguière, 1797; Species *Lingula* sp. (Lee and Campbell 1987) (see Figure 2).

Description and biology

Shell shape is oval-ovate with a glossy shell. Its different size on dorsal and ventral valve *Lingula* (Figure 2.D) is representing the dissimilar color of the shells, brownish or greenish (Figure 2.A, D, H). In posterior a point where pedicle occurs is found (Figure 2.G). The pedicle is a prolongation of the body like anchor. Pedicle length varies with striped texture, rough and hard, the colors a light brown to creamy. Pedicle has a sort of a transparent layer (cuticle) and the top part wrapped by the substrate. The sample was found to have two types of pedicle that are stubby and elongated.

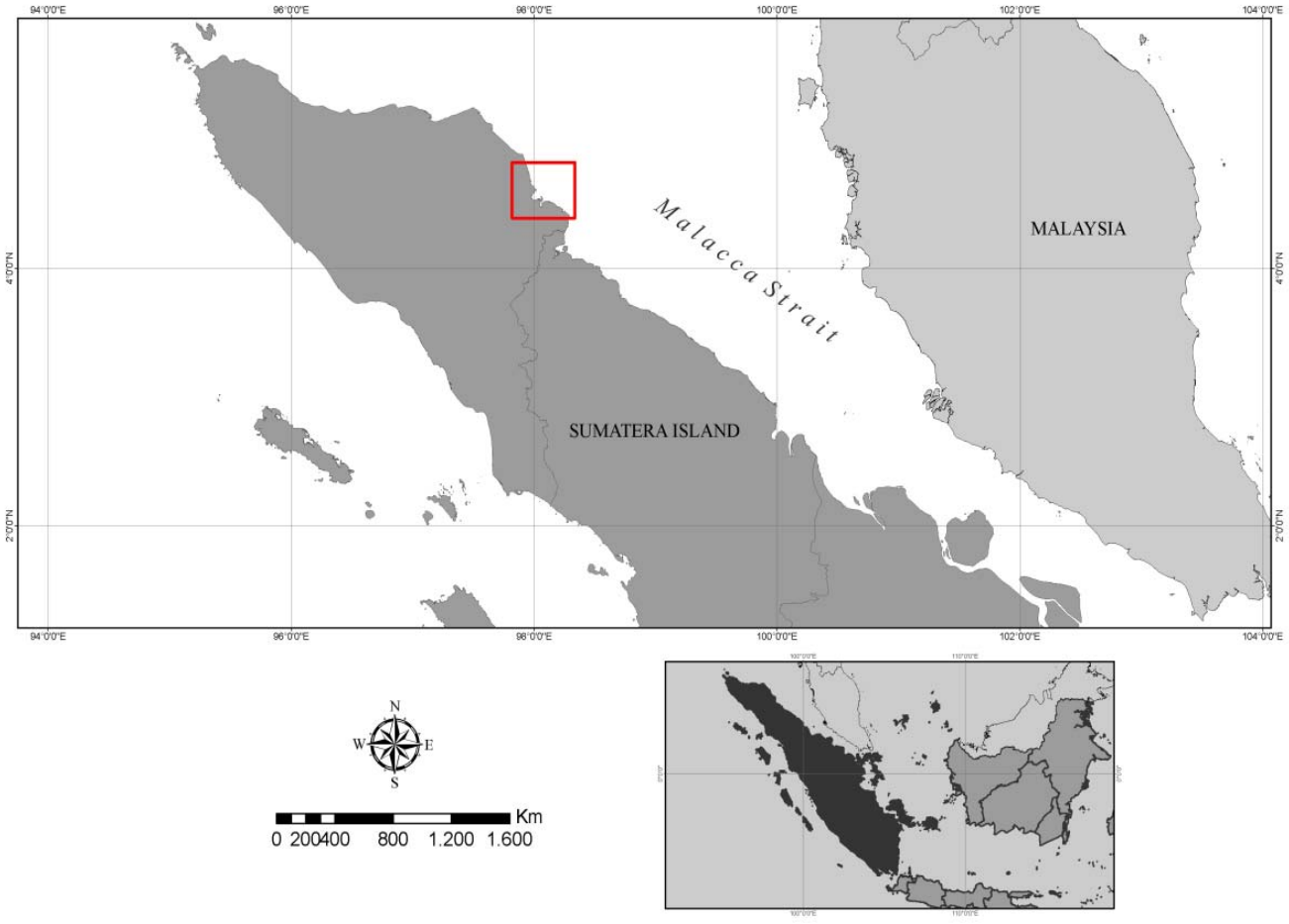


Figure 1. Research location showing the collection site of the brachiopod in the mangrove ecosystem of Lubuk Damar, Aceh Tamiang, Province Aceh-Indonesia. The red square indicates the sampling site

In each valve has a mantle with chaeta throughout shell (Figure 2.A, H). On the anterior have chaeta a longer, functioning as an inhalant and exhalant pseudo siphon (Figure 2.H). In the sampling locations small size *Lingula* was found too (Figure 2.C) and chetae on juvenile is evident. The inside of the body is lophophore which is the distinctiveness of this species (Figure 2.B, E). In this study, 74 individuals of *Lingula* sp. were obtained, and the total length, shell length, width of a shell and pedicle length ranged from 1-122 mm, 1-39 mm, 0.2-19 mm and 2-99 mm, respectively. While the weight ranged between 0.0001-6.543 g.

Shell

The size of *Lingula* sp. in the mangrove ecosystem of Lubuk Damar, Aceh was very diverse and bigger (3 mm-122 mm), if compared with the size of *Lingula* found in other studies, e.g. 22.5 mm-38.5 mm (Emig 1984), 45 mm-58 mm (Sundaram and Deshmukh 2011), 27.5 mm (Hutchins et al. 2003), and 24.84 ± 2.98 mm (Printrakoon et al. 2014). Shells of *Lingula* discovered in the mangrove ecosystem mostly had a light to dark green color (Figure 2.A, H) as in *Lingula anatina* reported from Subarnarekha estuaries, India (Mitra and Pattanayak 2013), but few specimens showed light brown to greenish color; similar to those found in New Caledonia (Bitner 2010) and in Ratnagiri, Maharashtra, India (Sundaram and Deshmukh 2011). Shell color distinction is probably an indication of species and environmental conditions. Observations also showed that the shell consists of two distinct parts. The anterior valve widens and posteriorly narrows which culminate in the appearance of the pedicle. The shape of shell reveal is oblong-elongate, (Figure 2.A, H) and smooth surface and *Lingula* sp. shells are thin and fragile, the shell divide by a dorsal and ventral valve. The ventral valve is more taper than dorsal on the posterior part. Clarke and Wheeler (1915) found that the brachiopod fell into two chemically distinct groups; one is calcareous and the other is highly phosphatic.

Emig (1979, 1984) and Samantha et al. (2014) explored the characteristics of the shell of *Lingula anatina* in West Bengal-Odisha coast, India, and they found their specimens had two dissimilar valves which emerge to be oblong-ovate, elongate, bilaterally symmetrical, endowed with parallel lateral margins, sharp beaks, and straight edges. Emig (2003) pursuant to genus *Lingula* characteristic from Sumatra is shell lengthen oval or subrectangular in broad outline, lateral margins subparallel, anterior margin amply spherical to lank.

Surface of the two valves was mostly found to be smooth but indicating growing process lines (Emig 1978, 1979; Hutchins et al. 2003). The outer of shell depicts flimsy growth lines (Figure 2.H). These shells have a ventral and a dorsal valve. Anterior valves have setae, two inhalant and one exhalant pseudo siphon (Emig 2003). *Lingula* has three low-siphonal ribs extend from beak to anterior margin of the shell (Emig 1979; Hutchins et al. 2003). Inhalant-exhalant pseudosiphon is shown in Figure 2H.

Pedicle

All of the *Lingula* sp. in the study site had longer pedicle size (1-2 times and more) than the shell length. The pedicle length on location is 2 mm-99 mm. Some juvenile sample length of the pedicle more than 3 times of the shell and some few completely lost of a pedicle. The length of pedicle size was found to be one and a half until 2 times the shell length similar (Emig 1978; 1979). The color of the pedicle is creamy to whitish (Figure 2.F) and substantially similar as described in Emig (1984). The pedicle described is fleshy in *L. anatina* (Emig 1979; 1984).

During the observation, some *Lingula* sp. position showed the pedicle on the bottom and shell upstairs in their habitat. This evidence is similar to the common findings that *Lingula* is a vertically digging brachiopod in soft sediments with shell position at the top of their burrows (Emig 1997, 2003; Hutchins et al. 2003). Yugan et al. (1991) mentioned that the pedicle of extant lingulids was composed of a deltoidal section banded to the pedicle valve, a cylindrical primary trunk, and a desolate bulb-like end anchored by a mucous secretion to the bottom of the burrow. The isolated bulb of the pedicle (foot like base of the organism) is firmly anchored into the substrate at the bottom of the burrow and is bestead by a mass of sand and organic particles take hold of together by sticky mucus. The pedicle of brachiopods is described attachment of generally accomplished tool of a stalk (Pechenik 2010), a stalk-like structure appearing between the two valves of most brachiopod, make easier attachment to a tough substrate but not for hard substrate (Carlson 2016).

Lophophore

Lophophore is a characteristic of *Lingula* (Figure 2.E, B), and is described as muscle endowed spiral feeding structure of ciliated tentacles around the mouth (Samantha et al. (2014a). The position of lophophore is on the anterior valve and/or on the open out shell. The shape is like a snail's dwelling, in white and has a tentacle at the edges (Figure 3.A, B). It is composed of two, right and left (Figure 2.E, 3.A). Lophophore consists of two long arms the circle to form a spiral (Figure 3.A), the detail part of lophophore (Figure 3.B). Emig (1976) showed lophophore as oval shaped round the mouth with a single line of tentacles, therefore the functions may be associated to feeding, respiratory, and protection. Emig (1976, 1992) stated that the lophophore had increasingly advantage than just being considered as a feeding mechanism absorbing alimentary particles and soluble nutrients. Its primary function is a water flow model that also acts as a respiratory organ.

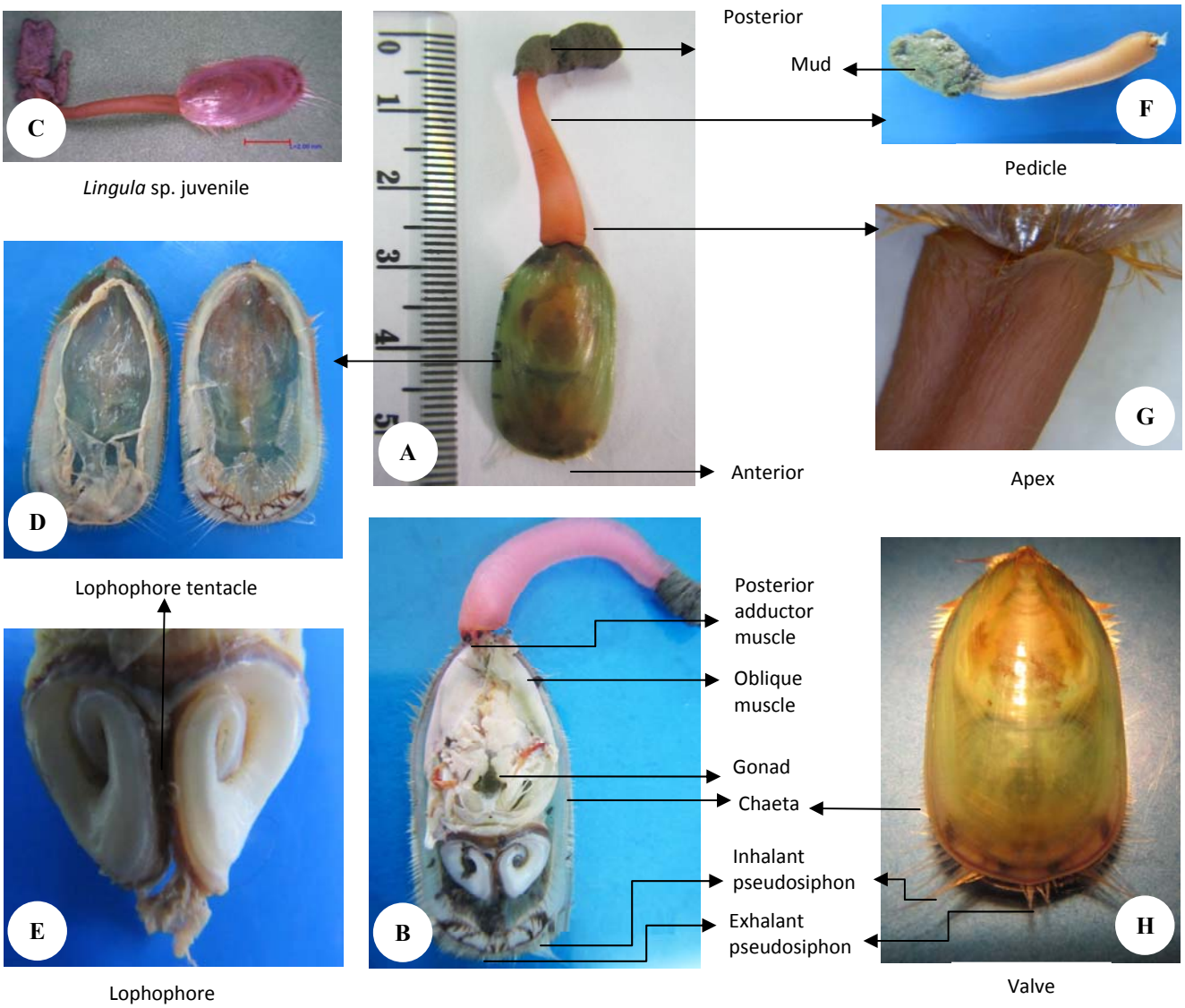
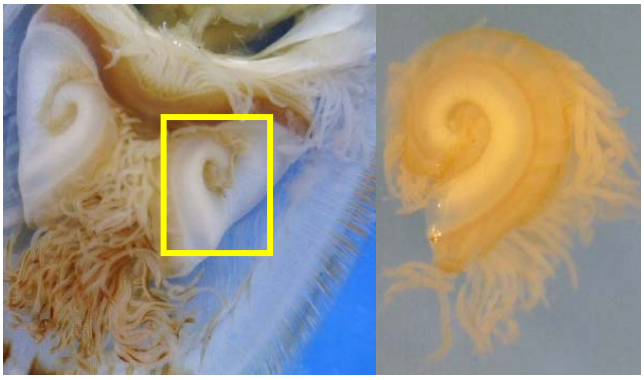


Figure 2. Morphology and anatomy of *Lingula* sp. collected from mangrove ecosystem in Lubuk Damar, Aceh Tamiang, Province Aceh, Indonesia. A. Body of *Lingula*, B. *Lingula* dissection, C. Juvenile, D. Valve, E. Lophophore, F. Pedicle, G. Apex, H. Shell.



A **B**

Figure 3. Lophophore. A. Lophophore coiling, B. The coiling part of lophophore

The lophophore is an organ of two arms that endure ciliated tentacles get used to filter feeding and respiration (Carlson 2016). Lophophore is characterized by the presence of muscle crease helical with ciliated tentacles (Samanta et al. 2014a), the function of cilia (tiny hair-like structures) produce a unidirectional current of water through the mantle cavity, extracting oxygen and food particles and transporting them to the mouth (Carlson 2016). According to LaBarbera (1981) the lophophore establishes a perpetual, laminar water flow, induced by rows of lateral cilia on the tentacles. Their movement draws water through the tight cleft between tentacles. There is no internal mixing and no recirculation of filtered exhalant current. Gametes and cesspool products are discarded from the mantle cavity by the same process (Carlson 2016). The spirolophe is characteristic of all alive inarticulate (Emig 1992; Hutchins et al. 2003).

Habitat

Observations conducted in areas of study acquired *Lingula* sp. of the distance from the highest tide-lowest tide was 0-500 m, from the mangrove canopy area until the beach. Substrate texture in location was muddy with 39.67% sand, 50.95% silt and 11.45% clay. Habitat of *Lingula* was reported with evenly density present on intertidal areas (Emig and Hammod 1981; Bitner et al. 2012; Samanta et al. 2014), which is partially exposed in middle intertidal muddy area during low tide. Superfamily Linguloidea was reported to share an infaunal way of life (Emig1984, 2003), and were found in sandy loam regions (Samanta et al. 2015). The variance found in this so that could be found on the substrate of silt up to coral (Savazzi 1991), in shallow waters by a depth of 20 m or more (Samanta et al. 2014).

The similar condition to the habitat of *Lingula* sp. in Subarnarekha India is that the substrate in most cases was soft muddy area, but black soil (decomposed) and sand mixed mud were believed to be preferential for the living. Juvenile's bed was established in fine soft mud (Mitra and Pattanayak 2013). On Fangchenggang mangrove in China *Lingula* occurred from areas with fine sand (>68%) with sediment particles on sandy loam and loamy sand (Printrakoon et al. 2014). In accordance with Hutchins et al. (2003), Lingulidae is exclusively infaunal (living in the sediment at the bottom of the ocean) in soft substrates from the intertidal zone.

In the study area, some other macrozoobenthos were also discovered, such as polychaetes (*Glycera, Maldane, Pista, Pistella, Sternapsis, Onuphis*, etc.), sipunculans, pelecypods (*Tellina, Siliqua, Anadara, Gastrana*, etc.), gastropods (*Solen, Cepridula, Dentalium*), crustaceans (*Pagurus, Uca, Scylla, Penaeus, Alpheus*), and many more (Darmarini 2017; unpublished data). This finding is similar with Mitra and Pattanayak (2013). There is probably indirect and direct relationships in habitat among those benthic animals with the brachiopod. Goto (2014) revealed a symbiotic relationship between the *Lingula anatina* and *Koreamya setouchiensis*. This mollusk embedded on the anterior part of the shell, perhaps to make use of water flow created by the host for filter feeding, estimation this bivalve may prefer to use exhalant by *Lingula*.

Feeding ecology and diet

Feeding habit of *Lingula* based on gut content analysis indicated the presence flake of mangrove leaves, planktonic materials, and detritus in the gut (Samanta et al. 2015). According to Hutchins et al. (2003) the food items of brachiopods include phytoplankton as diatom, superficial meiobenthos, colloidal organic matter. Therefore, the brachiopod can be included in suspension feeder (Emig 1984; Hutchins et al. 2003; Samanta et al. 2015; Temereva and Tsitrin 2015).

Distribution

Brachiopods live in all oceans (Emig 1997; Emig et al. 2013), and are greatly disparted geographically at depths ranging from intertidal down to more than 5000 m (Emig et al. 2013). They are is distributed considerably in Indo-West Pacific area (Emig 1997). *Lingula* has a widely distribution in Asia, Australia, Europe, Africa (Williams et al. 2000; Mitra and Pattanayak 2013), such as in Ambon, Indonesia (Jackson and Stiasny 1937), Singapore Island (Chuang 1961), Hokkaido Japan (Hayasaka and Hatai 1956), Mutsu Bay, Northern Japan (Emig 1984), Korea (Park et al. 2000; Hong et al. 2007), mangrove area in Ratnagiri Maharashtra, India (Sundaram and Deshmuk 2011), estuary Subarnarekha (Mitra and Pattanayak 2013), Thailand (Printrakoon and Kamlung-ek 2013), north-east coast of India (Samanta et al. 2014), China seas (Ricardson et al. 1989), Fangchenggang mangrove, China (Printrakoon et al. 2014), Nhatrang Bay, Vietnam (Temereva and Tsitrin 2015), Kaneohe Bay, Oahu, Hawaii, USA (Hunter et al. 2008), Atlantic (Williams et al 2000), Spain (Marques-Aliaga et al. 1999), France (Emig et al. 2007), South Germany (Bitner et al. 2009), New Zealand (Allan. 1936), Australia (Kenchington and Hammod 1977), New Caledonia, New Zealand (Bitner 2010), Madagascar (Emig 1977), Western Indian Ocean (Bitner and Logan 2016).

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Monthly range of adolescent orangutans (*Pongo pygmaeus wurmbii*) based on fruit availability in Tuanan Orangutan Research Station, Central Kalimantan, Indonesia

FAJAR SAPUTRA[1,3,♥], DYAH PERWITASARI-FARAJALLAH[2], SRI SUCI UTAMI-ATMOKO[3], TOMI ARIYANTO[3], MARIA A. VAN NOORDWIJK[4]

[1]Program of Animal Sciences, Department of Biology, Faculty of Mathematic and Natural Sciences, Institut Pertanian Bogor, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8622833, ♥email: fajarsaputra56@gmail.com

[2]Primate Research Center, Institut Pertanian Bogor; Department of Biology, Faculty of Mathematic and Natural Sciences, Institut Pertanian Bogor. Bogor 16680, West Java, Indonesia

[3]Primate Research Center, Universitas Nasional; Faculty of Biology, Universitas Nasional. Jakarta 12520, Indonesia

[4] Anthropological Institute and Museum, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

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Abstract. *Setyawan. 2017. Monthly range of adolescent orangutans (*Pongo pygmaeus wurmbii*) based on fruit availability in Tuanan Orangutan Research Station, Central Kalimantan, Indonesia. Biodiversitas 18: 1445-1452.* Adolescent orangutans become competitors with mothers supporting newborn infants. Thus, adolescent orangutans must coordinate with other orangutans in order to find fruit. How adolescent orangutans respond is reflected in the size and utilization of their home range area. The aims of our research were to determine (i) fruit availability, as an important component of orangutan diet, (ii) and the effect of fruit availability on home range use by adolescent orangutan. This research was conducted from August 2013 to July 2014 in Tuanan Orangutan Research Station, Central Kalimantan. The fruit trail method was used to estimate abundance of fruits (both from trees and lianas).Fallen fruit on census trails was assessed once per month, while focal animal sampling was used to estimate the home range by tagging a GPS point every 30 minutes during the tracking of an orangutan. In total, 62 tree and 15 liana species included in the known orangutan diet were fruiting during this study period. The highest period of fruit abundance for orangutan food occurred in November until January. Adolescent orangutans responded by increasing their monthly ranging area during this time of high fruit availability and decreased their range when fruit availability was low. Adolescent orangutans often used areas in their monthly range that had a high to medium abundance of fruit trees. When the number of trees bearing fruit decreased (low period), adolescent orangutans changed their monthly range to areas that consisted of medium to high abundance of liana fruit. Their ranging strategy optimized use of low quality habitat in order to survive in degraded forests.

Keywords: Adolescent orangutans, home range, fruit, tree, liana

INTRODUCTION

Bornean orangutans (*Pongo pgymaeus*) are one of the most critically endangered primates in the world (Ancrenaz et al. 2016). Bornean orangutans are widely spread over the island, mainly inhabiting peat-swamp and lowland forest. Bornean orangutans still have a larger population size than Sumatran orangutans, however, the number of Bornean orangutans is declining faster than that of Sumatran orangutans (Wich et al. 2008). Currently, deforestation is the greatest threat to orangutan populations. In the period from 1980 to mid-1990s, Indonesian forests suffered from conversion into agricultural, plantation, and residential land; forest fires; and unsustainable exploitation of forest resources (Soehartono et al. 2007). Moreover, human-orangutan conflict and killings occur in Borneo and is one of the serious threats to conservation of this species (Wich et al. 2008; Abram et al. 2015).

Pongo pygmaeus wurmbii is one of the three currently recognized subspecies of Bornean orangutan. This taxon is found in West Kalimantan (Indonesia) from the south part of the Kapuas River to the eastern Barito River in Central Kalimantan (with a few individuals in South Kalimantan province) (Goossens et al. 2009). This taxon has the largest population of the three Bornean orangutan subspecies, with 34,975 individuals in total (Wich et al. 2008).

Orangutans are frugivorous primates, meaning that a large proportion of their diet is composed of fruit (Galdikas 1988). Orangutans may also consume leaves, flowers, pith, cambium, soil, insects, small vertebrates (such as gray tree rat and slow loris) and (bird-) eggs. Orangutan diet is influenced by ecological factors (the habitat type and the habitat quality) and by orangutan status (wild or rehabilitant) (Russon et al. 2009). To fulfill their food and reproduction needs, orangutans range across their habitat (Singleton et al. 2009).

The accumulation of daily ranges results in a "home range". The home range of an orangutan is influenced by age, sex, and habitat (Singleton et al. 2009; Singleton and van Schaik 2001). Male orangutans tend to disperse from their birth area, while females are philopatric or tend to settle as close as possible to (and often overlapping with) their birth area (Arora et al. 2012; van Noordwijk et al. 2012). Adolescent female orangutans will adjust their

ranging in accordance with adult female orangutans already in the area. On the other hand, a male orangutan with broad cheek pads (an adult or flanged male) will permanently settle outside of his birth area. A male orangutan without cheek pads (an adolescent or unflanged male), sometimes stays in his birth area or returns to his birth area after ranging in another place (Morrogh-Bernard et al. 2011).

Fruit availability varies over time. Orangutans respond to this variable fruit availability by changing their ranging strategy and the composition of their food (Knott et al. 1998; Morrogh-Bernard et al. 2009). Independent adolescent orangutans will become competitors with mothers supporting newborn infants. This condition forces adolescent orangutan to adjust their ranging in response to other orangutans in order to find enough fruit. Orangutan responses to fruit availability are not only reflected in the size of their range, but also in how the home range is used (Wartmann et al. 2010).

The Tuanan Orangutan Research Station (TORS) is in a peat swamp forest inhabited by orangutans. In the past, this area suffered 'selective' commercial logging activity by companies as well as illegal logging by local communities (van Schaik et al. 2005). Thus, a lot of gaps in the forest canopy were made, which caused the growth of pioneer plants, such as lianas. Another ongoing impact from logging in TORS is the continued loss of water from the peat through canals which were dug and used to transport timber towards the Kapuas River. All these conditions can change the pattern of food plant production, which may affect orangutan home range use.

Currently, there is a lack of information about the home ranges of adolescent orangutans and their response to fruit availability. Therefore, the aims of the research reported in this paper were to identify (i) fruit availability from trees and lianas able to be used by orangutan as food, and (ii) the home range use by adolescent orangutans, based on fruit availability.

MATERIALS AND METHODS

Study area

The research was conducted from August 2013 to July 2014 in Tuanan Orangutan Research Station (TORS), Tumbang Mangkutub Village, Mentangai District, Central Kalimantan, Indonesia. The study area is a degraded peat swamp forest consisting of about 1,003 ha which is located approximately 0.4-1.5 km east of the Kapuas River. TORS is part of the Mawas Conservation Area managed by the Borneo Orangutan Survival Foundation (BOSF). The study area supports a high density of orangutans of ca. 4.25 individuals per km^2 (van Schaik et al. 2005).

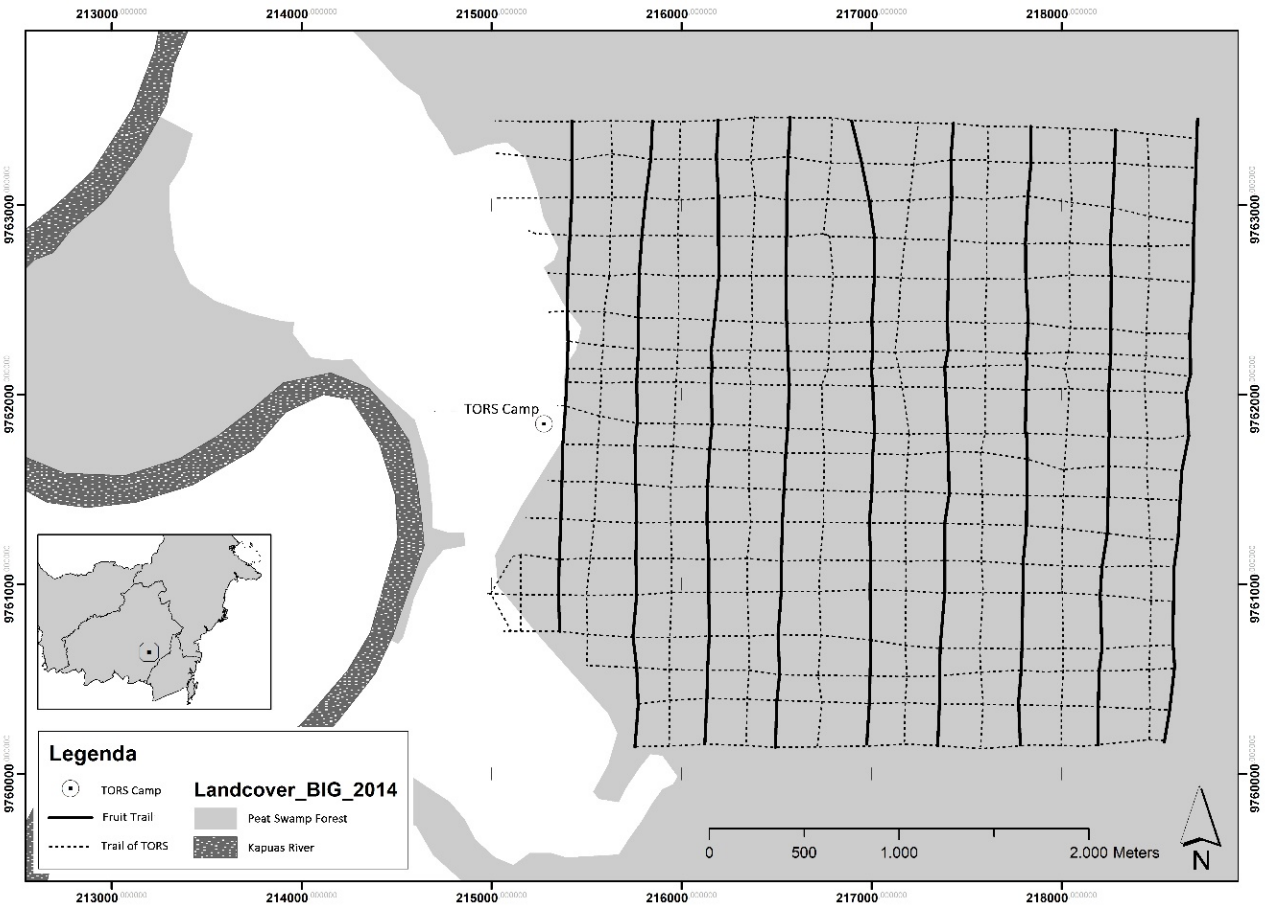


Figure 1. Fruit trail design (black lines) in Tuanan Orangutan Research Station, Central Kalimantan, Indonesia (2.151°S; 114.374° E).

Table 1. Adolescent orangutans as research objects

Name	Gender	Age	Independence level	Total number of waypoints (monthly data)
Milo	Female	13 years	Independent	745 (n = 5: 91, 200, 106, 144, 206)
Jip	Male	8 years	Dependent	469 (n = 4: 153, 114, 116, 86)
Ipsy	Female	7 years	Dependent	438 (n = 3: 113, 139, 186)

Study objects

Research objects were fruiting plants and adolescent Bornean orangutans. The data for fruiting plants were divided into two groups: lianas and trees. Three adolescent Bornean orangutans were observed: Jip, Milo and Ipsy. Jip and Milo are cousins (their mothers are maternal sisters), but Ipsy is unrelated to them (Table 1). The number of waypoints collected for each adolescent orangutan varied, see Table 1.

Observation of fruit availability

The *fruit trail* method was used to estimate fruit availability on a monthly basis along a designated trail (van Schalk et al. 1995; Buij et al. 2002). Observations were made on nine north-to-south trails, with a length of 3.0-3.2 km each (28 km in total) and approximately 400 metres distance between trails (Figure 1). Trails were designated to get data that represented the entire TORS area. When a fruiting plant was found, its coordinates were determined using a GPS device, and we recorded its species (all fruit species were identified first by local assistants, later verified by a botanist: Ambriansyah).

Observation of adolescent orangutan travel distance and monthly range

The focal animal sampling method was used to assess how far the adolescent orangutans travel and their monthly range use. We followed and observed an orangutan from wake-up time until night-time nesting for 2-10 days and then repeated this after an interval >40 days. Data collection was recorded by a research team who have passed an Inter-Observer Reliability (IOR) test. We obtained geographic locations using a hand-held GPS unit by tagging a point every 30 minutes and every time the orangutan made a nest **(**van Schaik and van Noordwijk 2013).

Data analysis

Fruit abundance is defined as the number of trees and lianas bearing fruit per kilometer along a trail. Fruit abundance is used to determine the fluctuation in fruiting plants every month (van Schaik et al. 1995). The relationship between fruit abundance of trees and lianas was tested through Pearson's correlational tests. Spatial patterns of fruit abundance (only fruit used by orangutans as food) every month was analyzed by the Kriging interpolation technique (Mkrtchyan2004) using ArcGIS ver.9.3 software. Our intention was to categorize the area based on productivity level each month (Ariyanto 2015).The three productivity levels (fruit sources) were: (i) low (white colour: tree 0-13.50 fruits/km and liana 0-11.50 fruits/km); (ii) medium (Grey colour: tree 13.51-27.00 fruits/km and liana 11.51-23.00 fruit/km); (iii) high (black colour: tree 27.01-81.00 fruits/km and liana 23.01-69.00 fruit/km).

The travel distances of the adolescent orangutans for one day were approximated by summing the distances between all half-hour locations for one day of pursuit. The monthly range of the adolescent orangutans was analyzed with the Kernel Density Estimator (KDE) method, which is a non-parametric analytical method widely used to estimate home range (Laver and Kelly 2008). Monthly range analysis used ArcGIS ver.9.3 software with ABODE v.5 extension. Potential relationships between the number of waypoints and home range size were tested through Pearson's correlational tests.

Effect of fruit availability on the home range use by adolescent orangutans was analyzed by determining the overlaps between maps of fruiting plants and maps of home range. The results estimated the area of utilization as a proportion (%) of the orangutans' home ranges, based on the productivity levels of fruit (trees and lianas) each month.

RESULTS AND DISCUSSION

Fruit availability

Our research recorded 113 species of fruiting plants in total, with the number varying between 29 and 44 species each month. The number of fruiting plants that was known to be orangutan food was 68% of the total number of species found. During the research period, the fruiting plants consisted of 62 species of trees and 15 species of lianas. This number of species used by the orangutans for fruit food during our research is fewer than has been observed previously, in the years since 2003 (Russon et al. 2009). On the other hand, comparison with data from the peat swamp forests in Sebangau, reveal no real difference in the fruiting plant species used as orangutan food between these two sites (Harrison 2009; Harrison et al. 2010).

The pattern of fruit abundance in TORS observed in this research did not fluctuate widely throughout the year. The fruit was always available, with the highest peak from November to January. The Kapuas River provides nutrient inputs for TORS. Peat swamp forests are low in nutrients but periodically may receive additional nutrients from aerial precipitation and seasonal river flooding (Page et al. 199; Vogel et al. 2015).This condition contrasts with what is observed in dryland forests dominated by

Dipterocarpaceae. There, mast-fruiting events result in wide fluctuations (Cannon et al. 2007; Wich et al. 2011).

In our study, there were more fruiting trees per km than fruiting lianas in all months except for August 2013 (Figure 2). There was no significant correlation between fruit abundance of trees and lianas (Pearson's correlation coefficient = 0.371; p>0.05; n = 24). The highest period of fruit tree abundance occurred from November until January, while the lowest period occurred from April until August. On the other hand, lianas had a different pattern, with the highest period occurring twice, from November until January and April until June, but the lowest period occurring from February until March and August until October (Figure 2). Highest and lowest monthly periods of fruit abundance in this research period were not different from what has been observed in previous research at TORS (Vogel et al. 2009; Ariyanto2015; Vogel et al. 2015; Vogel et al. 2016). Fruiting plant abundance every month is influenced by rainfall. Fruiting plant abundance is higher in the rainy season than in the dry season (Ariyanto2015; Harrison et al. 2016). Even so, the fruit availability pattern that we observed differed between lianas and trees. Lianas were seen in fruit more often per year than trees. This resulted in a very fluctuating pattern of fruit availability in lianas.

The spatial distribution of fruit abundance between lianas and trees differed. Fruiting trees were distributed uniformly throughout the habitat, so that the area of high-productivity changed location every month. On the other hand, the liana's fruit abundance was clumped in the western and southern area of TORS; thus, the high-productivity liana habitats were concentrated there every month (Figure 3). This condition is influenced by the vegetation conditions in TORS. The southern part is closest to a village and was (and still is) used for illegal logging. In the past, massive destruction, forest fire, and road construction occurred in the western area. Both conditions caused gaps which affect liana growth (Schnitzer and Carson 2001; Letcher 2014). In TORS, only the southern part of study area has liana-dominated gaps that leave tangles of lianas on the forest floor even after canopy closure (Letcher 2014).Lianas are pioneer plants, so they grow easily in degraded forests. Previous research has shown that lianas in disturbed forests (succession) have a higher density than in undisturbed forests (Ding and Zang 2009; Addo-Fordjour et al. 2012; Ledo and Schnitzer 2014; Barry et al. 2015).

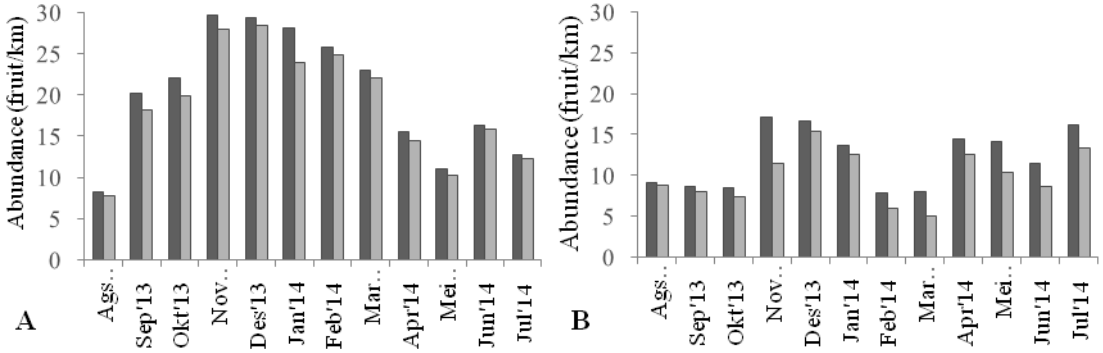


Figure 2. Abundance of fruiting trees (A) and lianas (B) each month in TORS (■ All fruit; ■ Fruit used by orangutan as food)

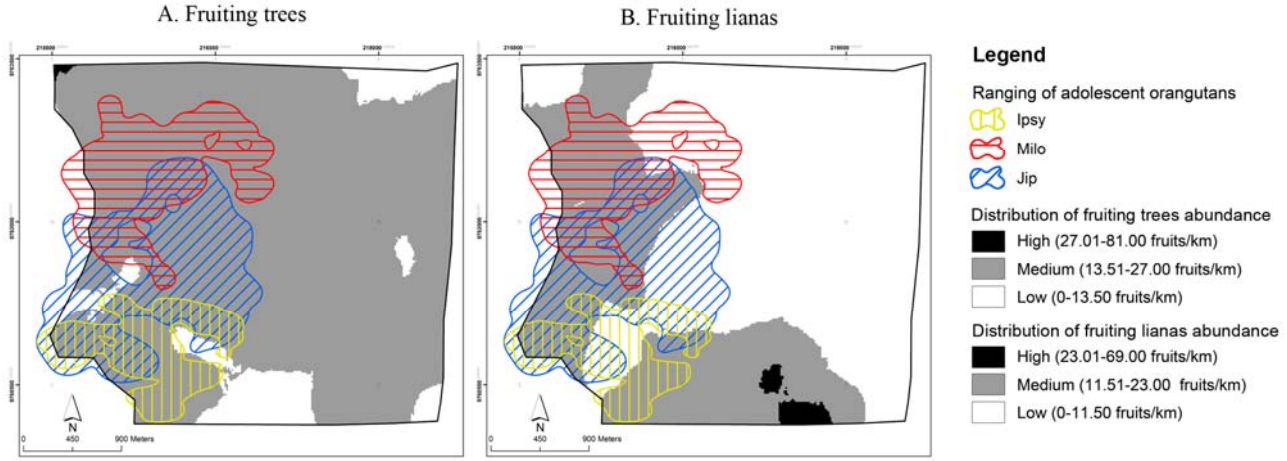


Figure 3. Overlap between home range and distribution of fruit plant abundance for one year (A. for trees and B for lianas). Note: The yellow, red, and blue depicted areas represent the calculated home ranges for the three orangutans Ipsy, Milo and Jip, respectively.

Lianas have an important role effect on forest dynamics, including succession; depressing tree regeneration (through competition that increases tree mortality) and altering tree allometry (Schnitzer and Bongers 2002; Alvarez-Cansino et al. 2015; Barry 2015; Dias 2016). Lianas also create bridges between trees that givea ccess to mobile arboreal animals, and provide food (leaves, flowers, and fruits) for animals like primates (Schnitzer and Bongers 2002; Setia 2009). The liana abundance distribution pattern, clumped in the western and southern area, thus provides benefits for orangutans in terms of greater accessibility via liana bridges and in providing a food source.

Adolescent orangutan home range and its response to fruiting plant availability

Travel distance is defined as the total distance an individual orangutan travels per day, from the moment it leaves its nest in the morning to the moment it builds the nest for the next night (Wartmann 2010). Home range is the area that is used by an orangutan to do its daily activities, such as feeding, reproduction, and parental care within a certain period (Galdikas1988). Jip and Ipsy were still always within 50m of their mothers, and as a result their traveling and ranging reflects their mothers' preferences. However, they were often feeding on other sources and made their own nests sometimes >10m away from their mothers'. The spatial analysis of travel distance in our study show that adolescent orangutans, carrying out daily activities in TORS traveled 936 m per day (on average; ±365; n = 64). Milo had the longest travel distance, followed by Jip and then Ipsy (Table 2). Meanwhile, home range for the study period showed that Jip had the largest home range, followed by Milo and finally Ipsy (Table 3). Jip's mother has a greater home range than other females (Wartmann et al. 2010; van Noordwijk et al. 2012). This results in Jip having a larger home range than Milo. Milo has a strongly overlapping home range with her mother (Mindy). Both of them range in the western part of TORS with about 178 ha (Mindy) and 169 ha (Milo). This shows phylopatry in female orangutans with a high intra-family tolerance (Wartmann et al. 2010; van Noordwijk et al. 2012). However, Ipsy's observed home range was smaller than for the other orangutans, but this may be because part of her home range is outside the TORS area and thus the number of waypoints sampled was small.

Monthly ranges varied in size every month (Table 3). There was no significant correlation between monthly range size and the number of waypoints (P-value > 0.05). This result showed that spatial analysis of monthly ranges using the KDE method was not affected by the number of points taken. KDE does not overestimate the home range (Seaman and Powell 1996; Laver and Kelly 2008; Wartmann et al. 2010). The results indicate that the monthly range for each month is fairly uniform and is influenced by food distribution.

The largest travel distance and monthly range for the adolescent orangutans occurred in December (Jip and Milo), and the smallest in March and August for Milo, and in May for Jip and Ipsy (Table2; Table 3andFigure 4). These conditions correlated with fruit availability in those months. According to Singleton et al. (2009), an orangutan's daily range is influenced by food sources. In our study, adolescent orangutans increased their travel distance and monthly range size in December due to high fruit availability. Orangutans did the opposite in March and May, as they decreased their travel distance and monthly range size due to low fruit availability. It is one of the orangutans' strategies to maintain energy balance (Knott et al. 1998; Morrogh-Bernard et al. 2009). Female orangutans of Jinak's family in TORS also use the same strategy to respond to the fruit availability (Wartmann et al. 2010). Generally, this strategy is used in a homogeneous habitat with a uniform gradation of fruit abundance (Singleton et al. 2009).

Table 2. Travel distance (metres) of adolescent orangutans

Adolescent orangutan	Travel distance								Mean
	Aug	Oct	Dec	Feb	Mar	May	Jun	Jul	
Jip		1152	1175			617	664		940.3
Milo	942	1365	1388		1082		703		1073.1
Ipsy				898		624		692	726.5

Table 3. Range area (hectares) within each month, and the home range across a whole year,for the threeadolescent orangutans

Adolescent orangutan	Monthly ranges								Home range (one year)
	Aug	Oct	Dec	Feb	Mar	May	Jun	Jul	
Jip	-	106.4	165.8	-	-	45.09	84.92	-	244.8
Milo	53.8	101.6	114.2	-	51.43	-	88.84	-	169
Ipsy	-	-	-	39.56	-	17.33	-	59.04	109.8

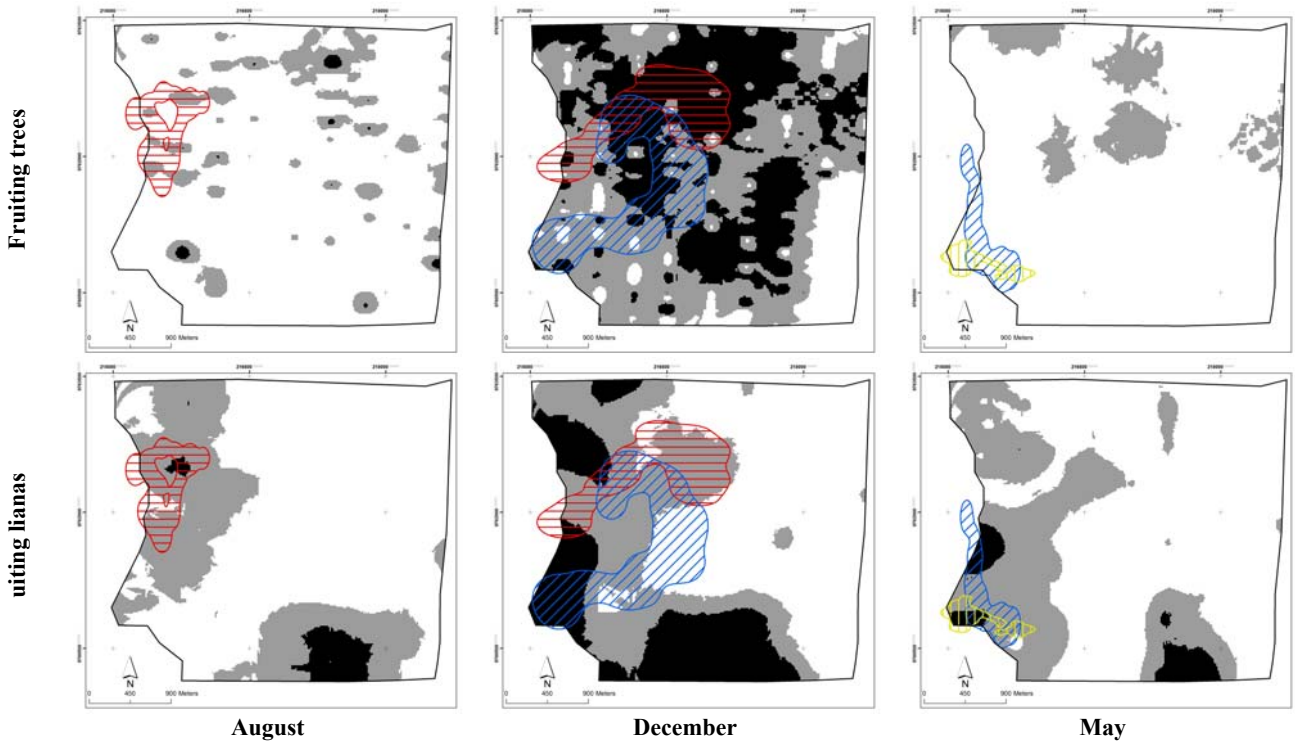


Figure 4. Orangutan monthly range overlapped with fruiting trees (top) and liana (bottom) in August (left), December (middle) and May (right). Note: See the complete legend in Figure 3; yellow, red, and blue depicted areas represent the monthly range areas for the three orangutans Ipsy, Milo and Jip, respectively

The orangutans' response to fluctuation in fruit availability was not only reflected in their home range size, but also in how they used the area in their home range (Wartmann et al. 2010). Our research showed that different utilization patterns occurred between trees and lianas by the adolescent orangutans. Jip, Milo and Ipsy were located in the western and southern areas of TORS, that consisted of medium to high abundance of liana fruit. However, the largest utilization in their home range was in area of medium abundance of fruit trees (Figure 3). The results showed that the area with medium to high abundance of tree fruit was preferred by the orangutans compared to the areas of abundant liana fruit.

Each month, adolescent orangutans used the part of their home range which was located in areas with a high to medium abundance of fruit trees. This condition occurred nearly every month, except during the period of low fruit tree availability (August and May). During the period of low tree-fruit availability, adolescent orangutans used a different area. They used areas which had a high to medium abundance of fruit lianas. Orangutans responded to a low period of fruit trees by not only decreasing their home range size, but also by changing the location of their home range to areas that had a medium to high abundance of liana fruit (Table 4). Liana fruits, leaves and flowers are an important food for frugivorous primates in TORS during the period of low availability of fruit trees (Vogel et al. 2009).Orangutan in TORS generally also consume inner bark, pith, young leaves, flowers as a fallback food (Bastian et al. 2010; Vogel et al. 2016).

Future research should focus attention on adolescent orangutans located in eastern TORS where the abundance of fruiting liana is low, and so adolescent orangutans maybe feeding on other alternative foods.

In conclusion, adjusting range use to fruit availability is an important survival strategy for orangutans. The process of responding adaptively to variability in fruit availability begins in adolescence. In our study, adolescent orangutans responded to months of low fruit availability (from trees) by decreasing their monthly range. In addition, they also changed the utilization area in their monthly range to locations that consisted of medium to high abundance of liana fruit. This ranging behavior is an adaptation that optimizes use of low quality habitat in order to survive in a degraded forest landscape.

Table 4. The proportions (%) of each orangutan's range within high, medium and low levels offruit productivity; from trees on the one hand and from lianas on the other; monthly data, as well as data across one year

	Tree			Liana		
	High	**Medium**	**Low**	**High**	**Medium**	**Low**
Jip						
October	0%	80.0%	20.0%	0%	27.3%	72.7%
December	39.5%	56.3%	4.2%	19.0%	45.4%	35.6%
May	0%	0%	100%	26.8%	71.3%	1.9%
June	0%	38.2%	61.8%	0%	8.0%	92.0%
1 Year	0%	94.6%	5.4%	0%	58.8%	41.2%
Milo						
August	0%	29.1%	70.9%	4.9%	85.4%	9.6%
October	0%	94.6%	5.4%	0%	61.7%	38.3%
December	72%	26.8%	1.2%	6.5%	79.8%	13.7%
March	0.3%	99.1%	0.6%	2.8%	27.8%	69.4%
June	0%	48.7%	51.3%	0.9%	37.0%	62.2%
1 Year	0%	99.6%	0.4%	0.0%	66.5%	33.5%
Ipsy						
February	18.9%	71.0%	10.1%	0%	0%	100.0%
May	0%	0%	100.0%	37.6%	60.2%	2.3%
July	0%	59.7%	40.3%	3.4%	56.2%	40.4%
1 Year	0%	96.7%	3.3%	0%	58.2%	41.8%

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Diversity of antibiotic-producing Actinomycetes in mangrove forest of Torosiaje, Gorontalo, Indonesia

YULIANA RETNOWATI[1,2,♥], LANGKAH SEMBIRING[3], SUKARTI MOELJOPAWIRO[3], TJUT SUGANDAWATY DJOHAN[3], ENDANG SUTARININGSIH SOETARTO[3,♥♥]

[1] Graduate Study Program of Biology, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sleman 55281, Yogyakarta, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo. Jl. Jenderal Sudirman No. 6, Kota Gorontalo 96128, Gorontalo, Indonesia. Tel.: +62-435-821125; Fax.: +62-435-821752. ♥email: yuliana_ri@yahoo.com
[3]Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sleman 55281, Yogyakarta, Indonesia. Tel./Fax.: +62-274-580839, ♥♥email: annisah-endang@ugm.ac.id

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Abstract. *Retnowati Y, Sembiring L, Moeljopawiro S, Djohan TS, Soetarto ES. 2017. Diversity of antibiotic-producing actinomycetes in mangrove forest of Torosiaje, Gorontalo, Indonesia. Biodiversitas 18: 1453-1461.* Actinomycetes for antibiotic production have been studied at various extreme environments. Mangrove forest of Torosiaje in Gorontalo Province, Indonesia has unique geomorphological conditions where the forest is surrounded by karst ecosystem consisting of fringe and overwash types. Therefore, the objective of this study was to analyze the distribution and diversity of antibiotic-producing Actinomycetes in various rhizosphere including different locations and mangrove species. Samples of rhizosphere soil were collected in the depth of 0-10 cm, which was then subjected to detailed physicochemical analysis. Actinomycetes were collected through heating pre-treatment (60^{o}C for 15 min) followed by culturing in the Starch Casein Agar medium supplemented with cycloheximide and nystatin. The screening process of antibiotic-producing Actinomycetes was based on Agar block method against pathogenic microorganisms. Grouping of Actinomycetes was determined by ARDRA fingerprinting analysis. The diversity of Actinomycetes was analyzed based on sequencing of 16S rDNA. The results showed that the distribution of Actinomycetes was found in overwash type, middle zone and upper zone of fringe type including rhizosphere of 7 species of mangrove. The highest population of Actinomycetes was found in rhizosphere of *R. mucronata* at the overwash type, and the lowest one found in rhizosphere of *R. apiculata* at the middle zone of fringe type. A total of 77 isolates amongst 167 isolate collection showed antibacterial activities. Forty seven representatives from 77 antibacterial-activities isolates were selected using ARDRA for partial characterization according to their phylogenetic diversity. Sequencing and analysis of 16S rDNA from selected-representative isolates displayed the presence of members associated with Actinomycetes genera such as *Streptomyces*, *Amycolatopsis*, *Saccharomonospora*, and *Nocardiopsis*. The member of genus *Streptomyces* such as *Streptomyces qinglanensis* and *Streptomyces champavatii* were distributed across locations. Genus *Saccharomonospora* and *Nocardiopsis* were mostly found at the overwash type, while *Amycolatopsis* was found at the upper zone of fringe type.

Keywords: Actinomycetes, diversity, fringe, mangroves, overwash

INTRODUCTION

Actinomycetes are Gram-positive bacteria that produce bioactive compound (Amrita et al. 2012). 70-80% of bioactive compounds are produced by Actinomycetes (Kanna et al. 2011), especially *Streptomyces* (Berdy 2005; Chaudary et al. 2013), and a part of bioactive compounds is produced by rare-Actinomycetes (Berdy 2005). The exploration of Actinomycetes that produce bioactive compounds has been focused on the extreme environment due to the rediscovery of bioactive compounds from terrestrial environment (Nolan and Cross 1988; Mangamuri et al. 2014). Mangrove forest is one of interesting ecosystems to explore Actinomycetes (Santhi et al. 2010; Baskaran et al. 2011; Khanna et al. 2011; Naikpatil and Rathod 2011; Ravikumar et al. 2011; Mangamuri et al. 2012; Mangamuri et al. 2014).

Mangrove ecosystem is a habitat of various of Actinomycetes. The Actinomycetes of mangrove ecosystem produce 122 bioactive compounds, where 73 of them have been identified as new kinds of bioactive compounds, and 49 compounds have not been identified, yet (Xu et al. 2014). The bioactive compounds from mangrove Actinomycetes demonstrate antibacterial activity (Sharma et al. 2011; Hunadanamra et al. 2013; Das et al. 2014; Phongsopitanum et al. 2014), anti fungal activity (Suthindiran and Kannabiran 2010; Sharma et al. 2011; Phongsopitanum et al. 2014; Das et al. 2014), and anti viral activity (Waksman 1984) and anti cancer activity (Hong et al. 2009; Suthindiran and Kannabiran 2010; Becerril-Espinosa et al. 2012; Ravikumar et al. 2012; Anupa et al. 2013; Xu et al. 2014).

Actinomycetes producing bioactive compounds in mangrove ecosystem are distributed on rhizosphere and non rhizosphere (Xiao 2009; Ravikumar et al. 2011), which rarely live as endophytics on stems and leaves of mangrove (Ravikumar et al. 2011; Gayathri and Muralikrishman 2013; Govindasamy and Franco 2013). The number of Actinomycetes population in rhizosphere are more than those in non rhizosphere. The various number of Actinomycetes is also depended on types of mangrove (Balagurunathan et al. 2010; Ravikumar et al. 2011;

Ramirez-Elias et al. 2014).

Mangrove ecosystem of Torosiaje is karst mangrove ecosystem including two types of forest, i.e., fringe and overwash types. The fringe type is divided into two zones, i.e. middle and upper zone. The middle zone was dominated by *Rhizophora apiculata* and *Bruguiera gymnorrhiza* while the upper zone consists of *Sonneratia alba*, *Avicennia marina*, *Xylocarpus* sp., and *Ceriops tagal*. The overwash type consists of *Rhizophora mucronata* and *Bruguiera gymnorrhiza* (Katili et al. 2014). Based on the uniqueness of Torosiaje mangrove ecosystem and some other important aspects; however, the diversity and distribution of Actinomycetes in this location had not been comprehensively explored, yet. Therefore, the objective of this study was to analyze the diversity and distribution of antibiotic-producing Actinomycetes in rhizosphere of various species mangrove located at mangrove ecosystem of Torosiaje, Gorontalo, Indonesia.

MATERIALS AND METHODS

Study area and sampling of soil rhizospheres. This study was conducted from April 2016 to April 2017. Samples of mangrove rhizospheres soil were collected from mangrove forest at Torosiaje, Pohuwato District, Gorontalo Province, Indonesia (Figure 1). The total of eight soil samples was collected from 3 different location i.e overwash type, middle zone and upper zone of fringe type, including 7 species of mangrove rhizospheres (*Rhizophora mucronata*, *Rhizophora apiculata, Bruguiera gymnorrhiza*, *Avicennia marina, Xylocarpus* sp*., Ceriops tagal* and *Sonneratia alba*). Sampling sites were located at 3 different coordinates i.e N. 00°.28'.21,9'';E.121°.26'.55,7"; N. 00°.28'.50,5"; E. 121°.27'.56,8" and N. 00°.28'.47,2; E. 121°.27'.56,2". Soil samples were collected on the depth of 0-10 cm by modified soil core with 4 replication.

Soil physicochemical characteristic analysis. The physicochemical analysis of soil samples (salinity, soil texture, soil pH, C-organic, total-N, N-NO_3, Fe, Mn, Zn, Cu, phosphor, K_2O and total S) was conducted at Soil Chemical laboratory, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia.

The isolation of actinomycete isolates. The initial treatment of rhizosphere soil samples was based on dry wet method on 60°C for 15 minutes in combination of sea water and aquadest (1: 1 v/v) (Mangamuri et al. 2012). Soil suspension was serial-diluted up to 10^{-6}. Soil suspension as much as 100 µL was inoculated in Starch Casein Agar (SCA) medium supplemented by 25 µg/mL of cycloheximide and 25 µg/mL of nystatin (Baskaran et al. 2011). Then incubated on 28 ± 2 °C for 10-28 days. Colony Forming Unit (CFU) was calculated and isolated in slant ISP 2 medium.

Screening of actinomycete isolates for potential antibiotics production. The screening of Actinomycetes for potential antibiotics production was based on Agar Block Method. The medium Muller Hinton agar got 5 holes and each hole was 5 mm diameters. After 28 days of age, actinomycete isolates cultured on ISP2 medium were then clipped around 5 mm diameters and moved to the holes. The Actinomycetes were incubated on 28°C ±2 for 3-7 days. The cell mass of microorganisms test (*Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*) on Nutrient Broth medium was equated by spectrophotometry on OD 0,6. Afterward, 300 µL of microorganisms test was inoculated in the around of actinomycete growing by surface plate method, then incubated on 37°C for 2 x 24 hours.

Extraction of genomic DNA. The spores of actinomycete isolates were suspended in aquadest. The suspension of spore was inoculated in 20 mL of ISP2 broth, then incubated in shaker incubator at 150 rpm for 4 to 7 days. Cell pellet in 20 mL of ISP2 broth was separated by centrifugation at 13.000 rpm for 5 min. The cell pellet was added with 20 mg of glass bead and 1 mL of buffer lysis (100mM Tris HCl pH 8, 50 mM EDTA pH 8, 2% SDS and 100 mM NaCl) then shaken out vigorously by vortex for 2 min. Proteinase K (20 mg/mL) as much as 10 µL was added then shaken out vigorously for 30 min. Then 60 µL of lysozyme (100 mg/mL) was added and incubated on 55°C for 30 min in water bath. The suspension was centrifugated at 13.000 rpm for 5 min, then cell pellet was discarded and the supernatant was resuspended by phenol (1: 1 v/v). The suspension of supernatant and phenol were shaken vigorously for 30 minutes, then centrifugated on 13.000 rpm for 10 min. The top layer was moved to new microtube and added by cold chloroform (1: 1 v/v), then shaken out slowly for 20 minutes. Then they were centrifugated on 13000 rpm for 10 minutes. The top layer was moved to new microtube and added with cold absolute ethanol (1: 1 v/v), and saved on -80°C for 2 hours or -20°C for overnight. The DNA genome pellet was separated by centrifugation at 13000 rpm for 10 minutes, cleaned up with 70% cold ethanol then dried for 2 hours. The DNA genome was suspended in 50 µL of TE (pH 8). The suspension of DNA genome was added by 3 µL of RNAase (10µg/mL) and incubated on 37°C for 1 hour in water bath. The genomic DNA was applied or saved on -20°C. The genomic DNA were examined by 2% agarose gel electrophoresis. The gels were stained by syber and visualized under UV transilluminator (Magarvey et al. 2004).

Amplification of 16S rDNA. The 16S rDNA was amplified from genomic DNA samples using eubacterial universal primers 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3'). The amplification was carried out in 25 µL volume by using 1 µL of genomic DNA as template with 12.5 µL GoTaq (R) Green Master Mix [buffer reaction, deoxynucleotide triphosphate (dNTPs), Taq DNA polymerase, and $MgCl_2$], 9.5 µL dH2O, and 1 µL universal primer 27F and 1 µL 1492R. The thermal cycling conditions were as follows: initial denaturation at 96°C for 5 min; 29 cycles at 95°C for 1 min, 54°C for 1 min, 72°C for 2 min and post-extension at 72°C for 10 min. The amplification reaction was performed by Bio-Rad thermal cycler (MyCycler, Bio-Rad, USA) and the amplified products were examined by 2% agarose gel electrophoresis (Mangamuri et al. 2012).

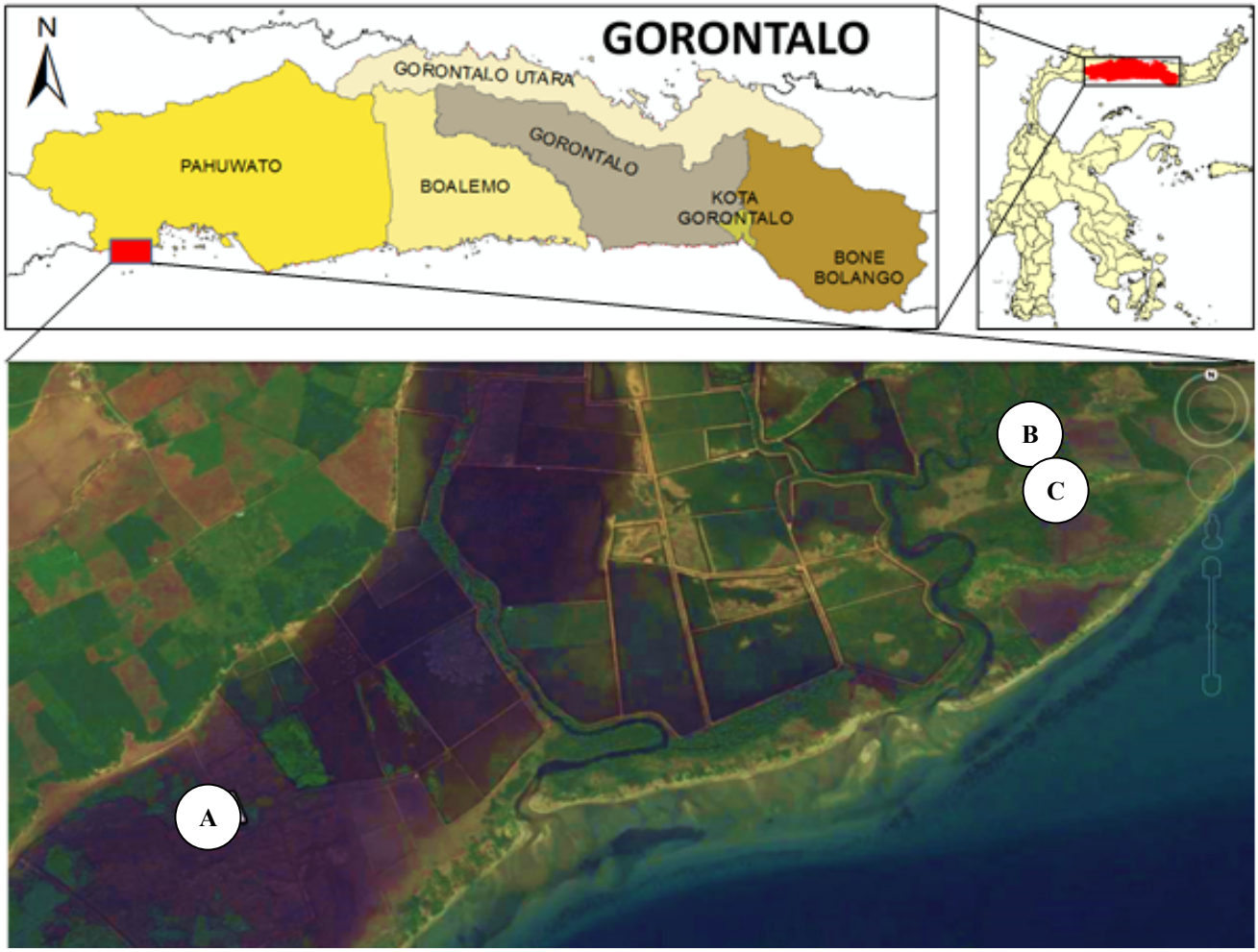


Figure 1. Location of mangrove forest of Torosiaje, Gorontalo, Indonesia. The alphabet A, B and C indicating the sampling site of mangroves soil rhizosphere at different mangrove forest type. A. overwash type (N. 00°.28'.21,9'';E.121°.26'.55,7"), B. middle zone of fringe type (N. 00°.28'.50,5"; E. 121°.27'.56,8"), and C. upper zone of fringe type (N. 00°.28'.47,2; E. 121°.27'.56,2")

Amplified ribosomal DNA restriction analysis (ARDRA). To identify the number of polymorphic groups and to select representative strains among the actinomycete isolates, aliquots of purified 16S rDNA amplicons were subjected to amplified ribosomal DNA restriction analysis. The purified PCR products were digested separately with *Hae*III and *Msp*I, in 10 µL reaction volume by using manufacture's recommended buffer and temperature. The digested restriction fragments were electrophorezed in 2% agarose gels using TE buffer. The gels were stained with syber and visualized under UV transilluminator. Strong and clear bands were scored in a binary data used for similarity and clustering analysis in numerical taxonomy analysis program package, MVSV Plus (Multivariate Statistical Package) Version 3.1A. Similarities among the isolates were calculated by Jaccard's coefficient, and the dendrogram was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method (Jose and Jebakumar 2012). If the strains formed a cluster defined at the 72% similarity level, they would be assigned to a single species (Meng Yuan et al. 2014)

16S rDNA sequencing and phylogenetic analysis. The PCR amplicons of 47 actinomycete isolates with representative ARDRA profiles were purified by using PCR product purification spin kit (BigDye® Terminator v3.1, USA) and sequenced by ABI PRISM 3730xl Genetic Analyzer develop by Applied Biosystems using same primary set as used in PCR amplification. All the sequences obtained from sequencing phase were analyzed and edited by using BioEdit soft-ware (Jose and Jebakumar 2012). Initially, all the 16S rDNA sequences were compared to sequences in GenBank by using the online service of Basic Local Alignment Search Tool to determine the approximate phylogenetic position. Sequences were aligned by using ClustalW with representative actinomycete 16S rDNA sequences, and a phylogenetic tree was constructed by using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 6.06 (Yuan et al. 2014).

RESULTS AND DISCUSSION

Physico-chemical characteristics of soil, population and distribution of Actinomycetes

Mangrove forest ecosystem is a habitat for various types of tolerant microorganisms to an extreme environmental conditions. Mangrove forest ecosystems are rich in organic, nitrogen and sulfur materials that required for the grow of microorganisms (Malek et al. 2014). Mangrove ecosystem of Torosiaje, Gorontalo consist of two type mangrove forest i.e., overwash type and fringe type. Fringe type divided into 2 zone i.e., middle zone and upper zone. Each of mangrove forest type and zone were show the specific physicochemical characters. The sediment salinity level was in range of 23,1 - 31,3 ppt. The physico-chemical characteristics of soil samples collected from overwash mangrove, middle and upper zone of fringe mangrove at mangrove forest of Torosiaje, Gorontalo were studied using standard protocols and summarized in Table 1. Several parameters, such as pH, salinity of soil, soil texture, macroelements and microelements have been measured. The results show that there were differences across all measurable characters in all three locations. Overwash type mangroves forest is washed periodically by tides of sea water so that it affects to the physicochemical character of the soil to be quite extreme, including salinity and high level of Fe, while the organic-C content was relatively low. Middle zone and upper zone in fringe type mangrove forests were shown the differences physico-chemical characters amongst both of them. The upper zone tends to be at least exposed by sea tides was shown sandy soil conditions with low salinity and neutral pH.

The physicochemical character of the 3 sampling sites were responded by actinomycete as one of the members of the mangrove ecosystem. The responses of actinomycete were indicated by the various number of population in the three locations. The highest population of Actinomycetes was found on overwash type, and the lowest one was found on middle zone of fringe type (Table 2). The periodically tides of overwash type is causes a continuously flow of macro-element and micro-element that supporting the growth of soil microorganisms. Otherwise, the geographical condition of the sloping fringe mangrove forest causes the middle zone is more exposed by the tides than the upper zone. This condition might cause the nutrient in the middle zone was more transported out of the zone.

Actinomycetes exploration at mangrove forest of Torosiaje is more emphasized on rhizosphere soil samples of 7 types mangroves at the depth of 0-10 cm. Nevin et al. (2000) and Mangamuri et al. (2011) claimed that the most Actinomycetes population were found in mangrove rhizosphere at the depth of 0-20 cm and apparently varied in each species of mangroves. The high population of Actinomycetes in the rhizosphere was due to the root exudation activities. That activity provided the space for actinomycete colonization and depended on the availability of amino acids and high concentrations of sugars and low concentrations of tannin content in rhizosphere (Atlas and Barta 1998; Badri and Vivanco 2009; Mangamuri et al. 2011). Mangamuri et al. (2011) also reported that the important nutrients such as nitrogen and potassium were found to be highest in the soil depths of 0-10 cm.

Table 1. Physico-chemical characteristic of soil samples from overwash type, middle zone and upper zone of fringe type at mangrove forest of Torosiaje, Gorontalo, Indonesia

Physicochemical characters of soil	Fringe type		Overwash type
	middle zone	Upper zone	
Salinity (ppt)	26.56	23.17	31.3
pH	3.16	7.46	6.04
Soil texture:			
Sand (%)	62	84	62
Dust (%)	31	10	26
Clay (%)	7	6	12
C-organic (%)	12.63	23.33	2.18
N (ppm)	102.94	246.65	610.40
Total-S (%)	0.38	0.20	0.43
$N\text{-}NO_3$ (ppm)	24.09	66.44	62.31
P_2O_5 (ppm)	63.33	21.31	3.09
K (me/100g)	1.74	3.59	1.23
Fe (ppm)	427.69	120.09	441.24
Mn (ppm)	4.76	4.04	8.08
Zn (ppm)	4.31	0.70	11.03
Cu (ppm)	0.37	0.76	1.07

Table 2. Population of Actinomycetes at 7 types mangrove rhizosphere

Mangrove forest type	Zone	Mangrove type	*Actinomycete* population (CFU/g)
Overwash	-	*R. mucronata*	$23,5 \times 10^6$
		B. gymnorhiza	$78,4 \times 10^3$
	Total on overwash		$11,8 \times 10^6$
Fringe	Middle	*R. apiculata*	$3,5 \times 10^1$
		B. gymnorhiza	$10,1 \times 10^3$
	Sub total		$50,7 \times 10^2$
	Upper	*S. alba*	34×10^1
		A. marina	$33,6 \times 10^3$
		Xylocarpus	$26,3 \times 10^2$
		C. tagal	$18,6 \times 10^2$
	Sub total		$96,1 \times 10^2$
	Total on fringe		$73,4 \times 10^2$

The isolation of actinomycete from mangrove rhizosphere using Starch Casein Agar medium supplemented with cycloheximide and nystatin was succeeded obtaining 167 actinomycete isolates. The type of mangrove vegetation affected the distribution and population of actinomycete (Balagurunathan et al. 2010; Ramirez-Elias et al. 2014; Ravikumar et al. 2011; Ravikumar et al. 2012). The result of study show that the number of actinomycete isolate was varied in the 7 types of mangrove rhizosphere (Figure 2). The highest number of isolate was found at *R. mucronata* rhizosphere and the lowest one at *R. apiculata* rhizosphere. This is probably due to the rhizospheres effect as a result of root exudation activity. The root exudates contains various compounds including amino acids, organic acids, sugars, phenols,

tannins, alkaloids and various secondary metabolites (Atlas and Barta 1998; Walker et al. 2003). Exudation activities of various compounds in plant roots can regulate the soil microbial community by mediating soil-microbial root interactions, altering soil chemical physical properties and inhibiting the growth of plant competitors (Badri and Vivanco 2009). Each of mangrove species was produce a specific kind of root exudates. The adult of *Avicennia marina* is produce root exudates with different levels of glucose and fructose composition (Lie et al. 2014) and root exudates of *Bruguiera gymnorrhiza* contain of aminopyrine, palmitic acid, stearic acid, di-n-propyl-ether, and 2.5-angidroglunonic (Kumar et al. 2009).

Screening of antibiotic-producing Actinomycetes based on agar block method

Mangrove forests are interesting ecosystems for the discovery of antibiotic-producing Actinomycetes (Santhi et al. 2010; Baskaran et al. 2011; Khanna et al. 2011; Naikpatil and Rathod 2011; Ravikumar et al. 2011; Mangamuri et al. 2012; Mangamuri et al. 2014). The extreme conditions of mangrove environment are expected to stimulate the actinomycete to produce antibiotics with specific character (Basilio et al. 2003). The bioactive compound were shown the activity as antibacterial, antifungal, antiviral and anticancer (Suthindhiran et al. 2010; Ravikumar et al. 2011; Amrita et al. 2012; Doroghazi and Metcalf 2013; Zotchev 2014). This study proves that amongst 167 actinomycete isolates, there were 77 actinomycete isolates that show the antibacterial activity against pathogenic bacteria. The highest number of antibiotic-producing actinomycete isolates was found at rhizosphere of *R. mucronata* in overwash type, and the lowest one at rhizosphere of *C. tagal* in upper zone of fringe type (Figure 3). While the high ability of actinomycete isolates to against pathogenic microbes was found in rhizosphere of *A. marina* and *Xylocarpus* sp. at upper zone of fringe type. That was shown that mangrove forest of Torosiaje, Gorontalo was potential to exploration antibiotic-producing Actinomycetes.

Amplification of 16S rDNA and ARDRA. PCR amplification of the 16S rDNA using a set of universal eubacterial specific primers: 27 F and 1492R yielded a single amplicon of ~1500 bp for 77 the antibiotic-producing actinomycete isolates. The amplicons of 16S rDNA were used to Amplified Ribosomal DNA Restriction Analysis (ARDRA) with restriction enzymes endonuclease *Hae*III and *Msp*I. Digestion of these amplicons with *Hae*III were generated almost similar profile for several of isolates. Whereas, when amplicons were digested by restriction enzyme *Msp*I, different number of fragments were produced about 5 to 9 fragments with varied sizes about 30 to 900 bp for different isolates.

The ARDRA pattern was used for dendrogram construction using UPGMA and Jaccard's coefficient algorithms (Figure 4). The 77 of antibiotic-producing actinomycete isolates were divided into 47 groups in a dendrogram inferred from ARDRA pattern obtained from *Msp*I restriction enzymes. The groups consisted of 1 to 5 actinomycete isolates. The groups formed on the dendrogram provide information about the presence of the same species found in different location and different kinds of mangrove. If the isolates formed a group/cluster defined at the 72% similarity level, they would be assigned to a single species (Meng Yuan et al. 2014).

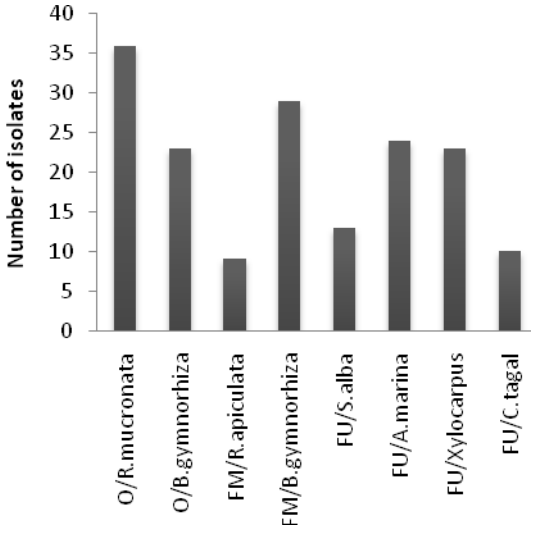


Figure 2. The number of Actinomycete isolates in 7 types of mangrove rhizosphere at mangrove forest of Torosiaje Gorontalo, Indonesia. O. Overwash; FM. Fringe/middle zone; FU. Fringe/upper zone

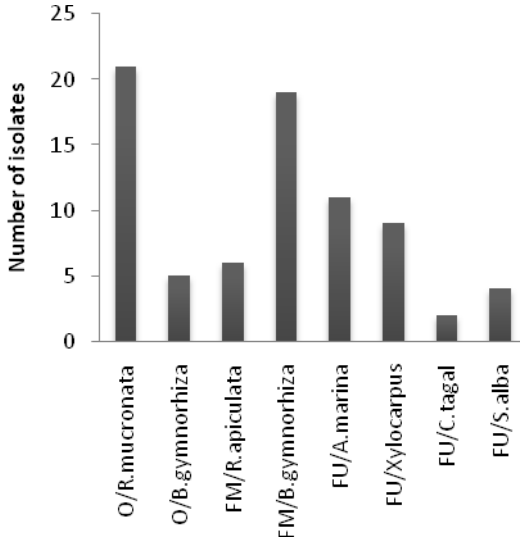


Figure 3. The number of antibiotic-producing Actinomycete isolates at 7 kind of mangrove rhizosphere. O. Overwash; FM. Fringe/middle zone; FU. Fringe/upper zone

Actinomycetes community composition and phylogenetic analysis. The 16S rDNA of 47 representative isolates that belong to different clusters established by ARDRA was sequenced and used to determine the diversity of isolates. Based on similar criteria of 98% at the 16S rRNA gene, the 47 isolates were sorted into 6 phylotypes, belong to 4 different genera. The four genera were *Streptomyces, Saccharomonospora, Nocardiopsis* and *Amycolatopsis* (Figure 5). The most abundant group of isolates was affiliated with the genus *Streptomyces*, represented by four ribotypes accounting for 95% of the total actinomycete population. The ribotypes ORm2-a was closely related to type strain *Streptomyces qinglanensis* (GenBank: NR_109303.1) with 99.9% sequence similarity, representing 83% of total isolates. The other ribotypes of *Streptomyces*, OBg1-3 (representing 5% of isolates) and FMRa1-1 (representing 7% of all isolates) were closely related to type strain *Streptomyces sanyensis* (GenBank: NR_116599.1) with 99% sequence similarity and *Streptomyces champavatii* (GenBank: NR_115669.1) with 97% sequence similarity, respectively. The second most dominant group in the isolates was the genera *Nocardiopsis*, representing 3% of total actinomycete isolates. Ribotype ORm2-f was closely related to type strain *Nocardiopsis lucentensis* (GenBank: NR_026342.1) with 99% of sequence similarity. Ribotype FUXy2-d and OBg4-e representing 1 % of total actinomycete isolates, respectively. Ribotype FUXy2-d was closely related to type strain *Amycolatopsis sacchari* (GenBank: NR_112696.1) with 94% of sequence similarity. While, ribotype OBg4-e was closely related to type strain *Saccharomonospora amisosensis* (GenBank: NR_109529.1) with 97% of sequence similarity.

The four genus of antibiotic-producing Actinomycetes were distributed at overwash type, middle zone and upper zone of fringe type of mangrove forest of Torosiaje, Gorontalo (Figure 6). Several studies have been conducted in various mangrove ecosystems around the world and managed to discover various new types of Actinomycetes from rhizosphere of mangrove, non rhizosphere and endophytes in stems, roots and leaf of mangrove (Mangamuri et al. 2011). Several types of members of the Actinomycetes group were found in the mangrove ecosystem. They were *Streptomyces avicenniae* sp. nov., (Xiao et al. 2009), *Streptomyces qinglanensis* sp. nov., (Hu et al. 2012). The result of study show that genus *Streptomyces*, especially *Streptomyces qinglanensis* was the most dominant species and distributed across locations. Several studies were reported that the Actinomycetes community at mangrove ecosystem dominated by *Streptomyces* (Kannan and Vincent 2011; Mangamuri et al. 2014; Malek et al. 2014). *Streptomyces qinglanensis* was established by Hu et al. (2012) as a novel species of the genus *Streptomyces* that isolated from mangrove sediment at Qinglan Harbor, Wenchang, Hainan, China.

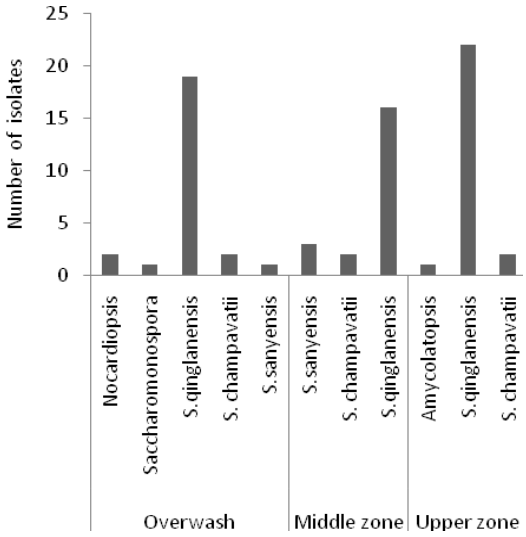


Figure 6. Distribution genus member of antibiotic-producing Actinomycetes at different location on mangrove forest of Torosiaje, Gorontalo

The genus *Nocardiopsis* and *Saccharomonospora* were specific found at overwash type, while genus *Amycolatopsis* at upper zone of fringe type. The genus *Saccharomonospora* which belongs to the family Pseudonocardiaceae was established by Nonomura and Ohara (1971). *Saccharomonospora amisosensis* was established by Veyisoglu et al. (2013) as novel species isolated from deep marine sediment at southern Black Sea coast of Turkey. Several members of genus *Saccharomonospora* were isolated from an extreme environments. *Nocardiopsis* strains are distributed ubiquitously in the environment. They are frequently isolated from habitats with moderate to high salt concentration such as saline soil or marine sediment (Sabry et al. 2004) and slattern (Chun et al. 2000).

In conclusion, distribution and diversity of Actinomycetes were explored from mangrove ecosystem at Torosiaje, Pohuwato District, Gorontalo Province, Indonesia. 167 actinomycete isolates were isolated from 7 types of mangrove rhizosphere. They were distributed at overwash mangrove, middle and upper zone of fringe mangrove. Among 167 actinomycete isolates, there were 77 actinomycete isolates shown antibacterial activity against pathogenic bacteria. The isolates which shown high antibacterial activity were found at rhizosphere of *A. marina* and *Xylocarpus* sp. The Actinomycetes that produce antibiotics were assigned to four genera i.e *Streptomyces*, *Saccharomonospora*, *Amycolatopsis* and *Nocardiopsis* on the basis of their16S rDNA sequences. This is the first step towards better understanding of actinomycete from mangrove ecosystem at Torosiaje, Gorontalo.

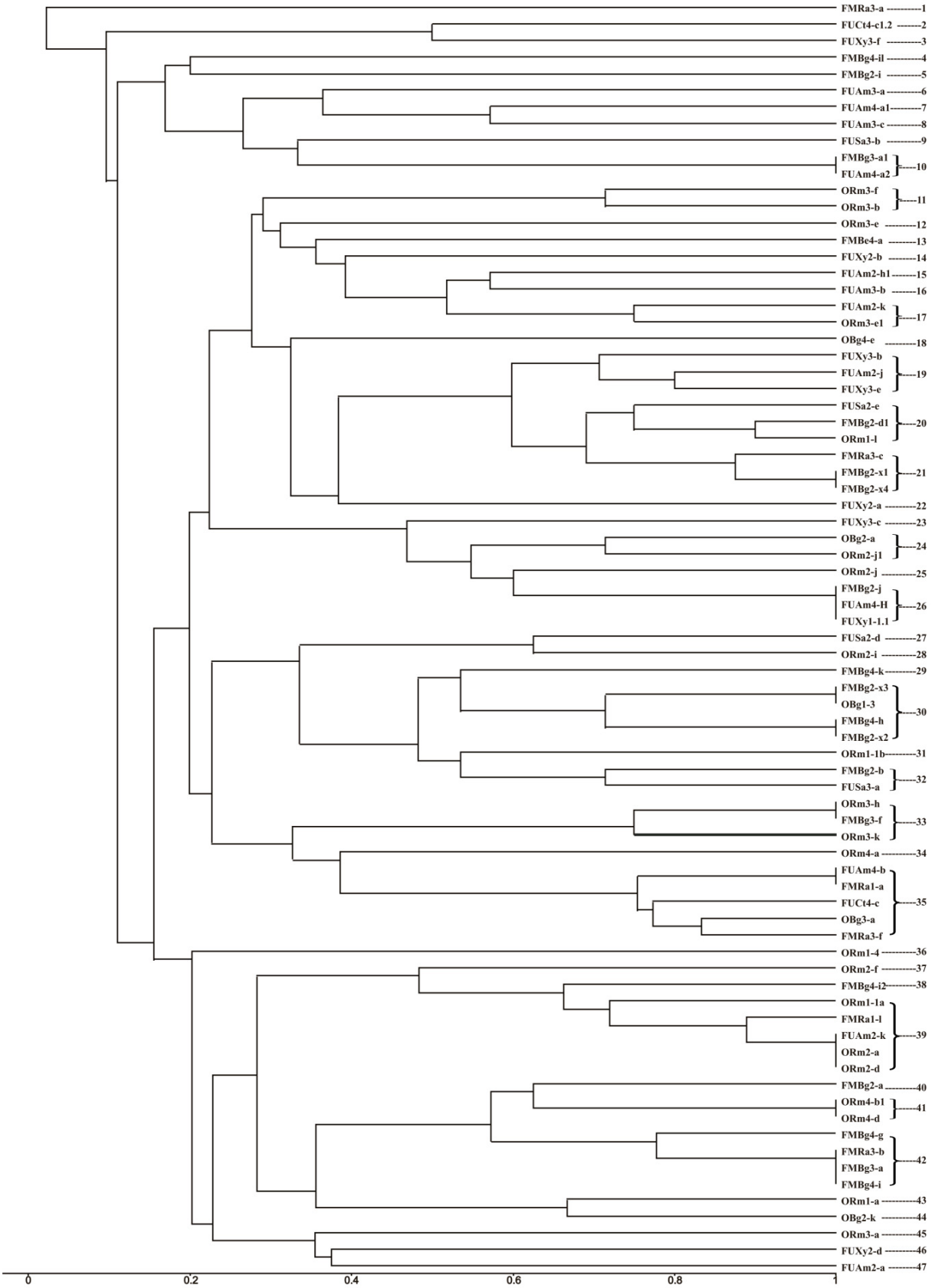


Figure 4. UPGMA dendrogram inferred from ARDRA patterns of *Actinomycetes* isolated from mangrove rhizosphere. The dendrogram shows the clustering of 77 antibiotic-producing actinomycete isolates generated from amplified ribosomal DNA restriction analysis with restriction endonuclease MSP1, using the UPGMA algorithm and the Jaccard's coefficient. Number 1 to 47 represent the forty seven clusters obtained in the analysis.

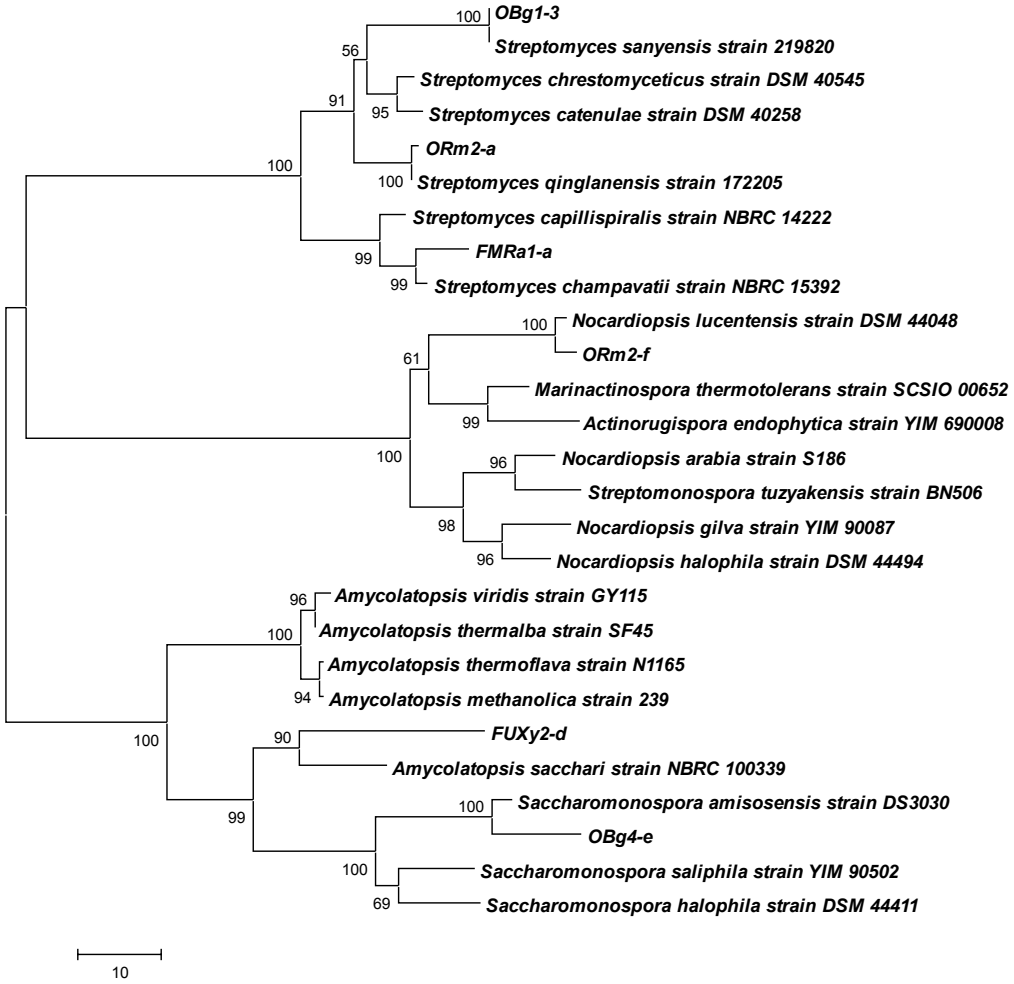


Figure 5. Neighbour-joining phylogenetic tree inferred from 16S rRNA gene sequences. The phylogenetic tree shows the phylogenetic relationship of isolates with related genera. Bootstrap values are expressed as percentages of 1000 replications. Bootstrap values, >50% are shown at branch points. Score bar represents 1 nucleotide substitution per 100 nucleotides

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Short Communication: The normal karyotyping result of Indonesian native breed bull qualified for artificial insemination

GATOT CIPTADI[1,♥], MUH NUR IHSAN[1], VERONICA M. ANI NURGIARTININGSIH[1], IRSANTI PUTRI ARDYAH[2], MUDAWAMAH MUDAWAMAH[3]

[1]Faculty of Animal Husbandry, Universitas Brawijaya. Jl. Veteran, Malang City 65145, East Java, Indonesia. Tel.: +62-341-553513, ♥email: ciptadi@ub.ac.id

[2] Program of Graduates, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang City 65145, East Java, Indonesia

[3]Faculty of Animal Husbandry, Universitas Islam Malang. Jl. Mayjen Haryono No.193, Malang City 65144, East Java, Indonesia

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Abstract. *Ciptadi G, Ihsan MN, Nurgiartiningsih VMA, Ardyah IP, Mudawamah M. 2017. Short Communication: The normal karyotyping result of Indonesian native breed bull qualified for artificial insemination. Biodiversitas 18: 1462-1467.* Bali and Madura cattle are important native breed in Indonesia, both descendants of wild banteng (*Bos javanicus*). These breeds are categorized as a small type beef cattle, and are indigenous, well adapted and, have high resistance to diseases. The aims of this research were to analyze dendrograms of these breed and karyotype breeding bull selected for Artificial Insemination (AI). The result showed that the 2n diploid number of chromosome of all cattle was 60 and they were considered normal, based on the number and structure of chromosomes. Fifty-eight acrocentric autosomes and 2 sex chromosome were observed, including a submetacentric X-large chromosome submetacentric and a small Y chromosome. Dendrogram analysis showed that Bali and Madura cattle were closely related and derived more from their ancestor wild banteng than from Java cattle. It is recommended to perform a chromosomal investigation of breeding bulls especially those used for freezing semen production and AI implementation.

Keywords: Abnormalities, Artificial Insemination, cattle, dendrogram, genetic, karyotyping

INTRODUCTION

In Indonesia, there are many native breeds, and unidentified breeds of ruminants that need to be characterized for their genetic potential, especially for standard genetic quality by karyotyping. Two important native cattle are Bali and Madura cattle, both of which are derived from banteng (*Bos javanicus*). Bali cattle are the result of banteng domestication in Java, Bali island and also in some areas of Southeast Asia and Australia. banteng is still found as wild cattle that live in several locations on the island of Java, such as at Ujung Kulon National Park in West Java province and Baluran national park forest at East Java. Bali cattle have spread and are raised throughout almost all provinces of Indonesia. Furthermore, Bali cattle expanded to Malaysia, the Philippines, and Northern Australia. Meanwhile, Madura cattle, a small type of beef cattle, are germplasm suitable, indigenous, and adapted to an arid environment and highly resistant to disease. They have been well developed on the island of Madura, East Java.

Basic genetic information about Indonesian native bull is relatively very limited.The analysis of chromosomes is one powerful method to characterize the genetic normality of animals. Karyotyping of breeding bull and their progeny should be done, because of the importance of chromosome abnormalities and their negative effect on production and reproduction of animals. Chromosomal aberration of a candidate bull can be identified and culled from a breeding program (Achmad et al. 2004). It is necessary that bulls qualified for artificial insemination (AI) must be screened out for any possible chromosomal abnormalities, because a commercial AI bull may be able to produce about 60.000 offsprings per year/bull (Ciptadi et al. 2014). Bali cattle are considered as important genetic resources to Indonesia, because of its high reproductive efficiency, good quality meat and carcass, high carcass percentage, excellent adaptability to the environment and particularly its ability to use a limited feed resource. Madura cattle is the result of cross-breeding of Bali cattle with Zebu (*Bos indicus*). The diversity of genotypes of Madura cattle is quite high. Chromosomal abnormality could be identified and the affected culled from the breeding population. Abnormal genetics of animals results in reduced fertility in both female and male carriers of the abnormality. Kosarcic et al. (2006) reported that numeric and structural changes on animal karyotype influenced on reproduction, phenotype expression, and selection program. Different aspects of reproductive disturbance include small litter, embryo mortality, frequent repeat breeding, abortion and mummified embryo, offspring with abnormalities and different kinds of sterility.

In Indonesia, AI implementation has been started intensively, especially using imported bulls (*Bos taurus*)

i.e. the Limousine breed. Therefore, it is important to execute chromosome analysis on these cattle. Limousine bull is very popular for smallholder farmers in the certain island of Indonesia to cross with local cattle i.e. Madura cattle resulted in very good vital statistic and phenotype of the F_1-cross. For the future this situation may be dangerous for maintaining the purity of the Madura breed. It is important to think that cytogenetic control is an important selective measure for choice of genetically healthy breeding bulls, especially for their use AI frozen sperm production. There is no information on the karyotyping of bull classified for AI in Indonesia.

The aim of this study was to perform an analysis of the chromosome and dendrogram of Indonesia native animal with focus on Bali and Madura cattle. This paper presents an analysis karyotyping of the numeric and early structural types of the chromosome of Indonesian native breeds selected for AI. Meanwhile, dendrogram analysis was taxonomical relationship analysis among breeds base on phenotypic specific characters of all Indonesian native breeds of Bali, Madura, PO, and Brahman, compared to Banteng.

MATERIALS AND METHODS

Animal materials

A karyotyping was performed on two main native breeds qualified for AI and sperm production. Karyotyping focused on Bali and Madura bulls (3 heads each) and Madrasin (Madura x Limousine cross) females. Dendrogram analysis was performed using specific phenotypic characters observed for parameters of four main local Indonesian breeds, namely Bali, Madura, PO, and Brahman, which were compared to Banteng. These bulls were reared in the AI Centre of Singosari, Malang, East Java, Indonesia.

Karyotyping

Karyotyping was performed by collecting blood samples from both 2 local breeds (Madura and Bali), 3 bulls each with different phenotypic characteristics. Chromosome analysis was also performed to Madrasin (Madura x Limousin cattle cross). The method for culturing blood cells used was adapted from a combination of several protocols (Miyake, 1985, Ahmad et al. 2004). Each animal's blood was collected in sterile heparinized tubes. A sample of 0.5 ml of blood/animal was added to 5 ml chromosomal medium (Karyo MAX, Gibco), placed in an incubator at 38 ^{o}C. After 70 hours, 1 ml colchicines was added to each culture tube and the tubes were kept for 2-3 hours, then were centrifuged at 1000 RPM for 10 minutes and the supernatant was discarded. After fixative solution added then a carnoy solution added to the cell pellet, cell then centrifuged at 1000 RPM for 10 minutes and the supernatant was discarded, this step repeated 3 times. Slides were prepared by dropping the cell suspension ±250µl on the glass slide and drying, then the slides were then stained with Giemsa stain, in order to identify the 30 pairs of chromosomes for normal metaphase scoring. Slides were examined under a high power phase-contrast microscope. The metaphase cells selected for the 10 best spreading metaphase chromosomes were micro-photographed and karyotyped. Each chromosome was identified according to International Standard Chromosome Analysis based on order from their specific banding pattern. Chromosomal analysis was performed with the help of cytovision software image analysis (Genus CytoVision Image ver 4.5.1, Genetrix, USA).

Dendrogram analysis

Dendrogram analysis of phenotypic 4 related breeds (Bali, Madura, PO, and Brahman) compared to banteng was done based on 7 main specific phenotypic characteristics, i.e. dominant skin color, horns, ears, muzzle color, foot color, hump and specific color a part of body. The number of animals used for dendrogram analysis of 4 local breeds (Bali, Madura, PO, and Brahman) is 3 heads each. Meanwhile, Banteng used as a reference is one comes from Baluran National Park, East Java. These consist of 16 sub-specific characters of Bali and Madura cattle compared to their ancestor of *Bos javanicus* from Baluran National Park and also possibly others breeds considered as their parents, namely Brahman and PO breeds, by Alogaritma: paired group (UPGMA), distance: Euclidean distance (d)=√ (x2-x1)2 + (y2-y1)2, Cophen Corr: 0.7862. This calculation was used to group members with two or more objects that have the closest similarities. The results were compared to the standard of karyotyping of *Bos taurus*, *Bos indicus* and *Bos javanicus*. Meanwhile, the dendrogram was analyzed descriptively.

RESULTS AND DISCUSSION

The dendrogram of among Indonesian native breed cattle

Based on their phenotypic characters, both the Bali and Madura breed were closely related and grouped in one group with their ancestor banteng (*Bos javanicus*) compared to PO breed, which grouped with Brahman cattle (Figure1). Both Madura and Bali cattle showed higher genetic distance with banteng in cluster A = banteng and Bali cattle displayed a genetic distance equal to 2,83. Meanwhile in cluster B = PO displayed genetic distance of 2.45 with Brahman cattle. The Madura cattle have a closer similarity to Cluster A (Table 1).

Chromosome analysis result in Bali and Madura cattle

In general, karyotyping of Madura and Bali candidate bulls used for AI and Madrasin (Madura x Limousin) cows categorized them as having normal chromosome numbers and structure (Figures 2, 3 and 4).

The 2N diploid numbers of chromosomes of both local cattle breeds were normal number of 60, 58 autosomal and 2 sex chromosomes in all cattle observed. It was observed that Bali and Madura bulls tested were normal. The karyotype analysis showed that the chromosomes of one cell and different individual each breed varied in size, shape, and position of the centromere within a biarmed

chromosome, in addition to the X and X or Y (Figure 2 and 3). The sex chromosome for bulls, the X-chromosome was the largest-submetacentric chromosome, while the Y-chromosome was the smallest-acrocentric chromosome. Some of the variation may be caused by physical factors during fixation or chromosome spreading (Figure 3 and 4).

Discussion

The results of our study of cattle chromosomes are similar to those of other researchers (Anis et al. 1990, Lio et al. 1995) that studied various breeds and reported that cattle have 60 chromosomes. All of the 30 pairs displayed G-banding patterns (shows in black and white after a photograph) characteristic of good quality chromosomes (Figure 1). With the help of cytovision software, the autosome and sex chromosome could be identified by their numbers, size and banding patterns. In a case of Madura cattle, chromosome analysis is very important, because in the past ten years, this breed has been crossed with a foreign breed of *Bos taurus*, i.e. Limousine to increase cattle production (Ciptadi, 2014), which may influence their genetics, reproductivity, and productivity. This cytogenetic test should be implemented, because of the importance of chromosome abnormalities that may have negative effects on production and reproduction performances. There are many types of chromosome abnormalities in domestic animals, and these abnormalities are closely related to the reproductive disorders (Miyake, 1996, Gallagher et al. 1999, De Luca et al. 2007, Anis et al. 1990). For instance, Schmutz et al. (1997) described reduced fertility in female carriers of the rob(1:29) translocation. The rob (1;29) translocation has spread to all European cattle breeds and, eventually, to the rest of the world through cross-breeding, with-out appearing ex-novo and the distribution of the rob (1;29) Robertsonian translocation was analyzed to determine whether this polymorphism contributes to the low reproduction efficiency observed in five endangered Andalusian (Spanish) cattle breeds (Serrano et al. 2013). Chromosomal abnormalities in the sex chromosome pair are widely associated with reproductive problems in horses (Anaya et al. 2017). Cytogenetic studies in Polish Black-and-White bulls showed the existence of two morphological forms of Y chromosome. Among the 22 karyotypically analyzed bulls, 12 had submetacentric and 10 metacentric Y chromosome (Parada et al. 2017).

This technique of karyotyping may be very valuable in the cytogenetical analysis of chromosomal abnormalities. Cytovision software is very helpful for producing more accurate results. With cytovision analysis tools counting/numbering of metaphase chromosomes with segmentation (split, overlapped, and joined) was possible. The cytogenetic analysis and control are an important selective measure for choosing genetically health candidate of breeding bulls or artificial insemination (AI) is important to perform. Based on the presence of a chromosomal aberration, a candidate bull can be identified and culled from a breeding program. For local Indonesian cattle, as well as other local animals, we proposed to use this test as a new criterion of selection to of candidate sires, especially for AI commercial sperm production, (Ciptadi et al. 2017). Ahmad et al. (2004) mentioned that screening of breeding bulls of different breeds through karyotyping is important, especially bulls maintained at semen production units or Artificial Insemination Centers. Karyotyping is one amongst different culling parameters. Chromosomal screening is beneficial in the selection of superior animals.

Table 1. Data on phenotypic characters of Indonesian native bull (for dendrogram)

Main Traits	Traits	Banteng	Bali	Madura	PO	Brahman
Colour	Major body	Dark Brown	Sorrel	Sorrel	White Greyish	White
	Front ankle	White	Sorrel	Sorrel	White	White
	Head	Cokelat Tua	Sorrel	Sorrel	White	White
	Whites around eyes	Yes	Yes	Yes	Darken	No
	Butt	White	White	Sorrel	White	White
	Back ankle	White	White	White	White	White
	Backline	White	Dark	Sorrel	Dark	White
	Whites around mouth	Yes	Yes	Yes	Yes	Yes
	Major hump	Dark Brown	Sorrel	Darken Sorrel	White	Dark
	Dark muzzle	No	Yes	Yes	Yes	Yes
	Dark nail	Yes	Yes	Yes	Greyish	White
	Around neck major	Yes	Yes	Yes	Yes	Darken
Horn	Shape	Curved	Slightly curved	Straight	Straight	Doward curved
	Size	Long	Middle	Short	Short	Short
	Types	Upward	Upward	Backward	Backward	Downard
Ears	Shape	Straight up	Straight up	Straight up	Lop-eared	Lop-eared
	Inner colour	White	White	White	White	White
Hump	Clearly	No	No	Yes	Yes	Yes
	Size	Small	Small	Middle	Big	Big
	Colour	Dark Brown	Sorrel	Darken sorrel	White	Dark
Dewlaps	Hanging	Yes	Yes	Yes	Yes	Yes
	Size	Small	Small	Middle	Big	Bigger

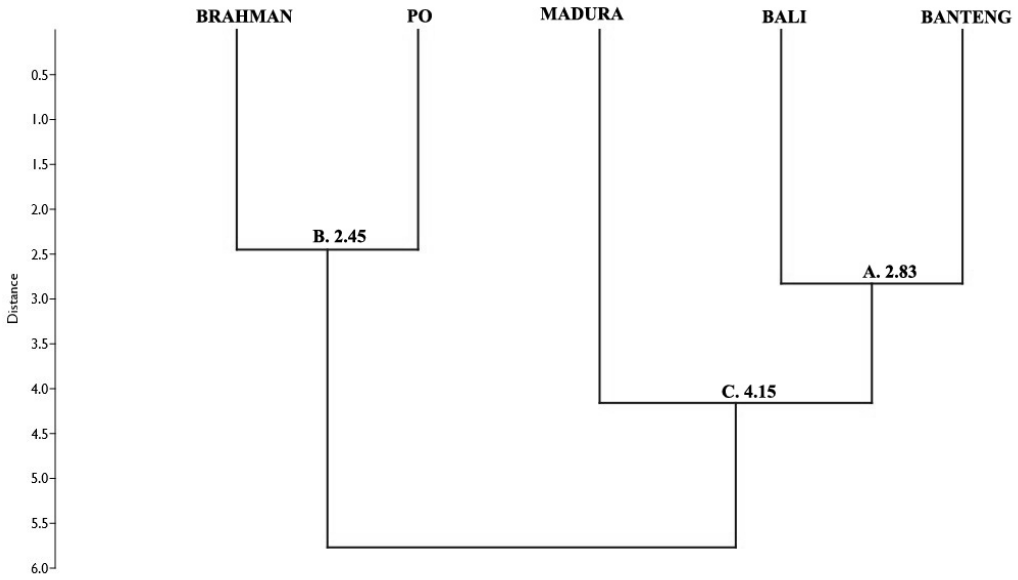
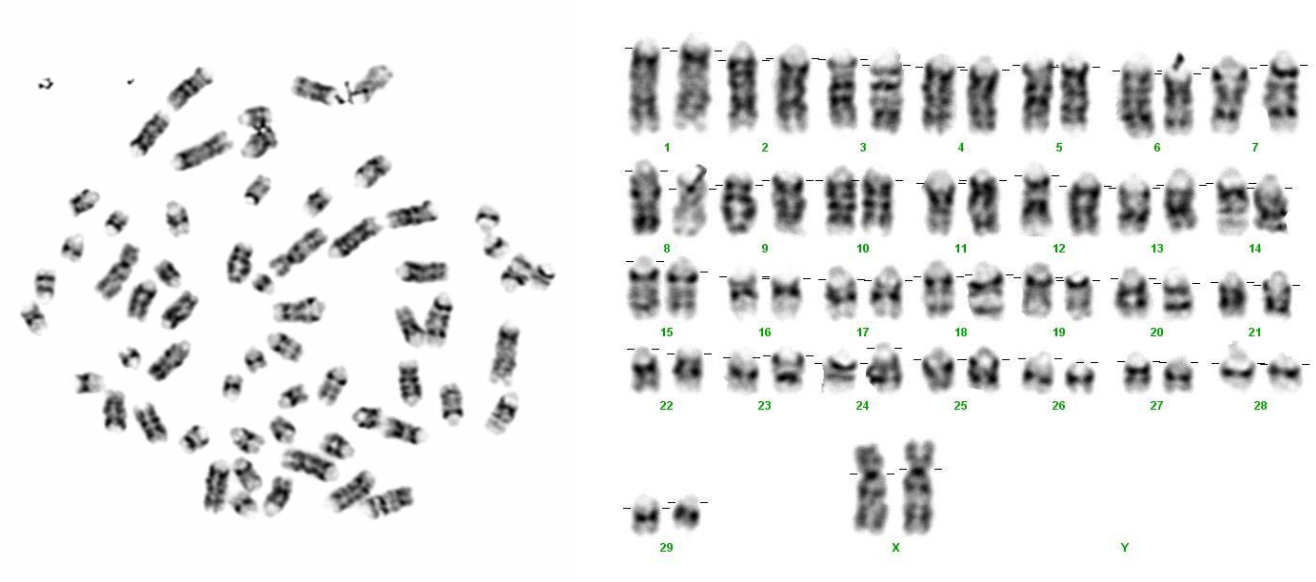


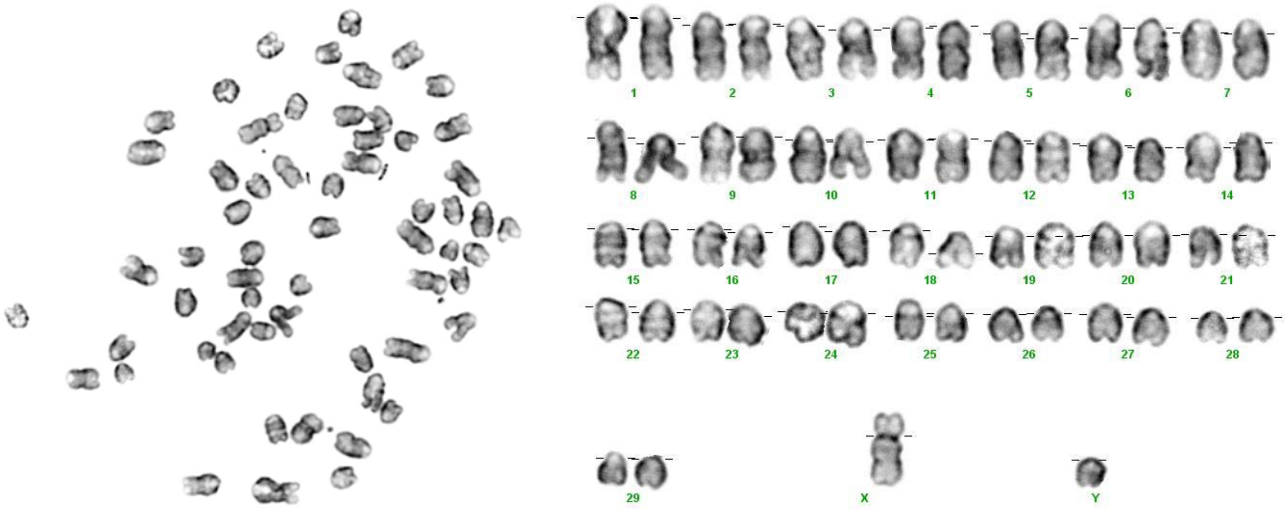
Figure 1. Dendrogram analysis of Bali and Madura cattle compared to other local breeds found in Indonesia namely PO (Java cattle) and Brahman. Madura and Bali's cattle were derived from banteng which may cross with Java cattle (Brahman and PO cattle). The genetic distance and clustering of each breed with wild banteng (*Bos javanicus*) are indicated that cluster A=2.83, B = 2.45 and C=4.15 respectively

The early analysis of G-banding preparation showed in some improper preparation; a clear banding pattern sometimes could not be obtained, because the bands were unclear or were too close each other. The technique of photography and manual karyotyping also provided less support for these analyses. Yamanaka (1977) suggested that in order to identify each chromosome for karyotype analysis in cattle, the number, intensity, width, and disposition of each band, as well as size of chromosomes should be considered. However, Ciptadi et al. (2017) suggested that in developing countries, Indonesia especially, where AI implementation has been started, at national or state levels especially in Artificial Insemination Center, it is necessary to introduce this karyotyping in selection programs, for chromosomal control of breeding males. Karyotyping is one amongst several culling parameters and could be used as an indicator of genetically normal bulls, because each can produce a large number of offspring per year/bull. Artificial Insemination is not only beneficial as a means of reproduction and raising the population but also aids efforts to improve the genetic quality of local livestock (Ciptadi et al. 2014).



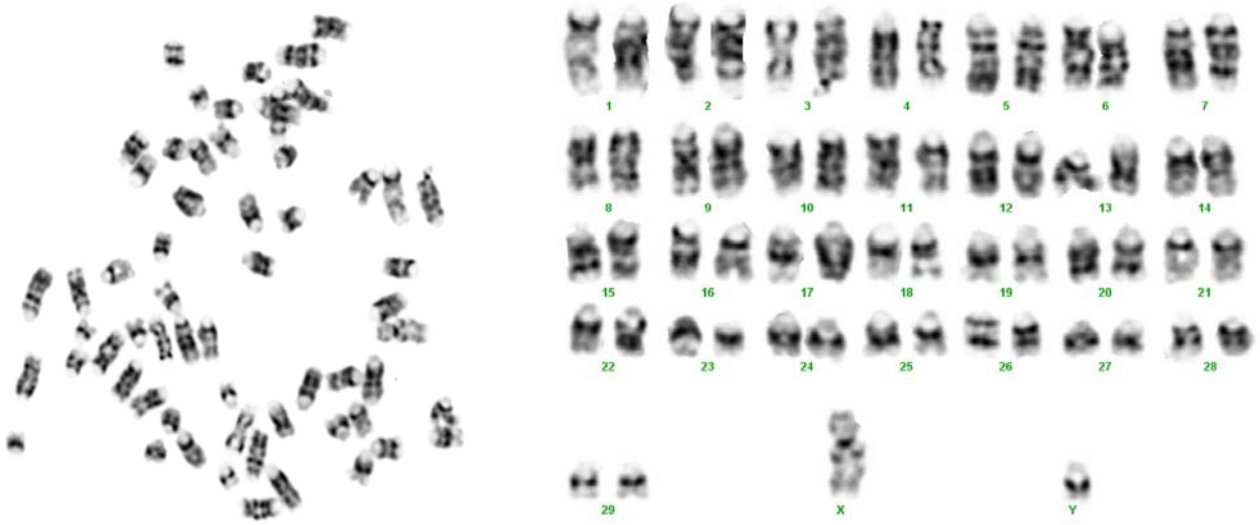
A **B**

Figure 2. Cattle chromosome (female) of F_1 Madura x Limousine cattle cross (Madrasin), showed normal numbers of 30 pairs chromosomes: A. Spreading chromosomes resulted in different size and shape of chromosomes. (1000 x). B. Karyotyping of images using cytovision software. The number of chromosomes was considered normal 2n = 60)



A **B**

Figure 3. Cattle chromosome (male) of Madura cattle, showed normal numbers of 30 pairs (2n=60). A. Spreading chromosome resulted in different size and shape of chromosomes (1000 x), B. Karyotyping of Madura bull. using software image of cytovision (Genetrix, USA)



A **B**

Figure 4. Cattle chromosome of Bali bull, showed a normal number of 30 pairs. A. Spreading chromosome (1000x) and B. Karyotyping of Bali bull

Abnormal genetics could contribute to reduced fertility in both female and male carriers of this abnormality. Kosarcic et al. (2006) reported that numeric and structural changes ion animal karyotypes influenced on reproduction disturbance, phenotype expression, and selection programs. The information obtained related to Madrasin cattle, a descendant of Madura and Limousine breeds, may be of benefit to a large number of cattle breeders or smallholder farmers to improve their animal genetics quality and productivity. Yimer and Rosnina (2014), mentioned that chromosome abnormalities in cattle could cause significant adverse effects on fertility through failure of production of viable gametes or early embryonic death that consequently leads to great economic loss. Chromosomal aberrations can occur as numerical errors or structural rearrangements usually without causing phenotypic abnormalities in carrier animals.

The dendrogram analysis indicated that banteng with Bali cattle form in one cluster (Cluster A), while PO and Brahman cattle form another closely related cluster (Cluster B). Madura cattle have a closer distance to Cluster A. Cluster B has the higher value similarity compared to

Madura cattle and cluster A. Historically, it was mentioned that Bali cattle and Madura were derived from banteng (*Bos javanicus*), which still exist in Java in part east of Baluran national forest parks, East Java province and west part of Ujung Kulon national park area, West Java province of Java island. It could be interpreted that Madura and Bali cattle was very possibly derived from banteng, which may have crossed with Brahman and PO.

It was concluded that the karyotyping performed on Bali and Madura's bull used for AI indicated that they fall in the normal category. It was very important to perform this chromosomal investigation of all candidate breeding bulls before using these bulls for commercial AI semen production. It is recommended to perform chromosomal investigation of all local candidate breeding animals of an important Indonesia local breed, especially those to be used for AI in the near future. It is necessary that candidate Bali and Madura bulls for AI must be screened to cull out any chromosomal abnormalities.

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Biological parameters of fish stock estimation in Cirata Reservoir (West Java, Indonesia): A comparative analysis of bio-economic models

ZUZY ANNA♥, ASEP AGUS HANDAKA SURYANA, INE MAULINA, ACHMAD RIZAL, PURNA HINDAYANI
Department of Fisheries Socio-Economics, Faculty of Fisheries and Marine Science, Universitas Padjadjaran. Jl. Raya Jatinangor Km 21, Jatinangor-Sumedang, Sumedang 40600, West Java, Indonesia. Tel. +62-22-87701519. Fax. +62-22-87701518. ♥email: suzyanna18@gmail.com

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Abstract. *Anna Z, Handaka AA, Maulina I, Rizal A, Hindayani P. 2017. Biological parameters of fish stock estimation in Cirata Reservoir (West Java, Indonesia): A comparative analysis of bio-economic models. Biodiversitas 18: 1468-1474.* Fish resources in reservoirs such as Cirata have a strategic value, especially for the surrounding community in terms of fulfilling the need for nutricious food, subsistence, and economic purposes. Unfortunately, until now there has been no serious attention from the government to develop and manage fish resources in these waters. From time series data it can be seen that there is a decrease in fish production, which is an indication of a decrease of fish stock, which has a negative impact on the wellfare of surrounding commmunity, as well as the sustainability of fish resources and their ecosystems. To meet the food safety and economic needs of the community, a healthy and sustainable fish resource is a must, characterized by sustainable inputs and outputs, so that the stock of fish resources will be maintained. In order to fulfill this need, a basic understanding of the biological and economic conditions of the dynamics of fish resources in these waters as a basis for sustainable management is needed. Research was conducted to calculate the biological parameters of fish resources through Fox, CYP, Walters Hilborn and Schnute algorithms, using quarterly catch and effort data from 2011 to 2015, collected from secondary and cross checking data. Furthermore, using price and cost data series, adjusted by consumer price index, the variable of input, output and economic rent of sustainable, optimal and open access management regimes were analyzed. The results of the study indicate that the most statistically appropriate model for estimating biological parameters in Cirata are the Fox and CYP models, and fisheries management using the optimal regime provides the most efficient results, where fewer inputs will result in the maximum profit. The research suggests the need for immediate enforcement of management rules based on sustainable management regimes through input or output restrictions on capture fisheries in Cirata Reservoirs.

Keywords: Biological parameters, fish stock, bio-economic models, sustainable and optimal

INTRODUCTION

Fish resources in reservoir waters are resources that contribute to the food security and the economy of the surrounding community. Capture fishery is one of the important activities related to fish resources that contributes the most to the surrounding community compared to capital-intensive cultivation activities. Capture fisheries actvities both subsistence, commercial, and recreational, also have a major impact on the growth dynamics, carrying capacity, availability, and sustainability of fish stocks in these waters, apart from the environmental conditions of the waters, and other external impacts. The sustainability of fish resources in these waters is not only of great significance to the local people who depend on these resources but is also important as one of the biotic configurations for the sustainability of the reservoir ecosystem (Angeler et al. 2005, 2002a, b, 2003 (see References); Zimmer et al. 2003; Bell and Kalff. 2001). Fish can be the counterweight to the aquatic system through its various functions in nature in the food web systems, even further into microclimate balancing and preventing the spread of infectious diseases due to the development of larvae of insects such as mosquitoes in waters, and controlling the development of aquatic weeds. (Van Dyke et al. 1984; Pauly et al. 1998; Sutton et al. 2014; Edmunds 2015; Sullivan et al. 2015).

Thus, the availability of an adequate fish stock will be very beneficial for the sustainability of the dam ecosystem. The exploitative behavior that is improper according to the rules, excessive, and tends to be destructive will greatly affect the availability of fish stocks in the waters. Utilization of water resources as a fish habitat that is also not right such as pollutive behavior, sediment accumulation and flood problems both from activities in the waters themselves and from the mainland that deliberately discharged into the waters will also greatly affect the reservoir's demands, riverine ecosystem, habitat and fish stocks in the waters (Saha and Setegn 2015a 2015b (see References); Grigg 2016; Gorenflo and Warner 2016; Xu et al. 2014; Chen and Tsai 2017 - not in References). Cirata Reservoir is an example of a water body that suffered decreasing fish stocks, characterized by the decreasing number of catches from year to year as a result of various production activities such as capture fisheries and cultivation, as well as non-production activities such as the disposal of liquid and solid waste into the waters (Anna, 2016) . There is a concern that this condition would cause the depletion of fish resources so that they become scarce and, more importantly, would cause extinction of important economic types of fish species, and even endemic fish that characterize the waters here.

The information on the biological parameter conditions of the fish stocks becomes important for stakeholders associated with reservoir management, as a basis for policy-making for the general management of integrated reservoirs and in particular the utilization of fish resources in the region. The data of the biological parameter values until now have not existed for this reservoir, especially for the total production and effort available those obtained from various bio-economic analyzes simultaneously. This research will calculate the value of biological parameters such as the intrinsic growth as well as the ability of environmental carrying capacity and capability of catching fish resources in Cirata Reservoir waters by using various models of analysis estimation of fish biology parameters. Those biological paramaters could be very usefull for further analysis of sustainable and optimal management of fisheries in the reservoir.

MATERIALS AND METHODS

This research was conducted in Cirata Reservoir using the data series of total capture fishery input and output every quarter from 2010 to 2016. The economic data of price and nominal average costs were obtained from cross section and adjusted to inflation to get real price per year. This economic data was obtained from interviews of 100 fishermen using various types of fishing gear used in these waters, namely gillnet, cast net, and hand line. Due to the difference in capability of the fishing gears, the standardization was done using gillnet as the base calculation, using technique developed by King (1995), as follows:

$$E_{jt} = j_{jt} D_{jt}$$

and

$$j_{jt} = \frac{u_{jt}}{u_{st}}$$

Where,

E_{jt} = Effort from gear j at t standardized

D_{jt} = Fishing days) of gear j at t period

J_{jt} = Fishing power of gear j at t period

u_{jt} = Catch per unit effort (CPUE) of gear j at t period

u_{st} = Catch per unit effort (CPUE) of gear based for standardized

The analysis method used to observe the dynamics of fish in Cirata is the bio-economic method by using estimation of production surplus model. The surplus production concept is a basic concept of the fishery knowledge based on the idea that the increase of fish population will be obtained from the small fish that produced every year, whereas the decrease of the mentioned population was due to the mortality, either because of the natural factor or by the human exploitation. This model needs the data catch (yield) and effort, two kinds of data that were collected and published in the fishery statistics. While in the dynamic pool, the parameters of the fish population was sorted and counted one by one, so the model is often called the analytical model. Ideally, the analysis should be done for every single species, but in the study case, the multiple species is treated as single species, due to the nature of the data. The use of this surplus production model has been widely used in various models of multi-species fisheries estimation and multispecies aggregate or lumped model (Mueter and Megrey 2006; Fogarty et al. 2012).

In the model of production surplus, the dynamics of biomass are described as the difference between natural production and mortality as illustrated in the following equation:

Biomas in t+1 = biomas in t + production - natural mortality

The above equation states that if the production exceeds the natural mortality, then the biomass will increase, otherwise if natural mortality is higher than production, then the biomass will decrease. The term surplus of production itself illustrates the difference or deviation between natural production and natural mortality. Furthermore, Hilborn and Walters (1992) argue that the production surplus represents an increase in the number of fish stocks in the absence of catching activity or in other words, the amount that can be caught if the biomass is maintained at a fixed rate. In the case of the introduction of catching, logistic model analysis is used to analyzed production function.

In this study, the production surplus model to be used is the equilibrium state model and parameter estimation model from Fox (1970), Clark et al. (1992), Walters and Hilborn (1976) and Schnute (1977) and the production surplus model to estimate the value of biological parameters of fish resources in Cirata Reservoir, is a logistic model developed that assumes that population growth is consistent with the proportion of the initial population, called the growth rate of intrinsic fish or r. While the rate of change of fish stock is formulated as:

$$\frac{dx}{dt} = rx\left(1 - \frac{x}{K}\right)$$

Where: x is the fish stock or the biomass, K is carrying capacity, and r is the intrinsic fish growth rate. By introducing the capture fisheries (h) to this stock growth function that is the stock function as well, catchability coefficient (q), and input (E), or:

$$h = qxE$$

Thus, in a long term, the fish resources equilibrium would be:

$$qxE = rx\left(1 - \frac{x}{K}\right)$$

Thus, the correlation between the catch per unit effort with effort can be obtained as follows:

$$\frac{h}{E} = qK\left(1 - \frac{q}{r}E\right)$$

Table 1. Formula for Model Parameter Estimations

Model parameter estimations	Formula
Fox	(Ut+1-Ut-1)/2Ut=rLn (qK)-rln (Ut)-qLn (Et)
CYP	Ln Ut+1 = a + b Ln Ut +c (Et+Et+1)
Walters - Hilborn	((Ut+1/Ut) -1)=r - r/Kq (Ut) -q (Et)
Schnute	ln (Ut+1/Ut)=r - r/Kq (Ut+1 + Ut/2) -q (Et+1 + Et/2)

From the above equation, the parameter estimations of r, K and q for the equation yield-effort model involve non-linear technique. By using $U_t = h_t/E_t$ formula, (CPUE or catch per unit effort), equation could be transformed into a linear equation so that ordinary least square regression method can be used to estimate the biological parameters of the above functions. Fox, CYP, Walters Hilborn and Schnute algorithms are used to measure the biological parameters of fish through the following equation:

The Fox Model (Fox 1975) is a development of production surplus model for parameter estimation using equilibrium models based on Schaefer model analysis, in a linear-non-linear (natural logarithmic) form. The Fox model has several different characters than the Graham-Schaefer model. The difference is that the Fox model states that biomass growth follows the Gompertz growth model, and the Catch Per Unit Effort$_t$ (CPUE$_t$) (what is CPUEt?) decline in the catch effort follows a negative exponential pattern, which makes more sense than the linear regression pattern. While the CYP model uses log model to estimate biological parameters, in a discrete form of logarithmic in natural logarithmic, which is a quite simple but logic in the natural system. In the Walters-Hilborn estimation model (Walters and Hilborn 1976), the production surplus model developed is the difference model, the discrete version. While on the Schnute model, which is also a modification of the Schaefer model, is using non linear analysis, log and also reciprocal. The Schnute model (Schnute 1977) presents another version of the dynamic, discrete in time, and deterministic model of production surplus from the Graham-Schaefer way. On the other hand, it provides a dynamic, stochastic time model, and is specific to the surplus production model as opposed to static, deterministic, and continuous models of other Graham-Schaefer models. The Schnute model is seen as a modification of the Schaefer model in discrete form. The parameters estimated from those models are intrinsic growth rate (r) refer to how much a population can grow between successive time periods, which often estimated with production models and plays an important role in evaluating the sustainability of different harvest levels and the capacity to recover after depletion; Carrying capacity (K) refer to the number of biomass of a particular species that the resources in the habitat can support (Myers et al. 2001); and Catchability coefficient (q), refer to fish caught per fish available per effort unit and per time unit. Catchability is also called gear efficiency (Hilborn and Walters 1992) or sometimes fishing power, and is strongly related to gear selectivity.

The use of these models is comparable, to see how the statistics perform and also to determine the approximate estimates of the biological parameter values. The data Regression analysis is done using Stata SE 12, and Minitabs. The calculation of the sustainable and optimal production and efforts was done using MAPLE 17.0. software and Microsoft Excell.

RESULTS AND DISCUSSION

Results

Cirata Reservoir include 18 species of fish from eight families, namely Cyprinidae, Bagridae, Eleotridae, Cichlidae, Pangasidae, Ophiocephalidae, Chanidae and Characidae (Jubaedah, 2004). While the abundance of fish based on the study of Wahyuni et al. (2014) (something not quite right in the start of this sentence) in observation of February, dominated by *Hampala macrolepidota* (40,74%) and *Oreochromis niloticus* (18,67%), March was dominated by Oxyeleotris marmorata (89,65%) and *Oreochromis niloticus* (19,89%), April was dominated by *Chanos chanos* (62.79%) and *Oreochromis niloticus* (20.48%), May was dominated by *Hampala macrolepidota* (14.81%) and *Oreochromis niloticus* (12.65%), June was dominated by *Aequidens pulcher* (36.47%) and *Amphilopus citrinellus* (30.18%), July was dominated by *Mystus nigriceps* (80.43%) and *Amphilopus citrinellus* (44.33%).

The data used in this study is quarterly, because that is what is available. In each quarter only production data varies, while effort is relatively fixed in every quarter of every year, and only changes in the next year. This is because data collection is only done once? per year. So the quarterly effort is assumed to be the same every year. From the quarterly production, Cirata fisheries shows significantly decrease from year to year. The average effort trend is increasing from year to year, with the total effort at the end of 2016 as much as 60984.94 trips, which comes from three types of fishing gear such as Handline, Castnet and Gillnet. The average trip of the fishing gear is - (what does this mean? once?) twice a day with a day of catching fish on average 25 days per month. Cirata fisheries is also characterized by a relatively low CPUE value, and relatively decreased throughout the year. By the end of 2016, the value of CPUE is 2 Kg per trip, as shown in Table 2.

The results of statistical performance analysis of the development of Fox model, Walters Hilborn, Schnute and CYP can be seen in the table 3 (do you mean Table 3? Indicate which table). It appears that the Fox model which specificly use Schaefer model for regression to get constant and E_t value, has a fairly good statistical performance, where CYP has the highest R^2 value of 81.4%, which means that the dependent variable can be explained by the explanatory variable of 81.4% and the rest by other variables outside the model. Furthermore, the R^2 value for the Fox model is 25.6, while other models have low R^2 values, even 0.0% for the Schnute model. Although the actual value of R^2 in the regression analysis of the bio-economic model which purpose is to obtain the parameter

Table 2. Cacht Per Unit Effort (CPUE)

Year	Quarterly	Total standard effort (trip)	Actual production (ton)	CPUE
2011	1	50215,87	328,55	0,007
	2	50215,87	341,79	0,007
	3	50215,87	335,04	0,007
	4	50215,87	337,29	0,007
2012	5	53476,47	319,16	0,006
	6	53476,47	315,19	0,006
	7	53476,47	315,64	0,006
	8	53476,47	295,39	0,006
2013	9	44176,47	224,32	0,005
	10	44176,47	147,32	0,003
	11	44176,47	128,32	0,003
	12	44176,47	165,32	0,004
2014	13	57661,22	243,78	0,004
	14	57661,22	166,78	0,003
	15	57661,22	147,78	0,003
	16	57661,22	184,78	0,003
2015	17	58840,93	129,44	0,002
	18	58840,93	117,44	0,002
	19	58840,93	166,44	0,003
	20	58840,93	170,44	0,003
2016	21	60984,94	135,54	0,002
	22	60984,94	106,27	0,002
	23	60984,94	131,77	0,002
	24	60984,94	129,83	0,002

value is not a very important issue, but the value of R^2 is expected not to be too low (below 25%) (Anna 2003; see reference in dissertation). When viewed from the P value, only Fox and CYP variables that have significant value, with 90% and 95% confidence levels. To determine the effects of all variables on F probability, as can be seen from the table, F probability has a value of less than 0.05 or 5% for Fox and CYP Models, meaning that all variables of independent variables jointly affect the dependent variables. While for Walters Hilborn and Schnute have a value of more than 0.5 both models, meaning that all variables jointly do not affect the dependent variables. For the Durbin-Watson statistic (DW), all model's values are in the range of -2 <DW <2, indicating that there is no interference autocorrelation on all models. The Varian Inflation Factor (VIF) values below 10 indicates that there are no multi-collinearity between variables. Look into the yield effort model resulting from the regression analysis, can be seen that the model that according to the original formulation model and following the right sign for coefficient are Fox. Walters Hilborn and CYP model, while Schnute does not have an appropriate coefficient sign. It means that Schnute model couuld not be consider to be the good model for the Cirata's Fisheries parameters estimation. From the regression analysis, it appears that the Fox model and the CYP Model have the best statistical performance.

By using various variations of the production surplus model, the estimation of biological parameters for fisheries in Cirata Reservoir is shown in the following table. The result shows that , if (meaning not clear here) it refers to the significance of the model, the Fox and CYP parameter is a relatively more suitable parameter that can be used in further analysis for fisheries sustainability in Cirata Reservoir. In addition, the value of biological parameters especially carrying capacity in the Fox (Schaefer regression) model close to reality. Referred to the amount of production per quarter, the average production is only around 100-300 tons.

For further analysis, finding sustainable yield and also optimal yield can be only done by using Fox and CYP models that have the best fits compared with other models. Optimization analysis is conducted on various management instruments, namely Maximum Economic Yields, Maximum Sustainable Yield, Open Access, and Optimal dynamic. In the optimal dynamic model, elasticity analysis is performed by using various discount rate values and the results for the Fox model can be shown in Table 5. The highest biomass will be obtained if the fishery is managed by using sole owner or Maximum Economic Yield (MEY), with biomass 459 Ton, with the most efficient effort of 33.309 trips. However, if the goal is to get the highest profit, the recommended instrument used is optimal dynamic with 5% discount rate. Currently, this discount rate is the lowest bank interest rate (BI Rate 2010-2016). Open access management are the lowest production value, the highest effort, the lowest biomass value, and zero profit value, so it is not a sugested instrument to be implemented. Thus, the management with a Maximum Economic Yields (MEY) instrument and also an optimal dynamic can be suggested for Cirata's fishery, so that fish resources can be optimal and sustainable.

Discussion

The results show that the most statistically appropriate model for estimating biological parameters in Cirata are the Fox and CYP models. This is in line with previous studies related to fish resources in tropical regions such as Indonesia with fish resources in multi-species waters and multi-gears, the use of Fox and CYP models is considered the most appropriate compared to using other models such as Walters Hilborn and Schnute (Pasisingi 2011; Sari 2012; Tinungki et al. 2004; Tinungki 2005; Triharyuni et al. 2014; Kekenusa et al. 2014). The two best model are also consider to be the appropriate model for multispecies aggregate or lumped model (Mueter and Megrey 2006; Fogarty et al. 2012).

The Fox model (Fox 1997) is basically a model that has its growth characteristics following the Gompertz model. In this model, the CPUE correlation and the production or catch results are in accordance to negative exponential pattern, meaning it makes more sense when compared to the linear regression model. In addition the Fox model also uses some logical assumptions such as that the population as a number of fish populations will not be extinct, and the relation between effort and catch is exponential with an asymmetric curve. While, the CYP model is a discrete form of logarithmic in natural logarithmic which is also simple but logical, it becomes the model that generates the best fit on biological parameter estimations of fish resources in Indonesia (Anna 2003; Fauzi and Anna 2005; Anna 2016). Thus, the parameter estimation model of Fox and CYP is

suggested to be applied in Cirata's fish stock assessment. The results of the analysis using both models show that the biological parameter values randomly ranged from 0.18-0.12; K between 815 tons to 4144 tons and q between 0.000016 up to 0.000008, relatively logical value if looking from the actual production conditions.

Table 3. Statistic performance for all models

Variable	Model											
	Fox (Schaefer)			Walters Hilborn			Schnute			CYP		
	Coefficient	P Value	VIF	Coefficient	P value	VIF	Coefficient	P value	VIF	Coefficient	P value	VIF
Constant	0.01299**	0.0000		0.4195	0.438		-0.094	0.88		0.5533*	0.0327	
Et	-0.00000017**	0.007	1,000	-0.0000058	0.509	1,357						
Ut-1				-33.8	0.237	1.357						
Et+Et+1										0.00000386**	0.0044	1.583
Ln Ut										0.8352**	0.000	1.583
(Et+Et+1)/2							0.00000072	0.943	1.465			
(Ut+Ut+1)/2							1.65	0.959	1.465			
R^2	29.1			6.9			0.00			81.4		
Adj R^2	25.9			0.00			0.00			79.6		
F Statistik	9.03			0.75			0.00			43.9		
Prob F Stat	0.007			0.49			0.97			0.000		
Durbin Watson	0.736			2.071			2.132			1.934		
Formulation	Ut = 0.0130 - 0.000000 Et			(Ut+1/Ut)-1 = 0.420 - 33.8 Ut-1 - 0.000006 Et			Ln Ut+1/Ut = - 0.094 + 1.6 (Ut + Ut+1)/2 + 0.000001 (Et + Et+1)/2			Ln CPUEt+1 = 0.553 + 0.835 Ln CPUEt + 0.000004 Et+Et+1		

Notes: * significant at the confidence interval 95 %, ** significant at the confidence interval 90%

Table 4. Biological Parameterization of all Models

Parameters	Fox	Walters Hilborn	Schnute	CYP
Intrinsic Growth (r)	1.217	0.420	0.094	0.180
Carrying Capacity (K) Ton	815.00	2139.87	79948.991	4144.41
Catchability Coefficient (q)	0.000016	0.000006	0.0000007	0.000008

Table 5. Fox model optimization analysis

Variable	Sole owner/MEY	Open access/OA	MSY	Optimal dynamic (i=5)	Optimal dynamic (i=6)	Optimal dynamic (i=7)	Optimal dynamic (i=8)
x (ton)	459.96	104.52	407.70	447.44	445.03	442.65	440.31
h* (ton)	244.07	110.92	248.15	245.79	246.07	246.32	246.56
E* (ton)	33308.64	66617.27	38205.88	34482.26	34707.95	34930.76	35150.75
µ (Profit)	2548.67	0.00	2493.58	52172.62	43662.68	37580.27	33015.14

Table 6. CYP model optimization analysis

Variable	Sole owner/MEY	Open access/OA	MSY	Optimal dynamic (i=5)	Optimal dynamic (i=6)	Optimal dynamic (i=7)	Optimal dynamic (i=8)
x (ton)	2167.82	191.23	2072.20	87.25	1577.03	1487.53	1401.04
h* (ton)	185.69	32.76	186.08	5.85	282.77	265.07	-515.60
E* (ton)	10191.79	20383.57	10684.79	7973.25	21334.59	21202.71	-43788.62
µ (Profit)	2370.28	0.00	2364.74	-1999.20	59701.17	47797.64	-80991.89

Optimization analysis on both Fox and CYP models shows that the management instrument that produces the most efficient condition is the Maximum Economic Yield (MEY). This management regime shows the highest biomass value, with the most relatively conservative effort, but high rent, although not as high as the dynamic management. This can be explained because the MEY is achieved in a condition where the marginal revenue curve cuts the marginal cost curve (Fauzi 2004; Fauzi and Anna 2005; Anderson 2010), so that the profit will be maximized. While in the open access condition, total revenue equals to the total cost, so the profit earned becomes zero. Thus, the management of MEY-based instruments is strongly recommended to be applied in Cirata Reservoir.

This MEY-based management can be operationally operated through input or output restrictions, depending on the socio-economic conditions of the community. If the community is still in need of fish resources as food and is subsistent, it is better to limit or impose quota (what is this?) to the output, by dividing the optimal production value in the MEY regime by the number of fleets or fishing gears available proportionately. While if the condition of the society is more prosperous and more commercial fishing activities occurs, then input restrictions can be suggested (Anna 2016). This is due to the nature of Indonesian fisheries and fishers characteristic, where input restriction will impacted to the poors and increase the number of unemployee, which often leads to potential conflict and social instability. The input limitation/quota can be done by referring to the optimum number of inputs (trip) in MEY, and adjusting to the number of available fleets, so the number of fishing gear or fleet that should be limited can be found, for sustainable fish resources.

However, this study still needs further research focusing on species, to determine the biological parameter values of each fish species present in this body of water using various models. Understanding the the biological parameter dynamics of any fish species present in these reservoir waters will be highly beneficial for sustainable management. In addition, there should also be an optimal measurement of input and output for each species of fish in the Cirata Reservoir, so that the management can be directly conducted on each species of fish to obtain the optimal and sustainable conditions.

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Short Communication: Ant (Hymenoptera: Formicidae) diversity as bioindicator of agroecosystem health in northern slope of Mount Slamet, Central Java, Indonesia

IMAM WIDHIONO♥, RIZKITA DINDA PANDHANI, DARSONO, EDI RIWIDIHARSO, SLAMET SANTOSO, LUCKY PRAYOGA

Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Soeparno 63, Purwokerto, Banyumas 53122, Central Java, Indonesia. Tel. +62-281638794, Fax.: +62-281-631700, ♥email: imamwidhiono@yahoo.com

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Abstract. *Widhiono I, Pandhani RD, Darsono, Riwidiharso E, Santoso S, Prayoga L. 2017. Short Communication: Ant (Hymenoptera: Formicidae) diversity as bioindicator of agroecosystem health in northern slope of Mount Slamet, Central Java, Indonesia. Biodiversitas 18: 1475-1480.* This study examined the diversity of ant assemblages in two different agroecosystems (organic and intensive farming) with maize as main crops on the northern slope of Mount Slamet, Indonesia from April to August 2015. The response of ant diversity to the different farming systems was evaluated. In total, 359 ants from 17 species in five subfamilies were collected: 13 species from the organic farm and 10 species from the conventional farm. Seven (41%) species were found only on the organic farm, four (23%) species were found only on the conventional farm, and six (35%) species were found on both. The Morisita-Horn similarity index (33%) showed that the similarity of the species composition in the two habitats was low. More individual ants were found on the organic farm than the intensive farm [287 (79.94%) *vs.* 72 (20.05%) individuals]. The diversity of the ants was affected by the density of weeds with organic farming. *Hypoponera* sp. had the highest Indicator Value (50.7), followed by *Aphaenogaster* sp. (28.9), *Diacamma* sp. (28.2), and *Odontoponera* sp. (27.6), however only *Odontoponera* sp can be used as bioindicator. Although the ant diversity was higher with organic farming than with intensive farming, there was no correlation with soil conditions. As a bioindicator, the Indicator Value reflects agroecosystem health better than does ant diversity.

Keywords: bioindicators, indicator value, intensive farming, organic farming

INTRODUCTION

Recent agricultural intensification and structural changes in the agricultural landscape have led to the over-use of agrochemicals, which in turn has caused a decline in farmland biodiversity. Intensive agricultural practices drive biodiversity loss with potentially drastic consequences for ecosystem services. Increased awareness of the high environmental costs of agricultural intensification has prompted a search for solutions that promote the preservation and restoration of natural resources (Mone et al. 2014). To advance conservation and production goals, agricultural practices should be compatible with biodiversity. In organic farming, crops are grown using organic waste and other biological materials along with beneficial microbes (biofertilizers) for increased sustainable production without spoiling soil health. Organic agriculture is a unique production management system that promotes and enhances agroecosystem health, including biodiversity, biological cycles, and soil biological activity, and this is accomplished using on-farm agronomic, biological, and mechanical methods that exclude all synthetic off-farm inputs (Jahanban and Davari 2012). Organic farming contributes to the preservation and enhancement of biodiversity, but it is unable to ensure sufficiently high production levels (Hole et al. 2005; Rundlöf et al. 2010).

Ants are important components of ecosystems, not only because they constitute a significant portion of the animal biomass but also because they act as ecosystem engineers. Ant biodiversity is incredibly high, and these organisms are very responsive to human impacts, which obviously reduce their richness. However, it is not clear how such disturbances damage the maintenance of ant services to the ecosystem. Ants are important in below-ground processes by altering the physical and chemical environments and affecting plants, microorganisms, and other soil organisms. Kinasih et al. (2016) found that the family Formicidae (ants) is dominant in coffee-pine agroforests compared with pine forests and is an important part of the litter decomposition process (Musyafa 2005). The diversity of ants is correlated with the above-ground vegetation as food resources and protects against environmental disruption (Rubiana et al. 2015). Organic farming results in higher weed diversity than does intensive farming (Mone et al. 2014). Widhiono et al. (2017) found that vegetation cover affected the diversity of endemic dung beetles. The diversity and abundance of ants differed significantly according to habitat type in Jambi (Yuniar and Haneda 2015), and ant diversity shows strong negative responses to agricultural practices such as fertilization, pesticide spraying, and burning (Uno et al. 2010)

Many ant species are very sensitive to microclimate fluctuations and habitat structure and respond strongly to

environmental changes (Paknia and Pfeiffer 2011). Rizali et al, (2013) found precipitation as the main environmental factors that affected ant communities. Increasing agricultural practices in intensive farming systems will decrease ant diversity, which alters the affected area (Andersen and Majer 2004). Ants are sensitive to disturbances and rehabilitation (Andersen et al. 2002), and diversity shows strong negative responses to agriculture intensification (Philpott 2010). Ants are considered suitable bioindicator species of ecosystem health because of their ecological significance in agroecosystems (Alonso 2000). Most habitats are likely to have specialized species, which occur when species diversity and abundance are sufficient, and these species serve as suitable terrestrial indicator species of habitat quality and changes. However research on ant diversity for agroecosystem health is very rare in Java and therefore this study was carried out to compare the diversity of ants between intensive and organic farming systems and analyze the potential of ants as bioindicators of agroecosystem health in each system.

MATERIALS AND METHODS

Study area

The study was conducted in Nyalembeng Village (7°10'01.76" S and 109°14',46.60"E) in the highland district on the northern slope of Mount Slamet, in Sub-district of Pulosari, District of Pemalang, Central Java Province, Indonesia. On the organic farm, the farmer applies only organic fertilizers such as farmyard manure, vermicompost, and biofertilizers. The farmer followed organic farming practices for the previous 3 years in the same field. The conventional farm, on which chemical fertilizers and chemical pesticides (herbicide atrazine 600 g/L with doses 1.5 L/ha) were applied, was located in the same village. This farmer used chemical pest control and crop management and applied synthetic fertilizers such as urea and phosphate. A 1-acre maize field area was selected for insect sampling on both the organic and chemical farms. Both fields were surrounded by maize cultivation (Figure 1)

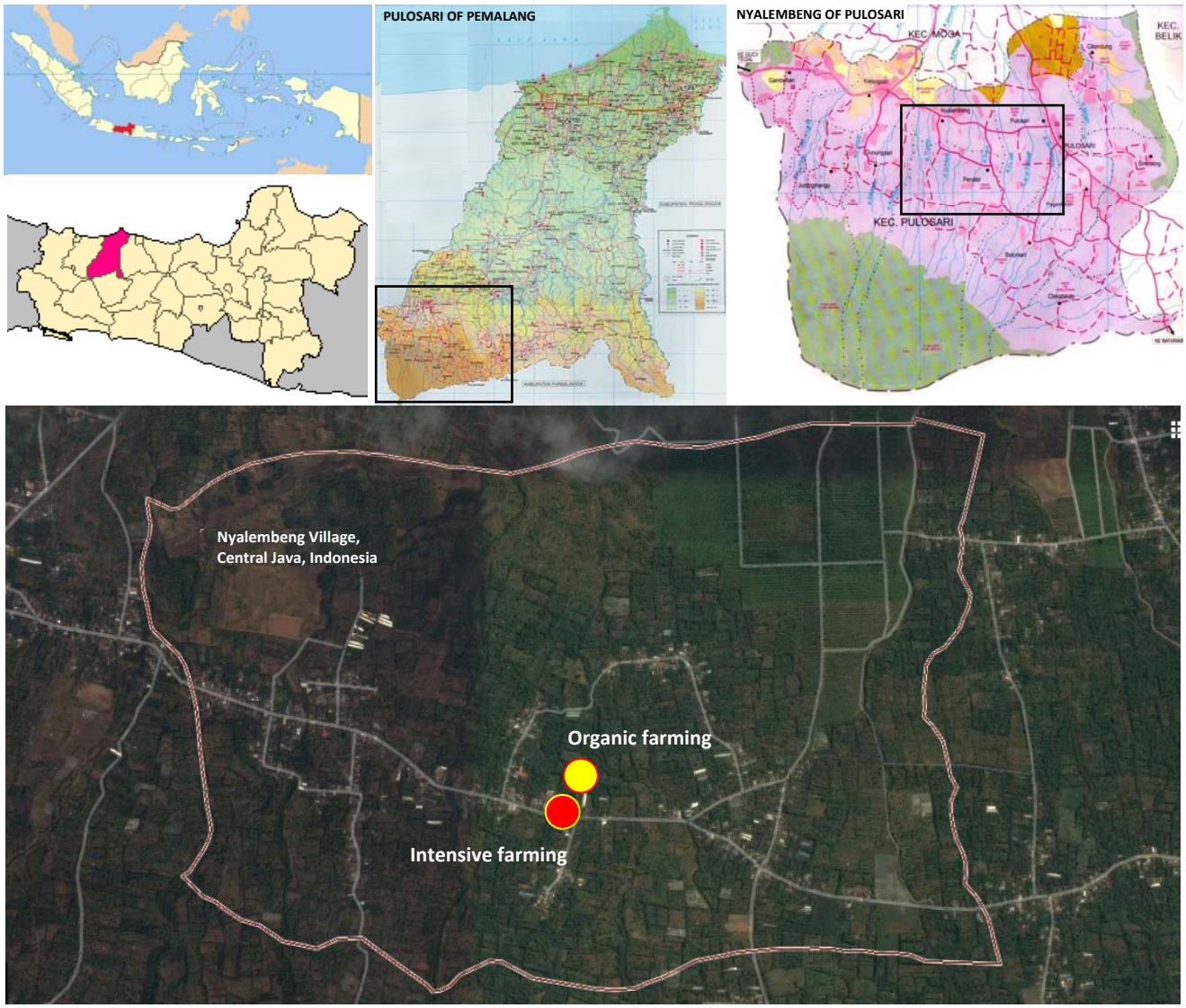


Figure 1. Sampling location in Nyalembeng Village, Sub-district of Pulosari, District of Pemalang, Central Java Province, Indonesia

Ant collection

Ants were collected from 10 random 1 m × 1 m quadrates in each farm using pitfall traps. Sampling was conducted five times from April to August 2015. Ants were identified using Fayle (2010).

Ant functional groups

Ant functional groups were classified according to Andersen et al. (2004), and Brown (2000) as follows: (i) Dominant Dolichoderinae (DD): genera *Iridomyrmex*, (ii) Subordinate Camponotini (SC): genera *Camponotus, Echinopla, Polyrhachis*, (iii) Tropical-climate Specialists (TCS): genera *Cladomyrma, Tetraponera, Myrmecina, Solenopsis, Dolichoderus, Myrmicaria, Vollenhovia, Epelysidris, Acanthomyrmex, Pristomyrmex, Anoplolepis, Acropyga*, (iv) Opportunists (O): genera *Tetramorium, Paratrechina, Paraparatrechina, Nylanderia, Cardiocondyla, Technomyrmex, Tapinoma, Aphaenogaster, Ochetellus*, (v) Generalized Myrmicinae (GM): genera *Pheidole, Crematogaster, Monomorium*, (vi) Specialist Predators (SP): genera *Pachycondyla, Odontoponera, Anochetus, Leptogenys, Platythyrea*, (vii) Cryptic species (C): genera *Mayriella, Ponera, Carebara, Hypoponera, Pheidologeton, Plagiolepis, Mystrium, Dacetinops, Calyptomyrmex, Amblyopone, Strumigenys, Procertium, Probolomyrmex, Eurhopalothrix, Centromyrmex, Cryptopone, Discothyrea, Protanilla, Cerapachys*

Measurement of environmental factors

For each farming system, five soil samples were collected, and two soil physical characteristics (soil humidity and soil temperature) and one chemical characteristic (soil pH) were measured. Vegetation in each plot was quantified as the measured weed density per square meter.

Data analysis

The total number of individuals of each ant species collected was recorded. Shannon's index (H'), Fisher's alpha (α), and the Simpson (D) and Evenness (E) indices were derived from data collected from both organic and conventional farming. PAST software was used to calculate the diversity indices.

The Indicator Value (IV) for each ant species was calculated using Dufrêne and Legendre's (1997) IV method (Dai et al. 2006) with the formula: IV = RA × RF × 10; where, RA is the relative abundance determined by the formula RA = n/N × 100%, where n is the number of individuals of a specific species recorded, and N is the total number of individuals recorded. Similarly, the relative frequency of occurrence (RF) was calculated as RF = f/F × 100%, where f is the frequency of detecting a particular species, and F is the total number of species recorded. The IV ranges from 0 (no indication) to 100 (perfect indication).

Simple correlation analysis was used to evaluate the impacts of environmental factors and vegetation complexity and density on ant diversity.

RESULTS AND DISCUSSION

Ant diversity and abundance were compared between organic and intensive farming. During the study, 17 ant species belonging to 16 genera and five subfamilies were identified from a sample of 321 workers. Of the subfamilies, Ponerinae was dominant in terms of species richness (5 species) and abundance, followed by Myrmicinae and Dolichoderinae (4 species each), Formicinae (3 species each), and Pseudomyrmecinae (1 species). Comparing the ant communities, more ant species were detected in organic farming (13 species) than conventional farming (10 species): seven species (*Odontoponera* sp., *Acanthomyrmex* sp., *Pristomyrmex* sp., *Tetramorium* sp., *Prenolepis* sp., *Cladomyrma* sp., and *Iridomyrmex* sp. (41%) were found only on the organic farm, comparing only four species (*Hypoponera* sp. 2, *Emeryopone* sp., *Technomyrmex* sp., and *Tapinoma* sp.) (23%) were on the conventional farm, and six species (*Diacamma* sp., *Hypoponera* sp. 1, *Aphaenogaster* sp., *Lepisiota* sp., *Dolichoderus* sp., and *Tetraponera* sp.) (35%) were found on both farms (Table 1, Figure 2).

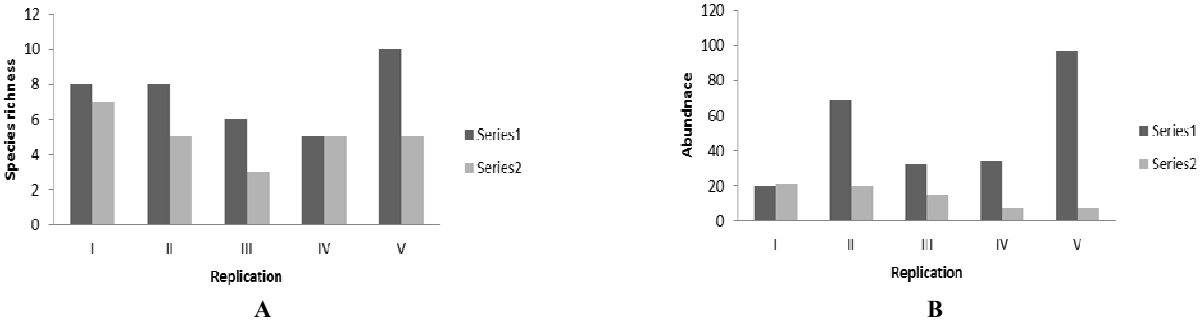
This result showed that more ant species and greater ant abundance were recorded on organic farms. This is in line with the result of Bharti et al. (2016) who has done the research of ants as bioindicators of ecosystem health in Shivalik Mountains of Himalayas. This finding could be explained by the intrinsic need for resources and biotope condition patterns required by each ant species, and because a more structured and complex habitat provides the best environmental conditions for all ants and relative habitat structure can induce major variability in ant diversity. In Jammikunta, India, ant diversity was affected by vegetation structure (Ravi et al. 2015), but Rizali et al. (2013) found that the main factor of dissimilarity of ants communities was precipitation rate. Vegetation structure may improve soil invertebrate abundance and the diversity, Kinasih et al. (2016) found that the diversity of soil macroinvertebrates was highest on a coffee plantation and lowest on a pine plantation. Agroforestry may improve soil invertebrate abundance and the diversity of monoculture pine forests through the creation of additional and alternative nutrition and microhabitats. However, Yudiyanto et al. (2014) obtained different results and found that the farming system on pepper plantations had no significant effect on ant diversity, while habitat conditions and the surrounding habitats had greater effects on ant diversity. In general, agricultural practices such as heavy grazing, irrigation, drainage, fertilization, mowing, conventional tillage, plowing, and reseeding reduce ant biodiversity and colony densities (Rubiana et al. 2015). Despite this reduction in biodiversity, ants seem to tolerate, recover, or re-invade the same areas after disturbance (Folgarait 1998).

Table 1. Species richness, relative abundance (RA%), relative frequency (RF%) , indicator value (IV) and functional groups (FG) of ants in organic farming (OF) and intensive farming (IF)

Family	Species	OF				IF			FG
			RA (%)	RF (%)	IV		RA (%)	RF %	IV	
Ponerinae	*Odontoponera* sp.	86	17.2	60	27.6	0				P
	Diacamma sp.	77	15.4	26.36	26.82	2	2.8	40	28.2	O
	Hypoponera sp.1	23	4.6	100	46.2	35	50.7	100	50.7	C
	Hypoponera sp.2	0				3				C
	Emeryopone sp.	0				1				C
Myrmicinae	*Aphaenogaster* sp.	50	10	100	17.1	20	28.98	100	28.9	O
	Acanthomyrmex sp.	2	0.4	0.6		0				TCS
	Pristomyrmex sp.	2	0.4	0.6		0				TCS
	Tetramorium sp.	2	0.4	0.6		0				TCS
Formicinae	*Prenolepis* sp.	3	0.6	1		0				TCS
	Cladomyrma sp.	5	1	1.7		0				TCS
	Lepisiota sp.	14	1.8	4.7		1				C
Dolichoderinae	*Dolichoderus* sp.	10	2	3.42		1				DD
	Iridomyrmex sp.	14	2.8	4.7		0				DD
	Technomyrmex sp.	0				2				DD
	Tapinoma sp.	0				1				DD
Pseudomyrmecinae	*Tetraponera* sp.	4	0.8	1.3		3				TCS

The density and complexity of weeds were greater in organic farming than in intensive farming. We found 28.2 individuals/m^2 and eight species on the organic farm and 8.6 individuals/m^2 and six species on the conventional farm (Table 2). This difference might result from the weeding method: hand mowing was used with organic farming, while herbicides were used with intensive farming. According to El Sebai and El Tawil (2012), most foliar pesticides are toxic to many non-target organisms, such as beneficial arthropods, including predators and parasitoids. The correlation analysis showed a strong relationship between ant diversity and weed density (r = 0.76) and a weaker relationship between ant diversity and weed complexity (r = 0.36). We hypothesized that organic farming, which involves relatively high weed density and complexity, creates a wider potential diversity of available ecological niches. Our findings were consistent with this hypothesis, since the ant species richness and diversity differed significantly with vegetation structure. Vasconcelos et al. (2008) found that vegetation structure and vegetation cover affected ant diversity in Amazonian savannah. Ant community structure also differed among the vegetation domains evaluated in Brazilian tropical dry forest (Silva et al. 2017).

The Species Richness (S), Shannon-Wieners Diversity Index ($H^{'}$), and Evenness (J) differed between the two habitats. There was less variation in the ant $H^{'}$ in the organic field ($H^{'}$ = 1.006-1.791) than in the intensive field ($H^{'}$ = 0.8532-1.494). Values greater than 1.00 indicate that the habitat supports the survival of ant species. J ranged from 0.4382 to 0.7496 in the organic field and from 0.5241 to 0.8911 in the intensive field. Examining the correlations between diversity and environmental parameters, there were weak correlations of diversity with soil pH (r = 0.23) and soil humidity (r = 0.37). This result was in accordance with Jacquemin et al. (2012), who found that soil properties weakly affected the subterranean ant distribution at small spatial scales.



A B

Figure 2. A. Species richness, B. Abundance of ants in organic farming and intensive farming

Table 2. Species richness and density of weeds in organic farming (OF) and intensive farming (IF)

Species	OF					IF				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Amaranthus spinosus	5	3	0	2	5	0	0	0	2	0
Ageratum conyzoides	0	1	4	4	1	0	1	0	4	1
Ipomoea sp.	2	5	5	3	4	2	0	5	3	0
Alternanthera philoxeroides	9	5	0	3	5	0	5	0	3	0
Synedrella nodiflora	2	3	6	2	6	2	3	0	2	0
Portulaca oleracea	5	3	5	5	5	5	3	2	0	0
Physalis longifolia	7	4	0	7	0	0	0	0	0	0
Galinsoga ciliata	4	5	2	0	4	0	0	0	0	0
Total	34	29	22	26	30	9	12	7	14	1
Means	4.25	3.62	2.75	3.25	3.75	1.12	1.5	0.875	1.75	0.25

Table 3. Diversity parameters of ants and the environmental factors in organic and intensive farming

Parameter	Organic farming					Intensive farming				
	I	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
Taxa_S	8	8	6	5	10	7	5	3	5	5
Individuals	20	69	32	34	97	21	20	15	8	8
Dominance_D	0.22	0.4068	0.2715	0.4948	0.2282	0.415	0.34	0.4756	0.25	0.25
Shannon_H	1.791	1.254	1.476	1.006	1.789	1.3	1.257	0.8532	1.494	1.494
Simpson_1-D	0.78	0.5932	0.7285	0.5052	0.7718	0.585	0.66	0.5244	0.75	0.75
Evenness_e^H/S	0.7496	0.4382	0.7294	0.5467	0.5981	0.5241	0.7027	0.7824	0.8911	0.8911
Soil PH	6.4	7	7	6.8	6.8	7	6.8	6.8	7	6.8
Soil humidity (%)	70	80	50	40	60	50	50	55	40	50
Weed complexity	5	5	4	4	6	3	3	2	3	3
Weed density	41	47	35	35	56	13	24	3	6	6

Of the 17 species detected in this study, only 4 were sufficiently abundant to calculate the IV, which was highest for *Hypoponera* sp. 1. (50.7), followed by *Aphaenogaster* sp. (28.9), *Diacamma* sp. (28.2), and *Odontoponera* sp. (27.6). However, according to Sitthicharoenchai and Chantarasawat (2006), only *Odontoponera* sp. can be used as a bioindicator species, because as a predator, it is very sensitive to habitat disturbances. The three other species (*Hypoponera* sp., *Aphaenogaster* sp. and *Diacamma* sp.) are generalist species and are usually abundant in all habitat conditions. Suputa et al. (2007) found *Odontoponera* sp. dominated in home yard and was an important predator of aphids and fruit flies in Yogyakarta. According to Read and Anderson (2000), one approach to analyzing changes in ant community composition is to focus on functional groups whose abundances vary with environmental stress and disturbance. In this study site (Nyalembeng), the result showed that both agricultural systems were dominated by generalists, this condition may because of the history of this field which has been construct to organic farming during recent three years (this sentence is not clear). A fairly large number of generalist ant species in both fields may exist because of the tendency of ant species to inhabit man-made habitats. This result was in accordance with Rubiana et al. (2015) who carried out research in four different habitats in Jambi. The use of such functional groups allows prediction of the community response to disturbances in the absence of species-level information and provides an ecological context for interpretations of compositional changes. Functional groups have been used successfully to monitor land-uses inducing marked ecological changes (Read and Andersen 2000).

In conclusion, the relative contributions of organic and intensive farming to ant diversity differed and were higher for organic farming. However, there were no correlations with soil conditions. In term of bioindicator attributes, ant functional groups are more sensitive in reflecting agroecosystem health than is ant diversity. However, in this study, we found that ant diversity was higher in organic farming than in intensive farming and conclude that organic farming leads to a healthier ecosystem than does intensive farming.

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Heavy metal tolerance in indigenous bacteria isolated from the industrial sewage in Kemisan River, Tangerang, Banten, Indonesia

WAHYU IRAWATI[1,♥], SEMUEL RIAK[1], NIDA SOPIAH[2], SUSI SULISTIA[2]

[1]Department of Biology, Teachers College, Universitas Pelita Harapan. Jl. M.H. Thamrin Boulevard 1100, Lippo Karawaci, Tangerang 15811, Banten. Tel. +26215466057, ext. 2615, Fax. +62216450655, ♥email: w.irawati3@gmail.com.

[2]Institute of Environmental Technology, Indonesian Agency for Assessment and Application of Technology (BPPT), Building 412 PUSPIPTEK Serpong, Tangerang Selatan 15314, Indonesia

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Abstract. *Irawati W, Riak S, Sopiah N, Sulistia S. 2017. Heavy metal tolerance in indigenous bacteria isolated from the industrial sewage in Kemisan River, Tangerang, Banten, Indonesia. Biodiversitas 18: 1481-1486.* The bacterial study is a part of human calling in preserving the earth. Many indigenous bacteria isolated from heavy metal contaminated sites had resistance to heavy metal toxicity and could be used for heavy metal removal. The aims of this study were to isolate heavy metal-tolerant indigenous bacteria from the industrial sewage of Kemisan River in Tangerang, Banten, Indonesia. The potency of bacterial isolates to remove heavy metals was also determined. The heavy-metal tolerance was determined by measuring the minimum inhibitory concentration. The potency of bacterial isolate for removing heavy metals from the medium was determined by an atomic absorption spectrophotometer. The results showed that there were eight heavy metal-resistant bacteria isolated from Kemisan River with minimum inhibitory concentration ranging from 7 mM to 11 mM. Isolate PbSI1 was the highest lead tolerant bacteria, and also tolerant to copper and zinc. The isolate was able to remove 91.25% lead, 73.38% zinc, and 98.57% copper from medium supplemented with the mixture of these heavy metals. The addition of 9 mM of lead in the medium affected the morphological appearance of isolate colonies i.e PbSI1 and PbSI3 to become darker which might occur due to the survival mechanism of bacteria by absorbing the lead inside the cells. The finding of this study indicated that isolate PbSI1 was a promising bacterium, which could be further developed for heavy metal removal.

Keywords: Bacteria, biosorption, copper, lead, removal, zinc

INTRODUCTION

The discharge of heavy metals into aquatic ecosystem has become a matter of concern in Indonesia. Indonesia has several sites contaminated by heavy metals and other residues from the mining industry. For example, most of industrial sewage containing heavy metal pollutants i.e lead, copper, and zinc were illegally introduced into Kemisan River, located in Tangerang, Banten, Indonesia. Andleeb et al. (2010) reported that the chemical water pollution has seriously damaged the ecology of surface and ground water, causing the dangerous effects on living organisms in a polluted area. According to Damini et al. (2013), most of heavy metals and their salts are undegradable or indestructible; moreover, they will remain permanently in the environment. Therefore, the remediation efforts to release heavy metals in the environment is very crucial and very challenging.

Efforts for removing metal ions from aqueous solution has been intensively conducted using technology approaches which mainly consist of physical, chemical and biological technologies. Conventional methods such as chemical precipitation, flocculation, membrane filtration, ion exchange and electrodialysis electrolysis, are often costly or ineffective for removing or reducing the pollutant concentration (Wang and Chen 2009). Bioremediation of heavy metals using microorganisms has received great attention especially for its potential application in industry. This is because of its nondestructive characters, cheap and economical usages (Rehman et al. 2007). Heavy metal-tolerant microorganisms may have a significant role in the soil treatment of metal pollutant. The ability of these microorganisms to detox the metal pollutant can be manipulated for bioremediation purposes especially for removing the heavy metal contamination in both wastewater and soil. Effluents containing heavy metals can be treated with these microorganisms by involving some processes including biosorption, bioaccumulation, and bioprecipitation (Rajbanshi 2008).

Biosorption using bacteria had been studied to sequester metal ions from aqueous solution, which was known as the cheap alternative method compared to conventional processes. This was due to the use of a low-cost sorbent material in biosorption process (Nameni et al. 2008). Bacteria having a potential to accumulate metal can be used to in the metal remediation by removing, concentrating and recovering metals from industrial effluents (Chowdhury et al. 2008). Some reports had shown that indigenous microbes could tolerate high heavy metal concentrations in different ways and may play a significant role in the restoration of a contaminated site (Ge et al. 2009). Isolation of bacteria from metal-polluted environment should be done to find the metal-resistant strain candidates which could be used for heavy metal removal and bioremediation purposes (Malik 2004). Therefore, the aims of this study were to isolate indigenous

bacteria with high tolerance to heavy metals from the industrial sewage in Kemisan River in Tangerang, Banten, Indonesia and also to study the potency of a bacterial isolate to remove heavy metals.

MATERIALS AND METHODS

Growth medium

Bacteria were isolated from industrial wastewater in Kemisan River, Tangerang, Banten, Indonesia. Bacterial isolate was grown in Luria-Bertani (LB) agar containing the following composition (per liter): tryptone 10 g, yeast extract 5 g, NaCl 10 g, and glucose 0.1 g. Stock of 1 M $CuSO_4$ (copper), 1 M Pb $(NO_3)_2$ (lead), 0.25 M $HgCl_2$ (mercury), and 1 M $ZnSO_4$ (zinc) sterilized by 0.2 m PTFE membrane. Appropiate concentration of heavy metal stock solution was added in autoclaved medium. LB agar was made with an addition of 2% pure agar (Irawati et al. 2015).

Bacterial isolation

One hundred microliter samples of industrial sewage was serially diluted and inoculated on each medium agar plate supplemented with 3 mM of copper, zinc, and lead. Cells culture was incubated at 37ºC for 72 hours. The growing colonies on medium were selected followed by purification to obtain a single colony. The morphology of the bacterial cells was observed by Gram staining followed by a light microscope observation.

The effect of heavy metals on bacterial growth

Initial inoculum was carried out as a starter by inoculating one loop of bacterial pure culture in 50 mL LB medium and incubated at 37°C on a shaker at 175 rpm. The growth of starter was monitored by measuring the optical density (OD) at λ 600 nm using spectrophotometer. Starter with OD of 0.6 was used for further growth observation. One mililiter of starter was inoculated in 50 mL LB broth medium. Each medium was supplemented with 3 mM of copper, lead, cadmium, zinc, the mixture of the heavy metals, and in medium without the heavy metals as a control. The cell cultures were incubated at 37°C on a shaker at 175 rpm. Bacterial growth was monitored by measuring optical density at λ 600 nm.

Heavy metals tolerance

The tolerance of bacterial isolates to copper, zinc, and lead were determined by Minimum Inhibitory Concentration (MIC). One loop of bacterial culture was streaked on agar plates containing 3 mM, 4 mM 5 mM 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM of copper, zinc until the isolate enabled to give colonies. The MICs value was determined after 48 hours of incubation at 37ºC (Rajbanshi 2008).

Biosorption of heavy metal

Biosorption is described as the concentration of copper which is removed from growth medium by concerning the initial copper concentration. Bacterial cells were grown in 50 mL LB broth containing 3 mM of copper, zinc, and lead and 1 mM of the mixture of heavy metals. Cell culture was incubated at 37°C with shaking at 175 rpm. After cell growth underwent logphase lasting, cells culture was centrifuged at 5000 g for 20 min at 4°C to separate supernatant from the pellet. The supernatant was digested with HNO_3 at 100°C for measuring the ability of bacteria to biosorp heavy metals. Heavy metals content was determined by using an atomic absorption spectrophotometer at 324.9 nm. Biosorption was calculated as the gap value between the total concentration of heavy metals added to the medium and the remained total concentration of heavy metals in the medium after bacteria were grown. Biosorption efficiency was calculated as (Ci-Cf / Ci) x 100% where Ci is the initial concentration and Cf is the remaining copper concentration in the solution (Moktar et al. 2011).

RESULTS AND DISCUSSION

Bacterial isolation

Kemisan River is one of heavy metals polluted areas in Indonesia caused by the increase of industrial activities around this site. Previous studies showed that many heavy-metal resistance in indigenous bacteria could be isolated from heavy metal-contaminated sites (Irawati et al. 2012). This study showed that eight heavy metal-tolerant bacteria were successfully isolated from Kemisan River designated as strains CuA, CuB, PbSI1, PbSI2, PbSI3, PbSI4, ZnSI1 and ZnSI2. Of all those strains, it showed that the MIC value of Copper-tolerant strains (CuA& CuB); Lead-tolerant strains (PbSI1, PbSI2, PbSI3, and PbSI4), and Zinc-tolerant strains (ZnSI1 and ZnSI2) were as follows: 10 mM $CuSO_4$, from 10 mM to 11 mM Pb $(NO_3)_2$, and between 7 mM to10 mM $ZnCl_2$ (Table 1). The variation of the metals tolerance might be due to the presence of different types of tolerance mechanisms which could be seen from the varied isolates (Ezzouhri et al. 2009). Interestingly, the heavy-metal tolerance of bacterial isolates found in this study (10 mM $CuSO_4$) was higher than *Sphingomonas* sp. and *Stenotrophomonas* sp. isolated from copper-polluted agricultural soil in Valparaiso Region, Central Chile. These bacteria were tolerant to copper with MIC value ranging from 3.1 to 4.7 mM (Altimera et al. 2012). In addition, copper-resistant *Pseudomonas* spp.and cadmium-resistant *Acinetobacter* spp. isolated from Guherwori sewage treatment plant showed resistance to heavy metals with MICs ranging from 2-4 mM (Rajbanshi 2008).

Six Gram-negative bacteria and two Gram-positive bacteria were successfully isolated from Kemisan River, Tangerang (Table 1). This is in accordance with the findings of previous study in which the majority of the isolates undergoing selection pressures in the presence of toxic compounds were Gram-negative followed by Gram-positive isolates accounting for only 20% (Brynhildsen et al. 1988). Adel et al. (2014) also reported that most of bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Chryseomonas* sp., *Burkholderia* sp., *Citrobacter* sp. and

Table 1. The heavy metals MIC of bacterial strains isolated from Kemisan River, Tangerang, Banten, Indonesia

Isolate code	MIC (mM)	Heavy metals	Type of gram
CuA	10	$CuSO_4$	-
CuB	10	$CuSO_4$	+
PbSI1	11	Pb $(NO_3)_2$	-
PbSI2	11	Pb $(NO_3)_2$	+
PbSI3	10	Pb $(NO_3)_2$	-
PbSI4	10	Pb $(NO_3)_2$	-
ZnSI1	7	$ZnSO_4$	-
ZnSI2	7	$ZnSO_4$	-

Note: + = Gram-positive, - = Gram-negative

Kluyvera sp. isolated from secondary effluent were 35.7% Gram-positive and 64.28% Gram-negative. In addition, Keramati et al. (2011) reported that each of Gram-positive bacteria isolated from dental clinic effluent had no tolerance to heavy metal.

Generally, the contaminated sites are sources of metal tolerant microorganism. The discharge of industrial wastewater containing heavy metals caused the increase population of heavy metal-tolerant bacteria. The higher concentration of these heavy metals remaining above threshold levels has a deleterious impact on the functional activities of microbial communities in soil. However, microorganism may develope tolerance against the elevated levels of these metals as survival mechanism toward the exposition of the higher concentrations of toxic heavy metals (Habi and Daba 2009). The interaction between heavy metals and microbes occurs in nature. Microorganisms can interact with metals via many mechanisms, some of them may be used as the basis of potential bioremediation strategies (Ahemad 2012). The interaction between metals and bacteria depends on the functional groups contained in the cell wall of Gram-positive or Gram-negative. The cell wall of Gram-positive bacteria naturally carries a negative charge because their phosphate group and teichoic acid will bind and then regulate the movement of cations across the membrane. Moreover, the outer membrane of Gram-negative bacteria, which consists of lipopolysaccharides, lipoproteins, and phospholipids, carries a strong negative charge (Tortora et al. 2005). Zaied et al. (2008) suggested that numerous chemical groups contributing to biosorption metal will bind hydroxyl, carbonyl, carboxyl, sulfhydryl, and phosphodiester groups. Velkova et al. (2012) assumed that carboxyl groups played a key role in Cu (II) removal from aqueous solution, followed by hydroxyl groups, phosphate, and amino groups. Carboxyl and hydroxyl groups have a negative charge and probably the electrostatic attraction, which may associate with the major mechanism involved in Cu (II) biosorption.

The strains of bacterial communities growth in medium containing lead, mercury, copper, and zinc were presented in Figure 1. The higher bacterial community was bacteria growing in medium containing lead, copper, zinc, and mercury, respectively. It indicated that Kemisan River was polluted by these heavy metals. Ahemad (2012) found that the higher concentration of these metals had a great effect on the microbial communities in soil in several ways including (i) the reduction of total microbial biomass, (ii) the decreased number of specific populations, or (iii) the changes of microbial community structure.

Two highest lead tolerant bacteria designated as isolates PbSI1 and PbSI3 with the MIC value of 11 mM were used for the further research. There was a difference of colonies color when the isolates PbSI1 and PbSI3 were grown in medium supplemented with 7-10 mM of lead. Based on Table 1, the MIC of isolate PbSI3 was 10 mM. It means that there was no colonies growth of isolate PbSI3 in medium with an addition of 11 mM of lead (Figure 2).

The colonies colors of isolates PbSI1 and PbSI3 in medium containing 9 mM of lead treatment were darker than that in the lower concentrations. It might be due to the survival mechanism of bacterial isolates by accumulating the lead inside the cells. The same results were previously observed in *Yarrowia lipolytica* which could grow at the high concentration of copper sulfate and also developed a brown pigment that could bind metal located in the cell wall (Ito et al. 2007). Bacterial isolates produced dark-colored colonies when grown in the presence of Ni^{2+}. Bacterial isolates that formed a dark color inside the colony might be caused by reduction and precipitation of the metals. It was considered that these isolates were to be Ni^{2+} biosorbents. No dark color was observed around the colonies of the sensitive bacteria (Abdel-Monem et al. 2010).

It was probably that the increase of lead concentration induced bacteria to develop resistance mechanism through the bioaccumulation of lead inside the cells resulted in the changes of colony appearance to become darker along with the increased concentration of lead. This result was similar to another findings in transgenic bacteria that tolerant to mercury namely *Escherichia coli* (Ruiz et al. 2011). It has been suggested that the appearance of transgenic bacteria expressing metallothionein and polyphosphate kinase, grown in high mercury concentrations demonstrated cell aggregation, precipitation, and darker color. These effects might also depend on the ability of transgenic bacteria to tolerate and to accumulate the high concentration of mercury due to the presence of metallothionein. This might also explain why the darker color of isolate PbSI1 appeared when it was grown in medium containing 8 mM of lead. This was due to the presence of metallothionein when the isolate was grown in high lead concentration.

The growth of isolate PBSI1 in medium containing zinc, copper, lead, and the mixture of these heavy metals was presented in Figure 3. The growth of isolate PbSI1 in medium containing heavy metals was slower than that in medium without the addition of heavy metals. Isolate PbSI1 grew well in medium containing lead which might happen due to the well-development of lead tolerance mechanism resulted in the lead-tolerant bacteria. The growth of isolate PbSI1 in medium containing copper was slower than that in medium with the addition of other heavy metal. Copper is a metallic element that crucial for cell metabolism; however, in extcessed concentration, it becomes toxic to all living organisms, including bacteria,

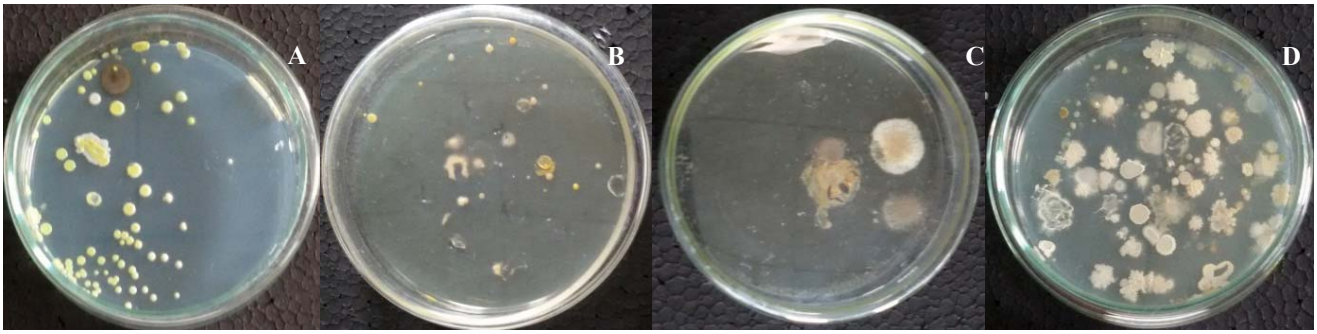


Figure 1. The growth of bacterial colonies communities isolated from Kemisan River, Tangerang, Banten, Indonesia on medium containing various concentrations of heavy metals: (A) 4 mM $ZnSO_4$, (B) 5 mM $CuSO_4$, (C) 0.1 HgCl2, (D) 2 mM Pb $(NO_3)_2$

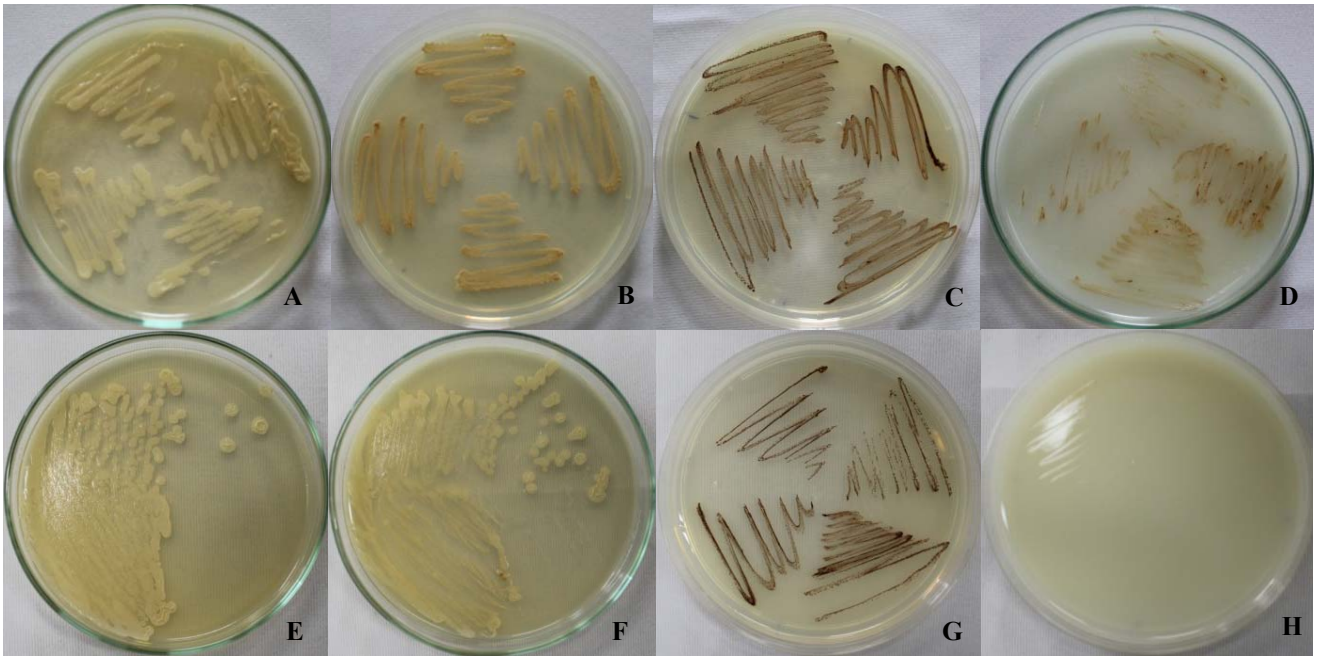


Figure 2. The growth of isolates PbSI1 and PbSI3 on medium containing various concentrations of lead. (A) PbSI1 7 mM, (B) PbSI1 8 mM, (C) PbSI1 9 mM, (D) PbSI1 10 mM, (E) PbSI3 7 mM, (F) PbSI3 8 mM, (G) PbSI3 9 mM, (H) PbSI3 10 mM

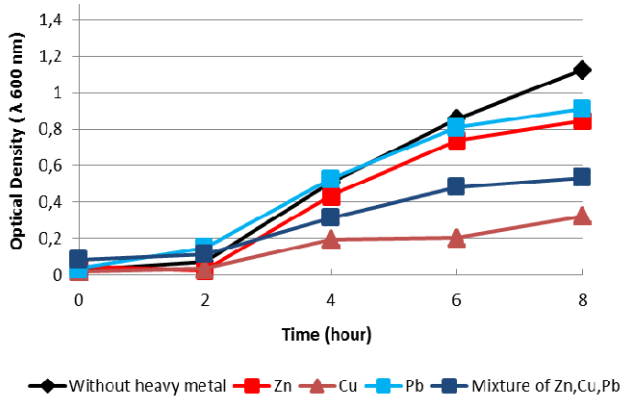


Figure 3. The growth of isolate PBSI1 in medium containing 3 mM of zinc, copper, lead, and 1mM the mixture of these heavy metals.

which mostly keep a tight hold of copper in the cells (Bondarczuk and Piotrowska-Seget 2013). Copper degrades iron-sulfur clusters of dehydratases through iron displayed, causing the inactivation of these crucial enzymes (Macomber and Imlay 2009).

The MIC test on isolate PbSI1 showed that this isolate was not only tolerant to lead, but also tolerant to zinc, copper, and the mixture of these heavy metals (data were not shown). It indicated that isolate PbSI1 had multiple tolerance to heavy metals. Rajbanshi et al. (2008) assumed that about 52.23% of bacteria had multiple resistance to three and more heavy metals. A previous study had revealed that 60% of bacterial isolates from a wastewater treatment plant had multiple tolerance to heavy metals. The bacterium which has multiple tolerance to many heavy metals can be used as a biosorbent efficiently, because polluted metal environment usually was contaminated by more than one type of heavy metals.

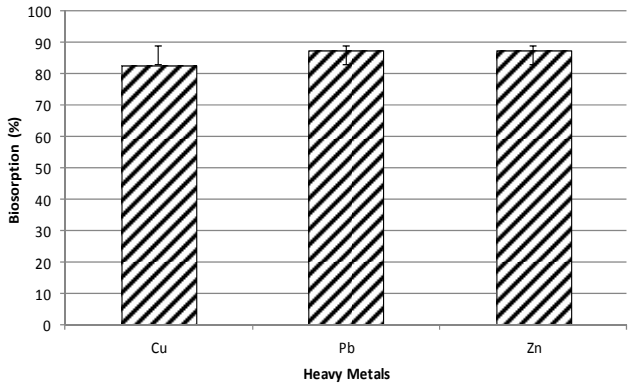


Figure 4. Biosorption percentage of isolate PbSI1 in medium containing 3 mM copper, lead and zinc

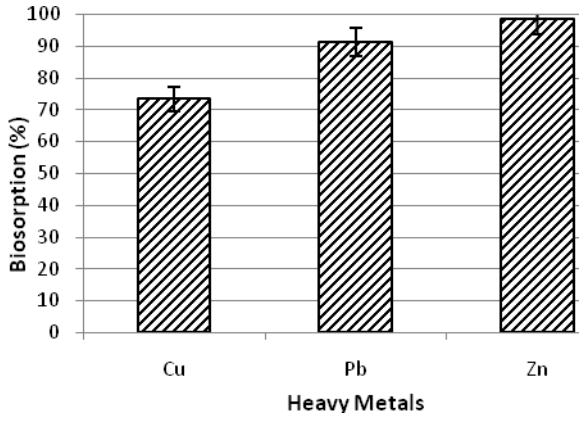


Figure 5. Biosorption percentage of isolate PbSI1 in medium containing the mixture of 1 mM copper, lead and zinc

The most of the bacterial genera has evolved numerous mechanisms to tolerate heavy metal ions and to protect their cell homeostasis against toxicity of heavy metals. Microorganisms can interact with metals via many mechanisms, such as biooaccumulation and biosorption processes (Ahemad 2012). According to Kadukova and Vircikova (2005), bioaccumulation is an intracellular metal accumulation by living cells. Sometimes, it called an active biosorption. Biosorption can bind heavy metallic species to live cells, an inert biomass or to a microbial extracellular polymer (da Silva Oliveira et al. 2007). A wide variety of living and dead biomass of bacteria have a capability to sequester toxic metals from waste streams, which offer an economical alternative for sorption technologies. Once, the toxic metals are adsorbed and/or transferred within organic materials; they can be removed from wastewater (Smith and Collins 2007). Isolate PbSI1 removed lead, copper, and zinc up to 87.68%, 82.53% and 87.69% in medium containing 3 mM of those heavy metals, respectively (Figure 4). The capablity of Isolate PbSI1 to remove heavy metlas was higher than previous study. Tolerant bacteria isolated from secondary effluents could remove 61.9% of lead and 74.61% of copper (Adel et al. 2014). *Bacillus* sp. isolated from an electroplating industrial effluent sample using copper for plating had an ability to remove up to 69.34% of copper (Rani et al. 2010). Biosorption capability of *Sienotrophomonas maltophilia* from primary solution was 22% of copper and 42.75% of lead (Parungao et al. 2007).

Isolate PbSI1 was also capable of removing lead, copper and zinc up to 91.25%, 73.38% and 98.57% from medium supplemented with 1 mM of these mixed heavy metals, respectively (Figure 5). This is a valuable finding since more than one type of heavy metals was contained in most of contaminated sites. It means that isolate PbSI1 is potential isolate which can be used for removing wastewater containing copper, zinc, and lead.

In conclusion, isolate PbSI1 has a great potential to be further developed. Indigenous bacteria may provide new information about the diversity and also has a potential role to remove heavy metals contamination from industrial sites. The potency of bacterial isolates to grow and remove heavy metals would be useful in the application of wastewater treatment plant where bacteria are directly involved as a bioremediation agent in biological processes for wastewater treatment.

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Short Communication: Occurrence and cluster analysis of palm oil (*Elaeis guineensis*) fruit type using two-dimensional thin layer chromatography

DENI ARIFIYANTO[1], MOHAMMAD BASYUNI[2,♥], SUMARDI[3], LOLLIE AGUSTINA PANCAWARASWATI PUTRI[4], ETTI SARTINA SIREGAR[5], IWAN RISNASARI[2], INDRA SYAHPUTRA[1]

[1]Graduate School of Agroecotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, North Sumatera, Indonesia
[2]Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Dharma Ujung No. 1, Medan 20155, North Sumatera, Indonesia. Tel./Fax: +62-61-820-1920. ♥email: m.basyuni@usu.ac.id
[3]Faculty of Pharmacy, Universitas Tjut Nyak Dhien, Jl. Rasmi No. 28, Medan 20123, North Sumatera, Indonesia
[4]Department of Agroecotechnology, Faculty of Agriculture, Universitas Sumatera Utara. Medan 20155, North Sumatera, Indonesia
[5]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. Medan 20155, North Sumatera, Indonesia

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Abstract. *Arifiyanto D, Basyuni M, Sumardi, Putri LAP, Siregar ES, Risnasari I, Syahputra I. 2017. Short Communication: Occurrence and cluster analysis of palm oil (Elaeis guineensis) fruit type using two-dimensional thin layer chromatography. Biodiversitas 18: 1487-1492.* The problems that have been faced by palm oil breeders are the length of time and high costs to discover the type of palm oil (*Elaeis guineensis*) fruit namely *Dura*, *Pisifera*, or *Tenera*, before using as a seed parent. These conditions are ineffective and add to the cost of maintenance and the production of seedling is more expensive. The present study describes the occurrence and cluster analysis of palm oil fruits using two-dimensional thin layer chromatography (2D-TLC). The leaves and fruit on each fruit mesocarp and shell, commercial seed on each fruit type were sampled through direct determination, plant nurseries, commercial seed and unknown type of palm oil. 2D-TLC chromatograms of hexane extracts showed diversity in palm oil fruits: *Dura* had ficaprenol-type polyprenol (C_{50}-C_{60}) and no carbon chain-length of polyprenol and dolichols (C_{85}-C_{100}) were found. In *Tenera* polyprenols of C_{45}-C_{60} and C_{90}-C_{100} occurred and dolichols of C_{85}-C_{105} as well, where polyprenols of C_{45} and C_{105} and dolichol of C_{105} found in *Tenera* were not detected in *Pisifera*. To confirm these findings, cluster analysis was drawn using the UPGMA method. The dendrogram demonstrated that the three types of palm oil were grouped to fruit type, suggesting that the occurrence of polyisoprenoids in palm oil fruits were chemotaxonomically significant.

Keywords: Chemotaxonomic marker, *Elaeis guineensis*, *Tenera*, Two-Dimensional Thin Layer Chromatography

Abbreviations: 2D-TLC: Two-Dimensional Thin Layer Chromatography, UPGMA: Unweighted-Pair Group Method with Arithmetic mean, MVSP: Multivariate Statistical Package, TL: total lipid, Pol: polyprenol, Dol: dolichol

INTRODUCTION

Palm oil is one of the main commodities of Indonesia and supplies over 18 million tons of vegetable oil in the world (Oil World 2015). Even though Indonesia is currently the largest producer and exporter of palm oil worldwide, it has still faced problems with the low productivity compared to other countries. High-quality planting material is needed to increase productivity. Determination of fruit type is one of the major problems of palm oil breeders before continuing crossing. At least 5-8 years are required for fruit type identification using conventional methods, which consume time, cost, and labor. Two approaches have been developed by researchers to determinate fruit type (Zhao et al. 2012; Rincon et al. 2013; Ritter et al. 2016). Firstly, the direct determination after the palm oil fruiting, but this way needs a long time to resolve the problems as previously described (Rincon et al. 2013). Secondly, the identification of palm oil fruits using molecular markers (Zhao et al. 2012; Ritter et al. 2016). Nonetheless, there are still issues on molecular markers that cannot differentiate the same fruit type of different origins (Ritter et al. 2016).

Several studies have shown that polysioprenoids can be used as chemotaxonomic markers (Roslinka et al. 2002; Kumari et al. 2013; Basyuni et al. 2016, 2017a). These studies suggest that the chain length of polyisoprenoid alcohol displays a distinct pattern that can be used to differentiate plants into genera and families. It is vital therefore to obtain information on the occurrence of polyisoprenoid in palm oil to determine palm oil fruit type and the chemotaxonomic significance of polyisoprenoids.

Many studies have reported the occurrence of polyisoprenoids from various plants (Ishinaga et al. 1990; Sagami et al. 1992; Kurisaki et al. 1997; Tateyama et al. 1999; Chouda and Jankowski 2005; Basyuni et al. 2016, 2017a). Two primary types of polyisoprenoid alcohols have been described on the OH-terminal (α-) isoprene structure. These include polyprenols (α-unsaturated) and dolichols (α-saturated) compounds (Basyuni et al. 2017). Two-dimensional thin layer chromatography (2D-TLC) has been used widely to analyze the polyisoprenoid compound that

relates to the development phase and different tissues of soybean (Ishinaga et al. 1990, 1992; Kurisaki et al. 1997), rubber and ginkgo (Tateyama et al. 1999), spinach leaves (Sakaihara et al. 2000), mushroom *Lentinus edodes* (Wojtas et al. 2004), sea fish (Ishiguro et al. 2014), and mangrove plants (Basyuni et al. 2016, 2017a). Despite the chemotaxonomic importance of polyisoprenoids, no information on polyisoprenoid distribution in palm oil has been previously available. The present study describes for the first time the occurrence and tissue distribution of polyisoprenoids in various tissues of palm oil with a particular reference to chemotaxonomic criterion.

MATERIALS AND METHODS

Chemicals

The dolichol (C_{90}-C_{105}) and polyprenol (C_{90}-C_{100}) standards were used as previously described (Basyuni et al. 2016, 2017), and used to identify the pattern of polyisoprenoid alcohol that was detected in this study. Silica gel 60 TLC plates and reverse-phase silica RP-18 HPTLC plates were purchased from Merck (Darmstadt, Germany). All of other chemicals and solvents were reagent grade (Merck, Darmstadt, Germany).

Plant materials

Samples of leaves and fruits from mature palm oils were collected from nurseries. Leaves in front number one (age approximately was 2-4 weeks after opening) were taken as samples from fruit type of *Dura* (D), *Pisifera* (P) and *Tenera* (T) of a mature plant. Leaves from nurseries of an unknown fruit type were also collected in the same manner as previously described. Samples of unknown fruit types of mature plants were also collected from a smallholder area. Fruit samples were gathered from mature fruits (about 5-6 months after pollination) of DxP, and TxT crossing, the mesocarp, and the shell were separated using clean knives. The hard shell was broken into the small pieces using a hammer. All Samples were collected when the average temperature of the environment was 25 °C and humidity was 83%.

Extraction of polyisoprenoid alcohols

The procedure of polyisoprenoid alcohol isolation was carried out as previously described (Sagami et al. 1992; Basyuni et al. 2016, 2017a). The samples of leaves, mesocarp of fruits and shells were dried using an oven at 60 °C for 1-2 days. The dried tissue was crushed into a fine powder using laboratory mills, then 5 g of each were immersed in chloroform/methanol (CM2:1; v/v), and then incubated in a water bath for 48 h. The supernatant was filtered, then dried using a rotary evaporator. The lipid extract of all samples was saponified at 65°C for 24 h in 86% ethanol containing 2 M KOH. The non-saponifiable lipids of each sample were evaporated and redissolved in hexane. All the samples extracted (50-100 mg) were applied to each TLC plate.

Investigation of Two-Dimensional Thin Layer Chromatography (2D-TLC)

First-dimension TLC was carried out on a silica gel glass plate (20x3 cm) with toluene: ethyl acetate (9:1) as a solvent for about 45 min as previously described (Basyuni et al. 2016). The polyprenol compounds will move slightly faster than dolichol family. The longitudinal edge of the first-dimension TLC and the concentration zone of a reverse-phase C-18 TLC were clamped with magnetic bars. The silica gel glass and C-18 (not sure what this means) of TLC plate were then developed in acetone for about 30 minutes to transfer all compounds that has been separated in the first-dimension into concentration zone of the reverse-phase TLC plate. To determine the family of a compound and its concentration, dolichols or polyprenols standards were added to the sample line and developed with a solvent system as previously described. The position of polyisoprenoid alcohol spots that had been developed using 2D-TLC were identified and visualized using iodine vapor. The chromatographic images were scanned using Canon E-400. The polyisoprenoid families pattern were determined by comparing the standard of dolichol or/and polyprenol with the occurrence of polyisoprenoid pattern in TLC samples plate.

The quantification of polyisoprenoid content in samples was carried out by comparing with dolichol and polyprenol standards. A standard curve of concentration of dolichol or polyprenol correlated to iodine-color estimation was drawn. The amount of dolichol and polyprenol were quantified using ImageJ ver. 1.46r (Schneider et al. 1992) in comparison with the standard curve that has been drawn previously.

Cluster analysis

Cluster analysis was performed on selected subsets of fruit type data consisting of 15 variables, including polyprenols and dolichols from 17 fruit type were log (10) transformed. From these data, dendrogram representing fruit type was drawn by cluster analysis using the unweighted-pair group method with arithmetic mean (UPGMA) and MVSP (multivariate statistical package) 3.22 (Kovach Computing Service). Euclidean distance was chosen as the criterion for cluster combination.

RESULTS AND DISCUSSION

The identification of fruit type from palm oil was performed by 2D-TLC to separate polyisoprenoids into polyprenol and dolichol families with different chain lengths. The summaries of the analytical results of the distribution of polyisoprenoids in palm oil are given in Table 1. The highest weight of total lipid was from fruit mesocarp ranging from 482-511 mg g^{-1} dry weight.The total lipid of leaves samples fluctuated between 20-66 mg g^{-1} dry weight. Total lipid content in fruits shell or seeds was varied between 26-102 mg g^{-1} dry weight.

In leaves, the highest total lipid content was *Tenera* fruit type ranging from 4.5-6.9 mg g^{-1} dry weight. On the other hand, *Pisifera* fruit type had total lipid content of 3.4-

4.2 mg g^{-1} dry weight. Total lipid content in *Dura* fruit type was the lowest compared to other fruit types, ranging from 3.0-3.8 mg g^{-1} dry weight. The distribution of polyprenols and dolichols in the plant tissue were classified into three types as previously reported (Basyuni et al. 2016, 2017a). In type-I, dolichol predominated over polyprenol (more than 90%); type-II displays the occurrence of both polyprenol and dolichol.

In type III, the predominance of polyprenol over dolichol by more than 90% was detected. Table 1 shows polyisoprenoid pattern in palm oil was only Type-II, without Type-I and-III. The presence of both polyprenols and dolichols agree well with previously reports (Kurisaki et al. 1997; Tateyama et al. 1999). The carbon-chain length of polyisoprenoid in palm oil as shown in Table 2 and Figures 1 and 2 varied according to each tissue even in the same species and formed a particular family with dominant molecule species (Tateyama et al. 1999; Basyuni et al. 2016). Therefore, the samples of this study were collected from the same ages and phases. Thus the composition of the carbon-chain length in each group was consistent and could be identified the differentiation among the fruit type (Tateyama et al. 1999; Basyuni et al. 2016).

Table 1. Distribution of polyprenol and dolichol in palm oil

Fruit type	Origin	Tissue	Status	TL (mg/g dw)	Pl (mg/g dw)	Pol (mg/g dw)	Dol (mg/g dw)	% in total lipid			% in Polyisoprenoid	
								Pl	Pol	Dol	Pol	Dol
Dura	Lame	Leaves	Parent palm	26 ± 6.8	3.0	1.8	1.2	11.5	6.9	4.6	60.0	40.0
Dura	Angola	Leaves	Parent palm	49 ± 3.7	3.3	1.9	1.4	6.7	3.9	2.9	57.6	42.4
Dura	Dabou	Leaves	Parent palm	42 ± 8.1	3.4	2.2	1.2	8.1	5.2	2.9	64.7	35.3
Dura	Unknown	Leaves	Parent palm	66 ± 6.6	3.8	2.1	1.7	5.8	3.2	2.6	55.3	44.7
Pisifera	Lame	Leaves	Parent palm	23 ± 5.4	4.2	2.7	1.5	18.3	11.7	6.5	64.3	35.7
Pisifera	Yangambi	Leaves	Parent palm	49 ± 3.7	3.6	2.5	1.1	7.3	5.1	2.2	69.4	30.6
Pisifera	Unknown	Leaves	-	37 ± 4.2	3.4	2.1	1.3	9.2	5.7	3.5	61.8	38.2
Tenera	Lame	Leaves	Parent palm	20 ± 11.2	5.1	3.6	1.5	25.5	18.0	7.5	70.6	29.4
Tenera	Yangambi	Leaves	Parent palm	41 ± 11.8	6.9	4.9	2.0	16.8	12.0	4.9	71.0	29.0
Tenera	Lame	Leaves	Commercial palm	34 ± 4.7	4.5	3.2	1.3	13.2	9.4	3.8	71.1	28.9
Dura x Pisifera	Lame	Leaves	Nurseries	23 ± 5.4	4.9	3.4	1.5	21.3	14.8	6.5	69.4	30.6
Dura x Pisifera	Lame	Fruit mesocarp	-	511± 78.1	3.9	2.4	1.5	0.8	0.5	0.3	61.5	38.5
Dura x Pisifera	Lame	Fruit shell	-	26 ± 14.1	2.8	1.6	1.2	10.8	6.2	4.6	57.1	42.9
Tenera x Tenera	Lame	Fruit mesocarp	-	482 ± 0.0	5.3	3.3	2.0	1.1	0.7	0.4	62.3	37.7
Tenera x Tenera	Lame	Fruit shell	-	102 ± 0.0	4.7	2.8	1.9	4.6	2.7	1.9	59.6	40.4
Unknown	Unknown	Leaves	Smallholders	27 ± 1.9	4.4	2.7	1.7	16.3	10.0	6.3	61.4	38.6
Unknown	Lame	Commercial seed	-	95 ± 25	4.5	2.8	1.7	4.7	2.9	1.8	62.2	37.8

Note: Total lipid are represented as the mean ± SD (n ± 2-3),-: unknown, TL: total lipid, Pol: polyprenol, Dol: dolichol

Table 2. Carbon-chain length of polyprenol and dolichol in palm oil*

Fruit type	Origin	Tissue	Status	Polyprenol	Dolichol
Dura	Lame	Leaves	Parent palm	50 55 60	85 90 95 100
Dura	Angola	Leaves	Parent palm	50 55 60	85 90 95 100
Dura	Dabou	Leaves	Parent palm	50 55 60	85 90 95 100
Dura	Unknown	Leaves	Parent palm	50 55 60	85 90 95 100
Pisifera	Lame	Leaves	Parent palm	**50 55 60**… 90 95 100	85 **90 95** 100
Pisifera	Yangambi	Leaves	Parent palm	**50 55 60**… 90 95 100	85 **90 95** 100
Pisifera	Unknown	Leaves	-	**50 55 60**… 90 95 100	85 **90 95** 100
Tenera	Lame	Leaves	Parent palm	**45 50 55** 60 … 90 95 100 105	85 **90 95 100** 105
Tenera	Yangambi	Leaves	Parent palm	**45 50 55** 60 … 90 95 100 105	85 **90 95 100** 105
Tenera	Lame	Leaves	Commercial	**45 50 55** 60 … 90 95 100 105	85 **90 95 100** 105
Dura x Pisifera	Lame	Leaves	Nurseries	**50 55 60** 65 … 90 95 100	85 **90 95** 100 105
Dura x Pisifera	Lame	fruit mesocarp	-	**50 55 60** 65 … 90 95 100 105	85 **90 95** 100 105
Dura x Pisifera	Lame	fruit shell	-	**50 55 60** … 90 95 100	85 **90 95** 100 105 110
Tenera x Tenera	Lame	fruit mesocarp	-	50 55 60 … 90 95 100 105	85 90 **95100 105** 110
Tenera x Tenera	Lame	fruit shell	-	50 55 60 … 90 95 100 105	85 90 **95 100105** 110
Unknown	Unknown	Leaves	Smallholders	45 50 55 60 … 90 95 100 105	85 **90 95 100** 105 110
Unknown	Lame	Seeds	-	50 55 60 … 90 95 100 105	85 **90 95 100** 105 110

Note: *The numbers refer to the carbon-chain length of the polyisoprenoid alcohols. The chain length of the main polyisoprenoid alcohols in each tissue are indicated in bold. Data are represented as three independent experiments

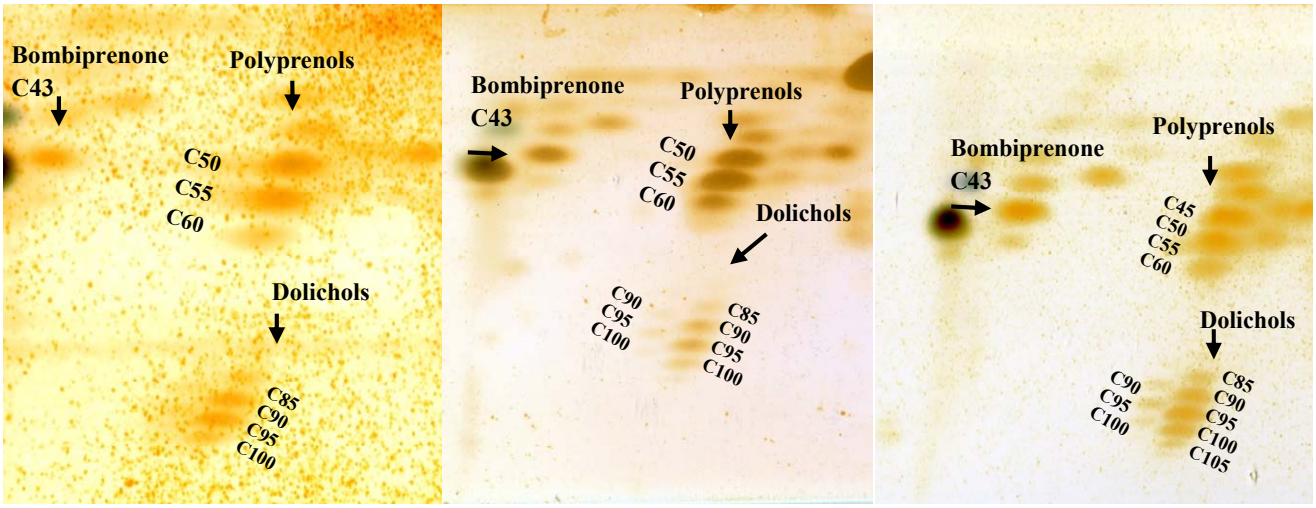


Figure 1. 2D-TLC chromatograms of polyisoprenoids from *Dura* (A), *Pisifera* (B), and *Tenera* (C). The Carbon number refers to the carbon-chain length of polyisoprenoid alcohols. Data are represented as three independent experiments

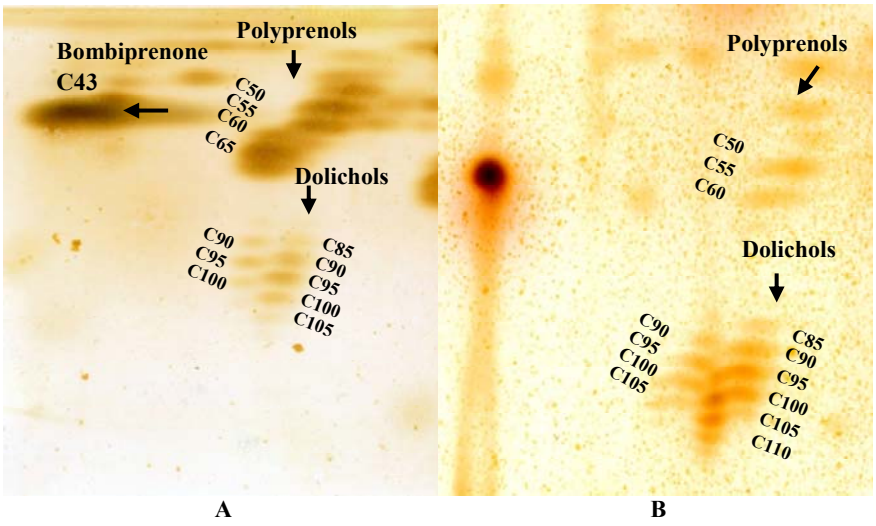


Figure 2. 2D-TLC chromatograms of polyisoprenoids from nursery DxP (*Dura* x *Pisifera*)(A) and fruit shell (B). The Carbon number refers to the carbon-chain length of polyisoprenoid alcohols. Data are represented as three independent experiments

The shorter carbon-chain of polyprenols (C_{50}-C_{60}) and dolichols (C_{85}-C_{100}) occurred in all *Dura* fruit types (Figure 1A), but long-chain polyprenol was not detected. The *Dura* fruit has a thick shell and the fruits are large. *Dura*'s fruit diameter is significantly distinguishable from other types (Basyuni et al. 2017b). This result indicated that the regulation of biosynthetic pathway of *Dura* fruit type might differ to the fruit type of *Pisifera* and *Tenera* (Swiezewska and Danikiewicz 2005).

As shown in Figure 1B, the *Pisifera* composition of polyprenol and dolichol was different in comparison to *Dura*. The differentiation of carbon-chain patterns between Lame and Yangambi was not detected. Shorter carbon-chain of polyprenol was (C_{50}-C_{60}) which is similar to *Dura*, also found in *Pisifera*. Bombiprenone compound identified in this fruit type. The main carbon chain in *Pisifera* was shorter polyprenol (C_{50}-C_{60}), longer polyprenol (C_{90}-C_{100}), and dolichol (C_{85}-C_{100}).

The polyisoprenoid family in *Tenera* fruit type (Figure 1C) was also differentiated compared to fruit type of *Pisifera* and *Dura*. Both Lame and Yangambi presented a similar pattern. Fruit type of *Tenera* has short-chain of polyprenols (C_{45}-C_{60}) which called as ficaprenol and much longer-chain polyprenols (C_{95}-C_{105}). Dolichol of *Tenera* was (C_{85}-C_{110}). The primary molecule of polyprenols was (C_{45}-C_{55}) and dolichol (C_{90}-C_{100}) which indicating that *Tenera* is differently regulated both of polyisoprenoid in their biosynthetic pathways (Sagami et al. 1992). *Tenera* seed from the view of palm oil plantation was superior and most planted variety worldwide; it is a hybrid of parent *Dura* (female) and *Pisifera* (male) (Basyuni et al. 2017b).

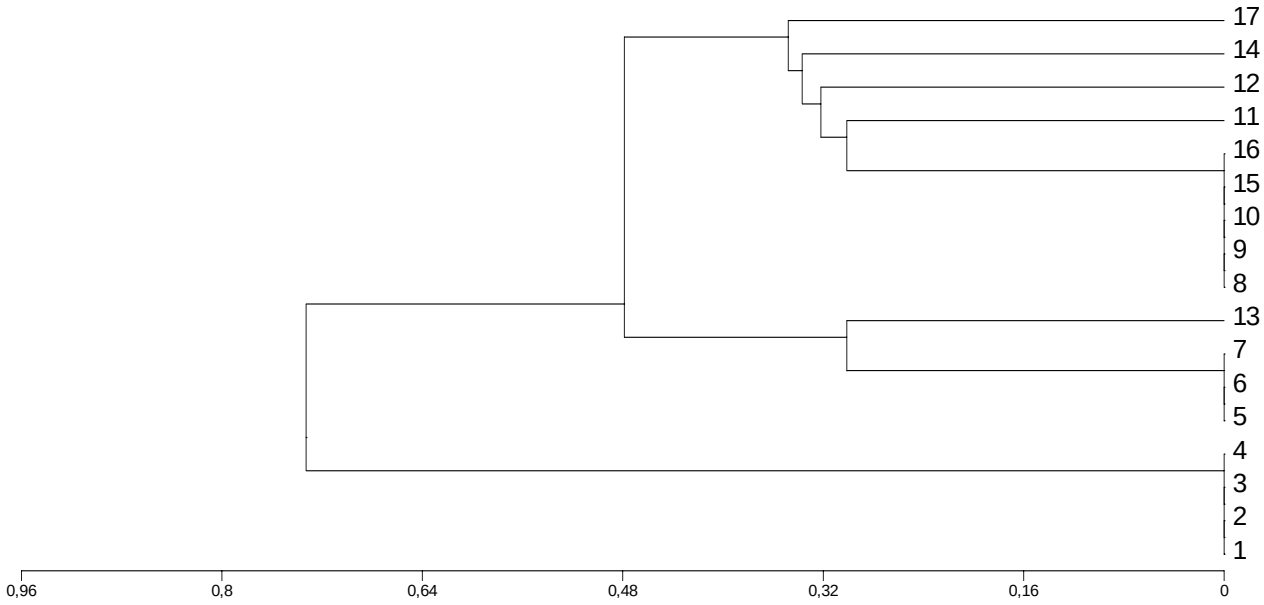


Figure 3. Dendrogram showing the relationship among 17 fruit types in palm oil from the fruit data of carbon-chain lengths of polyisoprenoids by log (10) transformation by Euclidean distance. Name of number for fruit type, see Table 2

The carbon chain polyisoprenoids from nurseries (Figure 2A) which are a hybrid between *Dura* and *Pisifera* has the composition of polyprenols (C_{50}-C_{65}) and (C_{90}-C_{100}), respectively, and dolichol (C_{85}-C_{105}). This structure was almost similar to the carbon-chain pattern of *Tenera* fruit type. The primary carbon-chain molecule of the nurseries plants was polyprenol (C_{50}-C_{60}) and dolichol (C_{90}-C_{100}) respectively. This study was indicating that the nurseries plants used both compounds in their biosynthetic pathways to produce shorter polyprenols and longer dolichols which are close to *Tenera* (Tateyama et al. 1999; Basyuni et al. 2016, 2017a).

Furthermore the fruit shell samples of palm oil crossing of *Dura* and *Pisifera* occurring short-chain of polyprenol (C_{50}-C_{60}) and longer polyprenol (C_{90}-C_{100}) and dolichol (C_{85}-C_{110}) (Figure 2B). Furthermore, the shell of *Tenera* x *Tenera* crossing has shorter polyprenols of C_{50}-C_{60} and longer polyprenols (C_{90}-C_{105}) and dolichol (C_{85}-C_{110}). The pattern of carbon-chain of commercial seeds has two family composition of polyprenols (C_{50}-C_{60}) and (C_{90}-C_{105}), and dolichols (C_{85}-C_{110}). These results are agreement with previous studies that the composition of polyisoprenoids is independently regulated in the plant kingdom, including in palm oil (Kurisaki et al. 1997; Tateyama et al. 1999; Basyuni et al. 2016).

The pattern of carbon-chain was analyzed and translated into binary data and visualized into dendrogram using UPGMA method. The dendrogram was expected to classify among the fruit type of *Dura*, *Pisifera,* and *Tenera*, respectively. Figure 3 shows that the samples of *Dura* were classified into a separate group with the similarity coefficient 0.78. Meanwhile, *Pisifera* also formed one group with similarity coefficient 0.32 and *Tenera* have similarity coefficient ranged 0.32-0.35. On the other hand, the fruit type of *Dura* was detectable to identify comparing to *Pisifera* and *Tenera*. Furthermore, similarity coefficient of 0.78 indicating that *Dura* fruit type has farthest evolution range compare to others fruits-type.

The similarity coefficient of *Tenera* and *Pisifera,* which are close to each other, shows that both fruit types have some identical carbon-chain patterns, and the differences between them could only be identified by the molecules of polyprenols namely C_{65} and C_{105}, respectively and having dolichol of C_{105}. These molecules belong to *Tenera* and did not occur in *Pisifera*.

The samples of unknown fruit type (No. 16 and 17, Table 2) were clustered into *Tenera*'s group as previously expected. This data indicated that samples number 16 and 17 as unknown fruit type could be confirmed as *Tenera* fruit type in the clustering analysis and carbon chain length. The present study demonstrated that 2D-TLC method could differentiate the fruit types of palm oil.

To conclude, 2D-TLC could be an alternative technique to identify fruit type of palm oil, in addition to molecular markers (Ritter et al. 2016). Diversity was noted in palm oil fruits: *Dura* had no carbon chain-length of polyprenol (>C90-C105) compared to *Pisifera* and *Tenera*. In *Tenera* polyprenols of C_{45}-C_{60} and C_{90}-C_{100} occurred, and dolichols of C_{85}-C_{110}, where polyprenols of C_{65} and C_{105} and dolichol of C_{110} were not detected in *Pisifera*. Our present data on palm oil polyisoprenoids confirmed the identification of palm fruit using 2D-TLC was clustered into appropriate palm fruit type and chemotaxonomically important.

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Gene flow and selection evidence of sandalwood (*Santalum album*) under various population structures in Gunung Sewu (Java, Indonesia), and its effects on genetic differentiation

YENI W.N. RATNANINGRUM[1,♥], SAPTO INDRIOKO[2,♥♥], ENY FARIDAH[3], ATUS SYAHBUDIN[4]

[1]Laboratory of Forest Seed Science and Technology, Faculty of Forestry, Universitas Gadjah Mada. Bulaksumur, Sleman 55281, Yogyakarta, Indonesia, Tel.: +62-274-550541; Fax.: +62-274-550543, ♥email: yeni.wnr@ugm.ac.id; yeniratnaningrum@gmail.ac.id;

[2]Laboratory of Forest Genetics and Tree Improvement, Faculty of Forestry, Universitas Gadjah Mada. Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel./Fax.: +62-274-545639, ♥♥ email: sindrioko@ugm..ac.id;

[3]Laboratory of Plant Physiology, Faculty of Forestry, Universitas Gadjah Mada. Bulaksumur, Sleman 55281, Yogyakarta, Indonesia

[4]Laboratory of Dendrology and Ethnobotany, Faculty of Forestry, Universitas Gadjah Mada. Bulaksumur, Sleman 55281, Yogyakarta, Indonesia

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Abstract. *Ratnaningrum YWN, Indrioko S, Faridah E, Syahbudin A. 2017. Gene flow and selection evidence of sandalwood (*Santalum album*) under various population structures in Gunung Sewu (Java, Indonesia), and its effects on genetic differentiation. Biodiversitas 18: 1493-1505.* Field observations on population structures and isoenzyme analysis were conducted to determine gene flow and selection evidence of sandalwood under various population structures in Gunung Sewu, and its effects on genetic differentiation. Sandalwood (*Santalum album* Linn., Santalaceae) is origin to the south-eastern islands but recently emerged as new landraces in Java Island, Indonesia. Results suggested that (i) natural barriers contributed to habitat fragmentation and disrupted gene flow among populations; (ii) gene flow affected selection processes regarding bottleneck effects and genetic drifts, which determined allelic richness and population diversity; and (iii) variation on gene flow and selection processes affected genetic differentiation among populations. Gene flow restriction and genetic drift occurred when population had lower genetic base, highly clonalized, fragmented, and/or more inbreeding in mating. Genetic differentiation was highest between populations within Timor island, and between Gunung Sewu (Java Island) and Sumba-Timor islands. Populations were not clustered based on geographical sites, but more by the similarity of genetic structures. Genetic differentiations were the combined effect of the differences on genetical processes regarding gene flow and selection events. Both differences existed due to differences on (i) population structures including landscape, clonality and parental genetic composition, and (ii) the disturbance histories of population, which affected the equilibrium between gene flow and drift. These findings emphasized the importance of larger gene flow and genetic base to naturally maintain genetical processes of sandalwood population under various landscapes structures.

Keywords: Clonality, gene flow, fragmentation, genetic base, genetic differentiation, *Santalum album*, selection

INTRODUCTION

Evolutionary processes such as selection and migration, along with ecological factors responsible for gene flow such as pollen and seed dispersal, contribute to the dynamics of genetic structure within and among populations (Craft and Ashley 2007; Pautasso 2009; Schmidt et al. 2009; Garfi et al. 2013). When populations become fragmented or isolated, gene flow may be disrupted, resulting in the alteration of mating pattern, loss of genetic diversity, and increased genetic drift and differentiation (Warburton et al. 2000; Byrne et al. 2003; Bottin et al. 2007; Moreira et al. 2015). Habitat fragmentation could also lead to reproductive isolation (Warburton et al. 2000; Torres et al. 2003), poor recruitment (Byrne et al. 2003), and population fitness reduction through inbreeding or pollen limitation (Lhuillier et al. 2006; Bottin et al. 2007; Agullo et al. 2015). Furthermore, the transformation history of landscapes (Craft and Ashley 2007; Schmidt et al. 2009; Garfi et al. 2013; Moreira et al. 2015) and the human activities (Warburton et al. 2000; Kettle et al. 2007; Agullo et al. 2015), as well as the occurrence of physical barriers i.e. mountains, cliffs or oceans (Byrne et al. 2003; Bottin et al. 2007; Craft and Ashley 2007; Rao et al. 2007; Dani et al. 2011; Cursach et al. 2012; Hmeljevski et al. 2014), may cause habitat isolation which restrict gene flow and lead to the genetic structuring. Recent genetic differentiation may also be caused by drift and gene flow in the past (Lutz et al. 2000). Habitat fragmentation might cause bottlenecks effects due to a low genetic base of the remnant parents, and the events of selection might play an important role (Bottin et al. 2007; Garfi et al. 2013; Moreira et al. 2015).

The Outer Arc of Banda Islands, now is the south-eastern parts of Indonesian archipelagos, has been believed as the origin of sandalwood worldwide (Harbaugh and Baldwin 2007; Rao et al. 2007). *Santalum album* Linn (Santalaceae), herein after referred to sandalwood, produce heartwood containing 1.5 to 5% of β-santalol, a strong, specific fragrances of oil. Its heartwood is widely used for wood carving, art, religious and medicinal purposes. Its oil is the materials of cosmetics, perfumes and aroma-therapy, and was considered containing anti-melanoma compounds (Rao et al. 2007; Dani et al. 2011; Anonymous 2012; da

Silva et al. 2016). However, significant genetic depletion was reported throughout the world, highlighting the forest fragmentation as the main factor responsible for sandalwood extinction (Warburton et al. 2000; Byrne et al. 2003; Suma and Balasundaran 2003; Lhuillier et al. 2006; Bottin et al. 2007; Rao et al. 2007; Dani et al. 2011; Indrioko and Ratnaningrum 2015a, 2015b; da Silva et al. 2016). In 1994, sandalwood was first listed as Vulnerable on the IUCN Red List due to habitat degradation as the results of population size reduction (IUCN 1994). Considering a rapid degradation, the Vulnerable status might be – raised up to Endangered, Critically Endangered or even Extinct in the Wild, and therefore the conservation status supposed to be re-evaluated (IUCN 2009). Since 2004, sandalwood was even considered Extinct in the Wild in most of its native in the south-eastern islands of Indonesia (Anonymous 2012).

In spite of significant degradation in their origin in the South-eastern islands of Indonesia, new landraces of sandalwood extended along geographical gradients in Gunung Sewu Geopark, a 1300 km^2 mountainous limestone zones in the central part of Java Island (Ratnaningrum and Indrioko 2014, 2015, 2016; Indrioko and Ratnaningrum 2015a, b). The occurrence of these new landraces sounds promising for any of rehabilitation efforts. However, they are naturally isolated due to the restriction of the up and down formation of various landscape types. Some of them are also fragmented due to any of geographical, evolutionary and disturbance histories. Many studies on sandalwood reported genetic structuring due to various population barriers (Byrne et al. 2003; Bottin et al. 2007; Dani et al. 2011) and/or mating constraints (Warburton et al. 2000; Rao et al. 2007; Tamla et al. 2012). Considering this, this study hypothesized that there are consequences of population barriers to genetic structures.

This study analyzed gene flow and selection evidence of sandalwood under various population structures in Gunung Sewu, Java Island, and its effects on genetic differentiation. The comparison was also made with those of its origin in Timor and Sumba Islands. Field observations which determined the population structure parameters, and biochemical marker gene analysis, were combined to answer the following questions: (i) Does natural barriers contribute to the habitat fragmentation, which restricts gene flow among populations?; (ii) Does the restricted vs. connected gene flow affect selection processes within population?; and (iii) Does the variation on gene flow and selection processes consequentially result in genetic differentiation among populations? Field inventory was conducted to measure population structures. Isozyme analysis was conducted to determine the allelic richness, which implies the existence of bottleneck effects and genetic drifts as the evidence of selection. Based on the allele frequency, the genetic distance was measured and the dendrogram was then constructed to identify genetic differentiation among populations. Based on the between-population genetic diversity, the migration rate was calculated to estimate gene flow among populations.

MATERIALS AND METHODS

Study area

Gunung Sewu is the mountainous limestone zones in Java Island, the central parts of Indonesian archipelagos, which grew through dissolution as the limestone was raised from the seabed about 1.8 million years ago. Recently Gunung Sewu areas stretched 85 km west-east covers three GeoAreas of Gunungkidul (Yogyakarta), Wonogiri (Central Java), and Pacitan (East Java) with an approximate area of 1300 km^2. Based on this geographical formation history, Gunungkidul GeoArea is now derived into three zonations: (1) Northern Zone, consisted of the Batur Agung Highland at 300m to 800m asl, and the lowland of Sambipitu formation at 200m to 300m asl; (2) Middle Zone, the lowland basin of Ledok Wonosari at 150m to 300m asl; and (3) Southern Zone, the Pegunungan Seribu Karst areas at 0m to 200m asl. Gunung Sewu possessed two seasons. Rainy season occurs during October to March and the dry season takes place from April to September. As it is adjacent to the Indian Ocean, Gunung Sewu areas has two types of climate -- *Aw*, the semi-arid to arid type that is characterized by a long drought; and *Am* which represents an intermediate condition between the tropical and sub-tropical climate (Simanjuntak 2002; Haryono and Suratman 2010). In Gunung Sewu, new landraces of sandalwood occurred at various types of landscapes which are extended along geographical zones (Table 1; Figure 1).

There are more than ten sandalwood landraces in the form of both planted and naturally regenerated stands. However, study was only conducted at seven landraces in Gunung Sewu which were having a clear history of establishment, naturally regenerated, able to reproduce sexually, and representing each of geographical zones. For Gunung Sewu regions, observation was made on three of sandalwood landraces (Bleberan, herein after refer to GSM1-Bleberan, GSM2-Mulo and GSM3-Wanagama) in the lowland of Middle Zone, two landraces (GSN1-Nglanggeran and GSN2-Sriten) in the highland of Northern Zone, one (GSN3-Bejiharjo) in the lowland of Northern Zone, and two (GSS1-Petir and GSS2-Botodayakan) in the karst area of Southern Zone of Gunung Sewu, respectively (Table 1; Figure 1). Each of Gunung Sewu Zones is separated by 25 to 40 km, but landraces within zone are separated by less than 10 km, with exception for Bejiharjo which is separated by more than 30 km away from any other landraces.

In order to determine the population structure and genetic parameter differences between the Gunung Sewu populations and those of its origin, this study also compared to the population and genetical data of sandal trees origin in Timor and Sumba Islands. It covered the remnant of the heavily harvested populations in Timor (the lowland of Soe, Tm-Soe) and Sumba (Sb-Sumba) islands, and the seed production area in the highland of Netpala (Tm-Netpala), Timor island. The data was gained from the previous researches (Ratnaningrum and Indrioko 2014, 2015, 2016; Indrioko and Ratnaningrum 2015a, b). In addition, the secondary (additional) data was collected

Table 1. The landscape and habitat characteristics of sandalwood populations

Population, width, altitude, climatic types	Landscape history and present habitat characteristics	Soil and rock units	Sandalwood history and present population characteristics
GSN1-Nglanggeran; 79.3 ha; 710-750m asl; *Am* type	A part of Nglanggeran Formation, Northern Zone of Gunung Sewu. Now it exists as the strong undulating mountainary landscapes, characterizing tropical mountain ecosystems.	Latosols with volcanic and sediment rocks, some with deeper solum.	Sandalwood was first documented in 1970's. Recently it occurs in groups of stands across the Nglanggeran mountain regions, in association with the tropical mountain vegetation. Habitat isdominated by the association of naturally regenerated mahogany, *Gliricidea* sp, and several *Garcinia* and *Eugenia* families.
GSN2-Sriten; 25 ha; 750-890m asl; *Am* type	A part of Semilir Formation, Northern Zone of Gunung Sewu. Now it exists as the strong undulating highland landscapes, characterizing tropical mountain ecosystems, and is isolated by mountainary physical barriers.	Latosols with volcanic and sediment rocks, mostly with deeper solum.	Sandalwood was first documented in 1960's. Recently it covers three biggest hills in an association with the tropical highland vegetation. Habitat is dominated by the association of naturally regenerated mahogany, *Gliricidea* sp, and several *Garcinia* and *Eugenia* families, and isolated by mountain physical barriers.
GSN3-Bejiharjo; 9.6 ha; 150-180m asl; *Aw* type	A part of Sambipitu Formation, Northern Zone of Gunung Sewu. Now it exists as the open dry-rocky hilly landscapes with caves and ground-rivers below. Representing the dryland ecosystems.	The association of red mediterrans and black grumosols with limestone rocks, mostly with the shallow solum	Sandalwood is a remnant of the 1970's planted stands. Fragmented due to heavy exploitation, urban and cave-tourism activities since 1990's. Now, it exists as a small-fragmented group of stands, and dispersely occurs in an open dry-rocky hills above the caves and ground-rivers. Sandalwood grows in an association with cajuputi and acacia regenerated from commercial plantation nearby. Younger sandal trees are largely derived from root suckers. Sites are dominated by dryland herbs such as grasses and *Eupatorium* sp.
GSM1-Bleberan; 52.9 ha; 150-170m asl; intermediate between *Aw* and *Am* type	A part of Wonosari Basin Formation, Middle Zone of Gunung Sewu. Now it exists as the catchment area of the ancient subterranean Oya River at the lowland basin landscapes. Representing the tropical lowland ecosystems.	The association of red mediterrans and black grumosols with limestone rocks, mostly with the deeper solum.	Sandalwood was first documented in 1970's along the catchment area of the ancient subterranean Oya River, at the lowland basin of middle zone. Sandalwood is dispersed widely along the riparian catchment area and nearby, in association with the tropical lowland forest vegetation which consists of more diverse vegetation including teak, mahogany, *Gliricidea* sp, *Schleicera* sp, cajuputi and acacia. Population is surrounded by several ex situ conservation areas, sharing the same river.
GSM2-Mulo; less than 1 ha; 150 m asl; intermediate between *Aw* and *Am* type	A part of Wonosari Basin Formation, Middle Zone of Gunung Sewu. Now it exists as the limestone cliffs landscapes. Isolated by cliffs barriers.	Latosols with limestone rocks, mostly with a very shallow solum.	Limestone cliffs ecosystems. Sandalwood was first documented in 1970's in the naturally regenerated stands surrounding the cliffs and nearby. Recently, small groups of sandalwood grow on the cracks of vertical limestone cliffs, at 3m to more than 50m belowground.

GSM3-Wanagama; 6 ha; 150-170m asl; intermediate between *Aw* and *Am* type	A part of Wonosari Basin Formation, Middle Zone of Gunung Sewu. Now it exists as the lowland basin landscapes.	Latosols with limestone rocks, mostly with deeper solum; well-managed.	Sandalwood was first introduced in 1967 using the seedlings from Timor. Ex situ conservation area is established in 1992-1993, consisting of seven provenances of Timor and Java Islands. Site is a part of Wanagama Forest Research Station which belongs to Universitas Gadjah Mada, well-managed for research activities.
GSS1-Petir; 78 ha; 70-100m asl; *Aw* type	A part of Wonosari-Punung Karst Formation, Southern Zone of Gunung Sewu. Now it exists as the karst hilly landscapes with open dry-rocky hills, strong undulating, characterizing the dry rocky-limestone ecosystems.	Latosols with limestone rocks. Solum is deeper at the basins, but very shallow at the limestone - rocky hills.	Sandalwood was first documented in 1960's in karst hilly areas, recently covering more than twenty open dry-rocky hills. Adult plants were mostly derived from root suckers. In the open-undulating areas, sandalwood grows in association with dry rocky-limestone vegetation including acacia and cajuput, but is dominated more by shrubs and herbs such as grasses and *Eupatorium* sp.
GSS2-Botodayakan; 6.75 ha; 100m asl; *Aw* type	A part of Wonosari-Punung Karst Formation, Southern Zone of Gunung Sewu. Now it exists as the karst hilly landscapes surrounded by a very dense teak forest, less undulating. The flat basin areas were bordered by the rocky-hills slopes barriers.	Latosols with limestone rocks, mostly with deeper solum.	Sandalwood was first documented in 1970's in karst hilly areas. Recently, small group of sandals grows in an association with dry-lowland forest vegetation, and is shaded by very dense teak canopies. Population is surrounded by a very dense teak forest and was bordered by the rocky-hills slopes barriers.
Tm-Soe; approx. 10 ha; 200m asl; Strong *Aw* type	Savanna landscapes in the lowland arid region of the southern part of Timor island. Previously it was sandalwood natural forests comprised more than 100 ha. Now it exists as a remnant of the heavily wild-harvested population.	Red mediterrans soils, mostly with the shallow solum.	Previously, it was sandalwood natural forests comprised more than 100 ha. Now it exists as a remnant of the heavily wild-harvested population. Sandalwood occurs in dispersed small groups of ramets, is heavily clonalized, and grows in an association with the lowland arid savanna vegetation.
Tm-Netpala; 4.09 ha; 1090m asl; *Aw* type	Seed production area in the highland arid region of Timor island. Less undulating, characterizing a highland arid ecosystem.	Ustic cambisol soils, mostly with the deeper solum.	Sandalwood seed production area is established in 1992-1993, consists of six provenances of Timor. It is located in the highland arid region in the middle part of Timor island. It is well-managed for seed production purpose.
Sb-Sumba; Strong *Aw* type	Sandalwood rehabilitated stands, using seedlings from Sumba, planted in the savanna landscapes in the middle region of Timor island.	Red mediterrans soils, mostly with the shallow solum.	Planted in 2013 with genetic materials collected from the remnant of sandalwood natural forests in the Sumba island. Now it exists as the three years old saplings of sandalwood rehabilitated plantation in Kupang, Timor island.

Note: GSN, GSM and GSS referred to the Northern, Middle and Southern Zone of Gunung Sewu (Java Island), respectively; Tm represented Timor island, and Sb is Sumba island.

from the annual reports of local forestry agencies and personal communication with key persons. Both Timor populations are separated by more than 50 km. Timor and Sumba Islands are separated by approximately 1000 km. Whilst, Timor and Java Islands are separated by more than 3500 km in distance (Figure 1). Timor and Sumba Islands are located in the south-eastern parts of Indonesian archipelagos. These islands represent arid regions with strong *Aw* climatic type, characterized by long drought, particularly at lower altitude. The altitude varies between 100m to more than 2000m asl. Following heavy exploitation which has had occurred since 1900's, recently there are only a few individuals remained, dispersed in small groups of stands. Conservation efforts have been made by establishing some of the rehabilitated stand, *in situ* and ex situ conservation areas, but most of them were constructed by the unknown sources of genetic materials, and therefore resulted in a very low survival rate (Anonymous 2012; Indrioko and Ratnaningrum 2015b).

Our previous study found a variation of population structures and genetic parameters among sandalwood populations in Gunung Sewu (Java Island), Timor and Sumba Islands (Ratnaningrum et al 2015; Table 2).

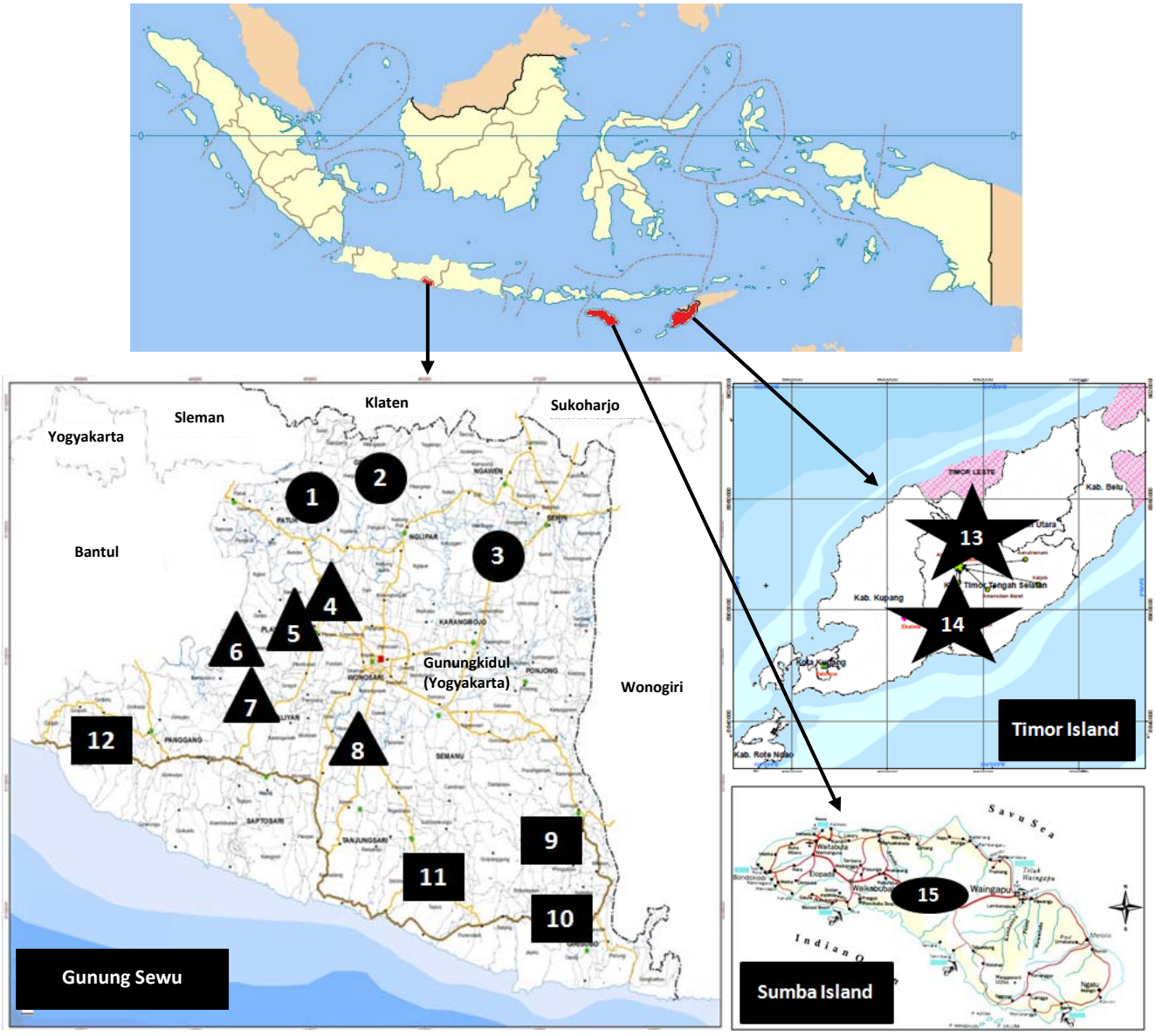


Figure 1. Study sites: sandalwood landraces in the Gunung Sewu Geopark, Java Island. The Gunung Sewu groups: the first group (Northern Zone): Nglanggeran (1), Sriten (2) and Bejiharjo (3); the second group (Middle Zone): Bunder (4), Wanagama (5), Banyusoco (6), Bleberan (7), and Mulo (8); and the third group (Southern Zone): Petir-Semugih (9), Botodayakan (10), Panggang (12) and Tepus (11) landraces, respectively. Within each Gunung Sewu zone, the landraces are marked by circles (Northern Zone), triangles (Middle Zone) and squares (Southern Zone) shapes, respectively. For comparison, the data was also obtained from sandalwood origin in Sumba Island (Sb-Sumba population, 15) and Timor Island (Tm-Soe population, 14 and Tm-Netpala seed production area, 13), respectively.

Table 2. Population structures, number of individuals, and genetic parameters measurements on eleven sandalwood populations in Gunung Sewu (Java Island), Timor and Sumba Islands (Ratnaningrum et al 2015)

Population	Number of individuals		Population structures		H_e		H_o		F_{IS}	
	Adult*	Seedling*	Clona-lity (%)	N_e (%)	Par.	Off.	Par.	Off.	Par.	Off.
Gunung Sewu:										
GSN1-Nglanggeran	1145 (50)	93 (39)	24.02	12.31	0.269	0.231	0.261	0.284	0.029	-0.231
GSN2-Sriten	330 (119)	50 (26)	20	15.76	0.308	0.296	0.317	0.348	-0.031	-0.175
GSN3-Bejiharjo	496 (116)	124 (23)	76.62	18.55	0.315	0.210	0.312	0.102	0.008	0.514
GSM1-Bleberan	1834 (99)	364 (205)	34.35	7.03	0.282	0.308	0.294	0.333	-0.042	-0.079
GSM2-Mulo	41 (35)	12 (0)	12.19	41.46	0.259	NA	0.243	NA	0.062	NA
GSM3-Wanagama	276 (120)	78 (41)	0	76.45	0.347	0.375	0.406	0.439	-0.172	-0.171
GSS1-Petir	9190 (55)	2945 (52)	77.48	50.92	0.210	0.219	0.153	0.117	0.271	0.464
GSS2-Botodayakan	151 (29)	72 (50)	25.83	17.22	0.367	0.306	0.324	0.313	0.117	-0.025
Timor:										
Tm-Soe	NA (21)	NA (0)	80	NA	0.044	NA	0.016	NA	0.638	NA
Tm-Netpala	344 (30)	NA (0)	0	63.95	0.240	NA	0.193	NA	0.197	NA
Sumba										
Sb-Sumba	NA (25)	NA (25)	80	NA	NA	0.209	NA	0.163	NA	0.223

Note: N_a = the mean number of allele per locus, *PPL* = the proportion of polymorphic loci, H_e = Hardy-Weinberg expected panmictic heterozygosity, H_o = observed heterozygosity, F_{is} = mean fixation index over all loci, the deviation from expected frequencies under Hardy–Weinberg equilibrium, Par. = Parents level, Off. = Offsprings level. NA = Not Assessed; * = number in parentheses is the amount of sampled individuals

Study species

Sandalwood is a long-lived, yearly flowering perennial of semi-arid and tropical region throughout south-eastern and middle parts of Indonesia. It is a shrub attaining a height of about 12 m and a girth of 10 to 30 cm (Rao et al. 2007; da Silva et al. 2016). It is entomophilous and often produced abundant flowers (Warburton et al. 2000; Tamla et al. 2012). Flowers are arranged in inflorescences consisted of 20 to 40 small-red single flowers. Generally, in Indonesia, sandalwood flowered twice a year: at the beginning of dry season in May to September, and at the beginning of rainy season in November to March, with 4 to 5 months flowering period. The previous study showed evidence of dichogamy in which pollen matures two days before stigma receptivity (Ratnaningrum and Indrioko 2014, 2015). Studies on sandals' mating systems have indicated highly outcrossing rate and self-incompatibility (Sindhu-Vereendra and Anantha-Padmanabha 1996; Tamla et al. 2012; da Silva et al. 2016). However, partially self-compatibility has also been reported for *S. accuminatum* (Warburton et al. 2000) and *S. album* (Suma and Balasundaran 2003; Ratnaningrum and Indrioko 2015), particularly under isolated condition. Despite its abundant flowers, the fruit production is very low (Sindhu-Vereendra and Anantha-Padmanabha 1996; Warburton et al. 2000; Tamla et al. 2012; Ratnaningrum and Indrioko 2016; da Silva 2016). Several studies reported high pollen fertility (88.4%) in *S. album* in India (Sindhu-Vereendra and Anantha-Padmanabha 1996); and high rate of pollination effectiveness (10 to 40%) in *S. album* in Java Island (Ratnaningrum and Indrioko 2015). However its reproductive success was very low, ranging from less than 5% in small populations (Sindhu-Vereendra and Anantha-Padmanabha 1996; Warburton et al. 2000; Byrne et al. 2003; Indrioko and Ratnaningrum 2015b) to about 20% in the bigger and wider genetic bases' populations (Ratnaningrum and Indrioko 2015; da Silva 2016). Sandalwood is mainly vegetatively reproduced by root suckers; the vegetatively propagated sproutings emerged from the horizontal roots, particularly under marginal condition (Warburton et al. 2000; Lhuillier et al. 2006; Dani et al. 2011; Herawan et al. 2014; da Silva et al. 2016). The clonality event may occur when most of off-springs in population were derived from a genetically identical individual, such a condition which has dramatically increased the level of selfing (Warburton et al. 2000; Charpentier et al. 2002; Indrioko and Ratnaningrum 2015a, b).

Procedures

Isozyme analysis

Genetic diversity was measured spatially across eleven landraces along geographical gradients in Gunung Sewu (Java Island), and several populations in Timor and Sumba Islands. To measure genetic diversity, at the beginning of rainy season in December 2015, juvenile leaves were sampled from randomly chosen individuals (N 208 individuals at GSN1-Nglanggeran, 110 at GSN2-Sriten, 104 at GSN3-Bejiharjo, 305 at GSM1-Bleberan, 35 at GSM2-Mulo, 161 at GSM3-Wanagama, 121 at GSS1-Petir, 129 at GSS2-Botodayakan, 57 at Netpala, and 21 at Soe). The flowering trees were of the first priority to be sampled.

Samples were wrapped, frozen in ice packs and taken to the laboratory for allozyme extraction and electrophoresis.

Previous study gained three enzymes, shikimate dehydrogenase (E.C. 1.1.1.25.), esterase (E.C. 3.1.1.) and diaphorase (E.C. 2.6.4.3.) which observed to be polymorphic. Zymogram phenotypes that were interpretable were found for only six loci, shikimate dehydrogenase *Skd-1*, esterase *Est-1, Est-2,* and *Est-3*, and diaphorase *Dia-1* and *Dia-2* (Indrioko and Ratnaningrum 2015a; 2015b). Electrophoretic procedures were conducted with vertical polyacrylamide gel electrophoresis procedures following David-Ornstein method (Nei 1987). The leaves were homogenized in modified extraction buffer and centrifuged at 15,000 rpm for 20 min at 0^0C. The supernatant was loaded onto polyacrylamide vertical slab (Sigma Inc., USA) gels and electrophoresed at 0^0C at 220 V and 100 mA current for about 3 h. After electrophoresis, the gels were stained using staining solution of each enzyme system, and the allozyme gels were genetically interpreted.

Measuring genetic differentiation, genetic distance and migration rate

At each of the allozyme locus, the frequency of each allele and the genotype were counted. The expected heterozygosity within population (H_S) for each locus and over all loci for each population in Hardy–Weinberg equilibrium was counted following the formula, $H_S = 1 - \Sigma\ p_i^2$, where p_i refers to the i's allele frequency of a given population. Total population heterozygosity (H_T) was counted following the formula: $H_T = 1 - \Sigma\ p_{it}^2$, where p_{it} refers to the i's allele frequency of the populations in total. To measure genetic differentiation, genetic diversity between population (D_{ST}) was calculated by reducing the value of total heterozygosity (H_T) to heterozygosity within a population (H_S). The mean proportion of total gene diversity at polymorphic loci due to differences between populations (G_{ST}) was measured by dividing D_{ST} to H_T. I, the genetic identity, was calculated following the formula, $I = (\Sigma\ x_i\ y_i) / \sqrt{(\Sigma\ x_i^2\ \Sigma\ y_i^2)}$, where x_i refers to the i's allele frequency of the x population, and y_i is the i's allele frequency of the y population. Genetic distance between pairs of populations (D) was calculated following the formula, $D = -\ ln\ I$. Based on the Nei's genetic distances between pair-wise populations, cluster analysis was performed following UPGMA algorithm and a dendrogram was then constructed (Nei 1987). Migration rate (N_em), the level of previous gene flow which contributed to the recent population structure, was counted following the formula, $N_em = (1 / G_{ST} - 1) / 4$ (Frankham et al. 2002).

RESULTS AND DISCUSSION

Results

The migration rate, evidence of selection and genetic differentiation

Gene flow among populations was determined by measuring N_em, the level of previous gene flow which contributed to the recent population structure (Table 3). GSM3-Wanagama, the most central population which also shares the same river with GSM1-Bleberan and several ex situ conservation areas nearby, tended to disperse seeds through both biotic agents and the stream flows. The seeds are widely dispersed by birds, in addition, some of seed deposits were observed along the river catchment areas in this study. Gene flow is largely occurred between GSM3-Wanagama and GSM1-Bleberan populations (N_{em} Wanagama-Bleberan 48 individuals per generation). However, low migration rate among populations within this Middle Zone (N_{em} GS-Middle Zone only 3 individuals) indicated the restricted gene flow, probably due to the significantly different landscape and genetic structures of GSM2-Mulo population.

Gene flow is larger within the Northern Zone populations (N_{em} GS-Northern Zone 19 individuals), showed clear evidence of inheritance between GSN2-Sriten and GSN3-Bejiharjo. Whilst, migration is restricted between the two of Southern Zone populations (N_{em} GS-Southern Zone only 3 individuals) despite their shorter distance (only separated 5 km away), it is probably due to the differences in the degree of isolation and genetic base. Overall, each zone of Gunung Sewu exchanged 3 individuals per generation, which is more or less similar to the gene flow observed between Timor and Sumba Islands. However, the gene flow between populations within Timor island, as well as those observed between Gunung Sewu (Java Island) and Timor-Sumba islands, is strongly restricted, since none of individuals were exchanged.

The evidence of selection was detected by measuring the allelic richness within populations (Table 3). As has discussed, the rare and missing alleles can be the indicator of bottleneck effects and genetic drifts, which might occur as a result of selection events. In this study, the highly genetically differentiated populations, which are in many cases related to the restricted gene flow, generally possessed a lower allelic richness and lost more alleles. The highly differentiated and restricted populations in Timor showed lowest level of allelic diversity (N_a 1.91, *PPL* 0.67), while those of Gunung Sewu were somewhat higher (N_a 2.30, *PPL* 0.91). The most connected populations in the Northern Zone of Gunung Sewu showed the most diverse alleles (N_a 2.36, *PPL* 0.97).

The Middle Zone populations in Gunung Sewu showed higher genetical differences than those of Northern Zone, and hence possessed lower allelic richness. These differences might attribute to the highly genetically and physically differentiated features of GSM2-Mulo, the cliff population. However, when GSM2-Mulo is removed from the measurements, the allelic richness of GSM3-Wanagama and GSM1-Bleberan are the highest (N_a 2.5, *PPL* 1). Moreover, rare alleles were less, and none of these alleles were missing at both of populations. GSM3-Wanagama, the most genetically diverse population, is the closest to GSM1-Bleberan with only less than 2 km of distance. Both populations experienced the similar environmental conditions, shared the same river, and possessed the highest migration rate (N_em 48 individuals per generation). GSM2-Mulo, the highly isolated population, possessed much lower allelic richness (N_a 2.0, *PPL* 0.67, respectively).

Table 3. The allelic richness, heterozygosity, genetic differentiation, and migration rate among populations of sandalwood, within and between islands of Java, Timor, and Sumba

	N_a	*PPL*	H_S	H_T	D_{ST} (%)	F_{ST} (G_{ST}) (%)	N_em (indiv.)
Among zones within Gunung Sewu regions	2.30	0.91	0.293	0.318	2.44	7.67	3
Among populations within Gunung Sewu Zones							
- Northern Zone	2.36	0.97	0.290	0.294	0.40	1.35	18
- Middle Zone	2.33	0.89	0.309	0.334	2.50	7.47	3
- Southern Zone	2.21	0.87	0.276	0.304	2.87	9.45	2
Among populations within Timor island	1.91	0.67	0.142	0.192	4.99	25.99	-
Between Timor and Sumba Islands	2.04	0.75	0.176	0.199	2.30	11.57	2
Between Gunung Sewu (Java) and Timor-Sumba islands	2.17	0.83	0.236	0.310	7.40	23.83	-
Average D_{ST} = 5.21 %							
Average G_{ST} = 16.79 %							
Average N_em = 1.24 individuals							

Note: N_a = the mean number of allele per locus; *PPL* = the proportion of polymorphic loci; H_S = heterozygosity within populations; H_T = total heterozygosity; D_{ST} = genetic diversity between population; F_{ST} (G_{ST}) = the mean proportion of total gene diversity at polymorphic loci due to differences between populations; N_em = migration rate

Genetic differentiation among populations. In general, the heterozygosity among populations (D_{ST}) was very low (Table 3). Within Gunung Sewu regions, only 2.44% of total heterozygosity existed due to the differences among populations. The three of populations within Northern Zone of Gunung Sewu performed very low diversity (D_{ST} GS-Northern Zone only 0.39%); whereas the three of Middle Zone populations showed higher diversity (D_{ST} GS-Middle Zone 2.49%) than those of Northern Zone. In contrast to the higher diversity of populations within Timor island (D_{ST} Timor 4.99%), the diversity between islands was somehow low (D_{ST} Timor-Sumba 2.30%). The genetic diversity was highest between Gunung Sewu (Java Island) and Sumba-Timor islands, with 7.40% of total heterozygosity, due to the differences among islands.

The genetic differentiation, represented by F_{ST} or G_{ST} (the mean proportion of total gene diversity due to differences between populations), ranged from 1.35% to 9.45% among populations within zones in Gunung Sewu; 25.99% among populations within Timor island; 11.57% between Sumba and Timor islands; and 23.83% between Gunung Sewu and the two of south-eastern islands. Genetic differentiation was highest between populations within Timor island (G_{ST} Timor 25.99%), which was even higher than those of between Gunung Sewu (Java Island) and Sumba-Timor islands (G_{ST} 23.83%). Only 7.67% of genetical differences existed among populations within Gunung Sewu regions. GSN1-Nglanggeran, GSN2-Sriten and GSN3-Bejiharjo, which were the three populations within Northern Zone, performed low genetical differences (G_{ST} GS-Northern Zone only1.35%).

GSM3-Wanagama, GSM1-Bleberan and GSM2-Mulo, the three of Middle Zone populations in Gunung Sewu, somehow showed higher genetical differences than those of Northern Zone. GSM3-Wanagama is the closest station to GSM1-Bleberan with only less than 2 km of distance, and both populations also shared the same river. When GSM2-Mulo is excluded from the measurements, the genetical differences between GSM3-Wanagama and GSM1-Bleberan are very low (D_{ST} 0.17%, G_{ST} 0.52%), and the migration rate is very high (N_em 48 individuals per generation). Both populations are separated from GSM2-Mulo by 15 km away. GSS1-Petir and GSS2-Botodayakan, the two of Southern Zone populations under karst ecosystem type, were also observed to show higher genetical differences (D_{ST} 2.87%, G_{ST} 9.45%) due to the landscape and genetical base differences. In contrast to the higher genetic differences observed within Timor island (D_{ST} 4.99%, G_{ST} 25.99%), these differences were somehow low between islands (D_{ST} 2.30%, G_{ST} 11.57 % between Timor and Sumba island).

Genetic distance

The dendrogram indicated a grouping of populations into three clusters. Populations within the same cluster expressed a high portion of same alleles and genotypes. The genetic similarity may be contributed to their similarity on the genetical and reproductive processes controlled by the landscapes structures and parental genetic base. However, there seemed to be less associative between geographical and genetic distance. Dendrogram clearly showed evidence that the populations were not clustered based on their geographical sites of occurrence, but more by their similarity on population structures with regard to the parental genetic base, degree of fragmentation and clonality.

In general, the more continuous, bigger and wider genetic-bases' populations allowed larger gene flow and

higher outcrossing rate. Moreover, the population structures similarity on the less clonalized conditions and higher parental heterozygosity led to a lower genetic differentiation, as it was reported on GSN1-Nglanggeran, GSN2-Sriten, GSM1-Bleberan, GSM3-Wanagama and GSS2-Botodayakan, which were grouped into the same cluster (*D* ranged from 0.008 to 0.032). Among populations within this cluster, GSN2-Sriten and GSS2-Botodayakan was separated along north to south gradient of more than 60 km away, and occupied significantly different geographical, altitudinal and ecological features. However, the genetic distance is surprisingly very close.

In contrary, the fragmented and isolated populations experienced lower gene flow and tended to have more inbreeding mating system, which led to the different genetical and reproductive processes, and therefore were significantly differentiated. This was a case with Tm-Soe, Tm-Netpala, GSN3-Bejiharjo and Sb-Sumba which were in the same clusters (*D* ranged from 0.005 to 0.024). In average, the first and second groups had a mean genetic distance (*D*) of 0.040. Another cluster consisted of two populations, GSM2-Mulo and GSS1-Petir (*D* 0.054). Both populations were located at the different zone of Gunung Sewu, and separated by more than 40 km in distance, and had different landscape and environmental types. GSS1-Petir seemed to be genetically farther apart from the rest of sandal populations due to the differences in the history of disturbance, degree of clonality and parental genetic base. Hence this population remained isolated and couldnot be linked to any other groups due to its genetical differences (*D* ranged from 0.094 to 0.149). In the case of GSM2-Mulo, the most isolated cliff landscape conditions were responsible for this genetical differences (*D* ranged from 0.068 to 0.117).

Discussion

Gene flow among populations

Fragmented and/or isolated conditions may result in a lack of gene flow and seed dispersal (Warburton et al. 2000; Lhuillier et al. 2006; Dani et al. 2011). Sandalwood performs outcross-mating and to avoid inbreeding depression they require sufficiently large populations for gene flow. Clear clustering on some of sandal populations regarded to their landscape differences, suggested that gene flow and outcrossing opportunities might have been restricted to those isolated populations. Gene flow via seed dispersal needs seed dispersers being both biotically agents such as birds or humans, and the abiotic ones such as water. In some cases, suitable habitats for sandalwood in the three zones of Gunung Sewu, as well as those of Timor and Sumba Islands, are spatially isolated, particularly those which experienced fragmentation for urban activities or natural barriers. Such an isolation will not be able to maintain a larger gene flow.

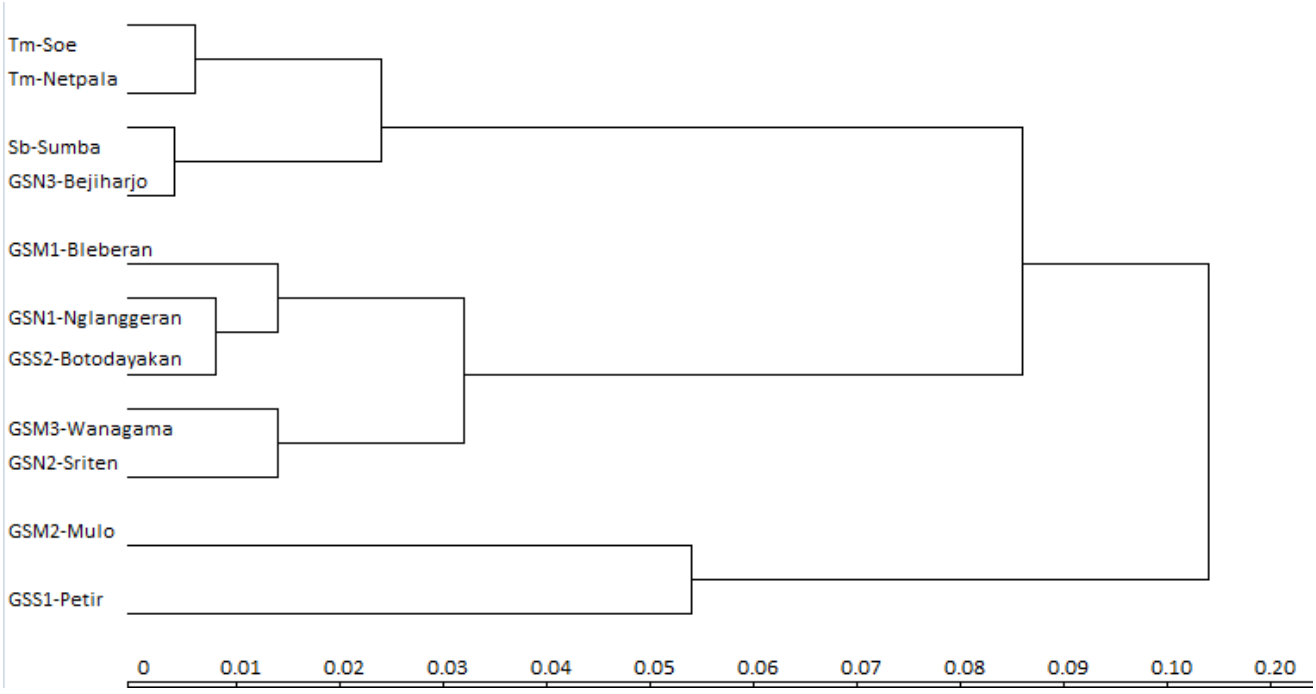


Figure 2. Dendrogram analysis for eleven sandalwood populations at different landscapes in Gunung Sewu (Java Island), Timor and Sumba Islands

A possible explanation for the low genetic differentiation, and in addition, a relatively high seedling recruitments, may be related to the pattern of dispersal by birds. Low genetic differentiation and more seedling recruitments were observed in populations which provide suitable environments for bird activities, such as GSM3-Wanagama, GSS2-Botodayakan and GSN2-Sriten which are surrounded by dense woody stands. Gene flow through pollen dispersal would be expected to be low in sandalwood as it is insect-pollinated (Sindhu-Vereendra and Anantha Padmanabha 1996; Dani et al. 2011), with optimum pollen distance is only 42 m (Ratnaningrum and Indrioko 2014). Isolated and small populations in sandals may disturb pollen transfer by its effects on restricted pollinator movements (Sindhu-Vereendra and Anantha Padmanabha 1996; Dani et al. 2011), inadequate flowers for rewards (Tamla et al. 2012; Ratnaningrum and Indrioko 2014), insufficient quantity of outcross-pollen donor and the incompatible pollen (Warburton et al. 2000). Seed dispersal in sandals is moderate as the seeds are widely dispersed by birds with a wider range of distribution (Byrne et al. 2003; Bottin et al. 2007) which can reach more than 5 kilometers in the distance (Ratnaningrum and Indrioko 2014). The wind pollinated and/or the long-distance bird-dispersal seeds, as of sandals, showed a lower association between genetic and geographic distance (Warburton et al. 2000). The negative value of fixation index was also represented the outcrossing, long-lived perennial species which have seeds dispersed through ingestion by animals (Moreira et al. 2015). Additionally, some extent of dispersal by stream river flow was also observed, particularly between populations which share the same river. Five birds species, *puyuh pepekoh* (*Coturnix chinensis*), *madu sriganti* (*Nectariana jugularis*), *cekakak sungai* (*Todirhampus chloris*), *kutilang* (*Pycnonotus aurigaster*) and *tekukur* (*Streptopelia chinensis*), reported as the dispersal agents of sandalwood in Gunung Sewu (Ratnaningrum and Indrioko 2014). This agent was also known to break seed chemical-dormancy and facilitate seed germination by their ingestion.

In contrast to many other results which reported rivers as the physical barriers (Cursach et al. 2012; Moreira et al. 2015), the Oya River in the Middle Zone acted as connecting agents for seed dispersal. Our observation detected very low genetic differences and much larger gene flow between GSM1-Bleberan and GSM3-Wanagama which share the same river. Hence the connecting role of both riparian dynamics and birds communities support the idea of a larger gene flow within the Middle Zone populations.

Populations separated by less than 10 km are linked genetically through pollen flow (Cursach et al. 2012). However, in this study, gene flow is restricted among some of isolated populations, even when they were within the same geographical zone. This is considered to be the effects of habitat fragmentation and isolation due to the geographical barriers, for example in the cliff and mountain ecosystems, which might limit gene flow (Cursach et al. 2012; Garfi et al. 2013; Moreira et al. 2015). Genetic structuring due to the habitat isolation by natural barriers was also reported on *Quercus macrocarpa* under savanna barriers in Illinois (Craft and Ashley 2007), *Lumnitzera littorea* in the tropical Asian mangrove (Pautasso 2009), *Santalum insulare* under the island ecosystems in insular Pacific (Lhuillier et al. 2006), and *S. album* in GSM2-Mulo population under rocky limestone cliffs habitat in this study. However, the small and naturally isolated populations of GSN2-Sriten and GSS2-Botodayakan could maintain genetic diversity and outcross due to a higher genetic base.

Evidence of selection

The lower genetic diversity and higher inbreeding rate in some of clonalized and fragmented populations is an indication that the gene flow is already restricted and reproductive fitness is already disrupted, since self-incompatible species are more vulnerable to the negative effects of habitat fragmentation (Lutz et al. 2000; Torres et al. 2003). This result confirmed theoretical models that the fragmented and isolated populations tended to loose more alleles and have lower heterozygosity, which provides evidence for a potential genetic bottleneck effects (Garfi et al. 2013; Moreira et al. 2015). As has discussed, the rare and missing alleles are regarded as criteria for measuring loss of diversity due to genetic bottlenecks. As described in the Result Section, both allelic diversity and heterozygosity decrease with an increase of isolation, and these loss is much more greater at the offspring level than those of parental ones. The events of selection (Garfi et al. 2013; Moreira et al. 2015), as well as migration (Cursach et al. 2012) and/or mutation (Torres et al. 2003), might explain such a situation.

The ability of a population to respond to selection is directly related to the level of genetic variation available for relevant adaptive characters (Torres et al. 2003). Therefore it is considered that species with a narrow genetic base will not be able to respond to changes in environmental pressures. According to the allelic diversity and heterozygosity, more continuous and larger genetic bases of sandalwood populations could maintain a significant level of genetic variation in such a condition which supports larger gene flow and outcrossing.

Genetic differentiation of sandalwood

The relation between geographical and genetic distance. This highest genetical difference between populations within Timor island, which was even higher than those of between Gunung Sewu (Java Island) and Sumba-Timor islands, might be attributed to the differences in the parental genetic materials and the degree of disturbances between Netpala and Soe populations. Netpala was established using several provenances of Timor and is well managed for seed production purpose. Whilst, Soe is the remnant of heavily harvested population which was damaged since a very long period.

A lower genetical difference among the three populations within Northern Zone may relate to the history of occurrence of GSN3-Bejiharjo population. GSN3-Bejiharjo population was introduced at 1970's with the unknown sources originated from Nglipar District, a site

that is recently recognized as the GSN2-Sriten population (The Head of RPH Gelaran Bejiharjo, *pers. comm.*). This finding showed evidence that GSN2-Sriten was the ancestor of GSN3-Bejiharjo population in the past, and therefore both Northern populations shared the same allele frequencies and distributions. The differences of past condition from recent condition had a relation with the differences in landscapes structures and anthropogenic disturbances, which resulted in the different genetical processes. Another population in Northern Zone, the GSN1-Nglanggeran mountain, were connected to GSN2-Sriten population and altogether formed the Batur Agung highland zones. Both GSN1-Nglanggeran and GSN2-Sriten were less disturbed, influenced by similar *Am* climatic type and the highland environment, and having similar soil types, and therefore were shared similar evolutionary and genetical processes.

It seemed that it is not the geographical distance which caused the genetic differences among the three of Middle Zone populations, as well as the two of Southern Zone populations in Gunung Sewu. This higher genetic differentiation was caused more by their differences on landscapes structures and parental genetic base. If GSM2-Mulo, the cliff-isolated landrace, is included in the measurements, then the genetical differences is much higher (D_{ST} 2.50%, G_{ST} 7.47%, N_em 3 individuals per generation). On the other hand, differences were somehow lower between Timor and Sumba island. These findings revealed some evidence that both islands might share the same sources in the past, experienced similar evolutionary and disturbance histories, and therefore shared a large portion of same genes.

Many landscape-genetical based studies reported isolation and genetic differentiation by distance (Lhuillier et al. 2006; Bottin et al. 2007; Schmidt et al. 2009). However, differentiation among sandalwood populations was neither related to population size, nor to the geographic distance among populations. Grouping of population in dendrogram was not based on their geographical sites of occurence, but more by the similarity on clonality level and parental heterozygosity, which led to similar mating systems and reproductive processes. As reported in many cases, isolated species are more differentiated due to the restricted gene flow (Byrne et al. 2003; Pautasso 2009; Dani et al. 2011), the possible dominance of the founding event (Bottin et al. 2007; Garfi et al. 2013), the isolation from source populations (Lhuillier et al. 2006), and the stochastic processes due to their limited size (Botting et al. 2007; Moreira et al. 2015). Overall, the lack of a marked effect on differentiation to geographic distance indicates a dominant role of genetic drift relative to gene flow, as has also reported with sandalwood in other studies (Bottin et al. 2007; Schmidt et al. 2009; Garfi et al. 2013).

Similar result was also reported with *Santalum insulare* in Pacific islands where dendrogram did not clearly separate the archipelagos, and populations were grouped based on the similarity of genetic structures (Lhuillier et al. 2006). In *S. australocaledonium* in New Caledonia, some of highland and lowland populations were gathered on the same branch due to the low and non-significant F_{ST} (Bottin et al. 2007). In a long-lived, monoecious tree species *Quercus macrocarpa* in the north eastern U.S., the savanna landscape barriers did not restrict gene flow, hence the differentiation among populations were not correlated to distance (F_{ST} 0.027) (Craft and Ashley 2007).

Timor and Indian *S. album* populations had a mean genetic distance (*D*) of 0.447, which is very large compared to intra-specific distances in other species (Brand 1994). The average of *D* between western and eastern populations of *Acacia albida* in Africa was 0.273, and they were considered as different races (Brand 1994). In contrast, a very low genetic distance was observed in 16 populations of *Quercus ilex* along Mediterranean gradients (*D* only 0.023) (Craft and Ashley 2007). The continuous populations performed lower genetic distance, as reported in the connected populations of *S. spicatum* along Western Australia (*D* ranged from 0.006 to 0.073) (Byrne et al. 2003). While for *S. australocaledonium* in New Caledonia, the populations were strongly differentiated by the marked genetic drift, limited gene flow, and natural selection due to contrasted environment condition on the different geographical regions (Bottin et al. 2007).

The effects of population structures to the genetic differentiation. Landscape structures regarded to different rock outcrops, slopes and natural barriers may lead to the differences of the genetical and evolutionary processes within population (Torres et al. 2003). As reported in this study, the genetical difference among populations was related to the differences on landscapes structures and anthropogenic disturbances. More continuous, undisturbed and bigger population tended to exchange larger gene flow per generation. In contrast, gene flow was restricted in the isolated and disturbed populations. According to the *D* and F_{ST} values, GSS1-Petir and GSM2-Mulo appeared to be the most differentiated populations. The significant geographic barrier is the rocky limestone cliffs in GSM2-Mulo, which might have determined the genetic differentiation in the Middle Zone. The disjunct and isolated limestone cliff population of GSM2-Mulo is genetically different from others. Most of adult trees flowered abundantly, performed moderate level of diversity, and the inbreeding coefficient was closed to zero which indicated a random mating. However they failed to produce mature fruits due to the pollinators and pollination difficulties in the cliffs environments. Some of mature fruits might have survived, however the unavailability of seed beds on the dry, rocky vertical walls did not allowed the survived seeds to germinate. Genetic differentiation related to landscapes was also reported for many other species within a wider habitat range. Some of landscape features which may act as geographic barriers are rivers (Moreira 2015) and cliffs (Cursach et al. 2012; Garfi et al. 2013; Hmeljevski et al. 2014; Agullo et al. 2015). For example, in *Saxifraga aizoides*, large spatial gaps existed between small remnant populations within regions, resulted in the considerable genetic differentiation (Lutz et al. 2000). Pollinator and pollination limitation were also reported with *Ranunculus weyleri* under cliff granitic rocks environment, resulted in a very low reproductive success (Cursach et al. 2012).

The genetic differentiation is considered high when G_{ST} > 30% (Young et al. 2001). In this study, the G_{ST} was highest between populations within Timor island (G_{ST} 25.99%), which was even higher than those of between Gunung Sewu (Java Island) and Sumba-Timor islands (G_{ST} 23.83%). Results on genetic differentiation were similar to *S. insulare* which experienced similar isolation and clonality problems (Lhuillier et al. 2006). In *S. insulare,* 58 % of the trees were clones, and the genetic structure was characterized by high levels of differentiation between archipelagos (27 % of the total variation) and islands (G_{ST} 50%). RAPD-based study on the three small, fragmented populations of *S. album* in Southern India reported highly genetic structuring due to a restricted gene flow (F_{ST} 54.9%) (Dani et al. 2011), a same case to those occurred in five isolated populations of *S. lanceolatum* in south-eastern Victoria (D_{ST} 19%; F_{ST} 76%) (Warburton et al. 2000). In contrary, the more un-isolated and bigger populations allowed gene flow to occur among sites, and therefore they shared the same alleles and possessed lower genetic differentiation. Ten provenances of *S. album* in India and two in Timor performed lower level of genetic differentiation, indicated some extent of gene flow in the past (F_{ST} 28.6% and 17.2%, respectively) (Brand 1994).

The highly varied F_{ST} due to the fragmentation differences, as reported in this study, was also observed with sandalwood in India (Rao et al. 2017). In the bigger and continuous populations of *S. album* in Peninsular India, a high gene flow occurred (F_{ST} 3.4%) (Rao et al. 2007); in contrast to their counterparts in small, fragmented populations in Southern India which possessed highly genetic structuring due to a restricted gene flow (F_{ST} 54.9%) (Dani et al. 2011). Similarly, *S. spicatum* in Western Australia, in which their undisturbed and bigger populations could maintained their equilibrium and gene flow, was reported to have a low value of F_{ST} (8.3%) and genetic distance (*D* 0.031) (Byrne et al. 2003).

The genetic structuring of plants in isolated habitats may also be influenced by the founder effects (Torres et al. 2003; Craft and Ashley 2007). From a historical perspective, particularly the Tm-Soe and GSS1-Petir populations have heavily disturbed and experienced founder events since a long time period (Indrioko and Ratnaningrum 2015b). This event may still dominate the genetic processes and lead to the low levels of recent genetic diversity. A very low genetic base due to long periods of heavy exploitation, which resulted in a high genetic differentiation, was also reported for *Acacia nemorosa* and *A. columnaris* in New Caledonia (Kettle et al. 2007), and the remnants of heavily harvested *S. album* in southern India (Dani et al. 2011). In other cases, genetic differentation due to many kinds of disturbances were observed in the Brazilian tropical dry forests fragments which were under severe human threats for a long time period (Moreira et al. 2015), the disturbed populations of *Pinus squamata* in tropical Yunnan of China (Zhang et al. 2005), the fragmented populations of *Geum urbanum* due to extensively agricultural activities in central Europe (Schmidt et al. 2009), and the isolated populations of *S. lanceolatum* due to grazing in south-eastern Victoria (Warburton et al. 2000).

The results of the study on several populations of sandalwood, which covers both *Am* and *Aw* environments across geographical gradients in Gunung Sewu (Java Island), Timor and Sumba Islands, suggested that (1) natural barriers contributed to the habitat fragmentation and disrupted gene flow among populations; (2) the gene flow affected selection processes regarding to the bottleneck effects and genetic drifts, which resulted in the differences in allelic richness and population diversity; and (3) the variation in gene flow and selection processes consequentially resulted in genetic differentiation among populations. Gene flow restriction and the considerable genetic drift occurred, particularly when the population had a lower genetic base, highly clonalized, fragmented, and/or several inbreedings in mating system. In contrast, more continuous, undisturbed and wider genetic bases' population tended to exchange larger gene flow per generation. Such populations also performed higher genetic diversity and outcrossing rate which minimized loss of rare alleles due to genetic drift. Some exceptions were also made for several isolated, but less-clonalized and higher genetic bases' populations, which were able to maintain higher genetic diversity and to outcross under gene flow restriction.

The genetic differentiation was highest between populations within Timor island, and between Gunung Sewu (Java Island) and Sumba-Timor islands. But there was less association between geographical and genetic distance. Populations were not clustered based on their geographical sites of occurrence, but more by their similarity of genetic structures. Genetic differentiations among sandalwood populations might be best explained by the combined effect of the differences on genetical processes, with regard to the gene flow and selection events. Both differences existed due to the differences in (i) population structures including landscape types, degree of clonality and genetic composition at parents level, and (ii) the disturbances histories of the population, which contribute to the equilibrium between gene flow and drift. This findings emphasized the importance of larger gene flow and populations' genetic base to naturally maintain the genetical processes of sandalwood population under various landscapes structures.

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Economic valuation for tembawang ecosystem, in Sanggau District, West Kalimantan, Indonesia

EMI ROSLINDA♥, SITI MASITOH KARTIKAWATI, RABUDIN
Faculty of Forestry, Universitas Tanjungpura. Jl. Imam Bonjol, Pontianak 78124, West Kalimantan, Indonesia. Tel.: +62-561-765342, 583865, 732500, Fax.: +62-561-765342. ♥email: eroslinda71@gmail.com

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Abstract. *Roslinda E, Kartikawati SM, Rabudin. 2017. Economic valuation for tembawang ecosystem, in* Sanggau District, *West Kalimantan, Indonesia. Biodiversitas 18: 1506-1516.* The tembawang ecosystem in West Kalimantan (Indonesia) spreads over especially in Sanggau District region. *Tembawang* is a system of land used by customary community of Dayak ethnic which is traditionally managed and communally used. The objective of this study is to determine the total economic value (TEV) of the tembawang ecosystem. Survey methods were used in this research. 70 respondents were selected by census method. Total economic values in this research were restricted on direct use value, indirect use value and option value. Based on market price (biomass) and opportunity cost (hydrological role), the economic values were calculated. The results showed that total economic value of Tembawang Ampar was IDR 263,837,082 year^{-1}, which was equivalent to IDR 36,900,291 year^{-1}ha^{-1}. They are IDR 33,545,791 year^{-1}ha^{-1} from direct use value, IDR 3,072,738 year^{-1}ha^{-1} from indirect use value, and IDR 281,762 year^{-1}ha^{-1} from option value. Total economic value of tembawang was rather low. The low of economic value was due to limited benefits assessed in this study. Whereas in fact, there were many benefits from tembawang obtained by the community. Conserving tembawang ecosystem will not be achieved by simply considering the conservation of species in tembawang, but the preservation will need to encompass a wide range of natural resources and the potential services provided by them.

Keywords: Community, conservation, economic valuation, market price, tembawang

INTRODUCTION

The *tembawang* ecosystem in West Kalimantan (Indonesia) spreads mostly in Sanggau District region. Based on the tembawang description, tembawang is like a forest ecosystem which supports almost all the needs of a human being through its contribution of tangible and intangible values. So far, the economic value of the tembawang is generally assessed from the value of fruit production only, while the value of the environmental services of the tembawang is not considered properly. This has resulted in the underestimation of the total value of the tembawang leading to the conversion of tembawang areas into other more tangible profitable usages, and mismanagement of them. Environmental services of tembawang have no intangible benefits which are difficult to quantify and it will give rise of externalities. According to Hartwick and Oliver (1998), public externalities occur when public goods are consumed without proper payment. To suppress these externalities, there is a need to make a valuation of tembawang values.

The economic valuation of tembawang ecosystem aims to provide the economic value of resources used based on the real value from the society's viewpoint. Economic valuation offers a more comprehensive assessment of the various goods and services produced by the ecosystem so as to contribute more in-depth information for decision-making in sustainable forest management (Munasinghe et al. 1993 Barbier et al. 1997; Napitupulu et al. 2012; Kildow and Gino 2014; Roslinda and Yuliantini 2014; Malik 2015).

Economics as a tool in conducting economic valuation is the science about making a choice. The economic valuation of natural resources can be defined as the study of allocation of natural resources like water, land, fish and forest (Fauzi 2004). Benefits which could be provided by natural resources to meet human needs are many, but limitations in science and technology, as well as rigid institutional arrangement, has impeded the types and amount of its utilization so that obtained benefits are still very low (Darusman 2012). To make choices of several alternatives on environment management are more complex than the selection of options in the context of purely private goods (Harahap 2010). The economic valuation can be defined as the attempt to assign quantitative values to the goods and services provided by the ecosystem. The economic value of any goods or services is generally measured in terms of what we are willing to pay for the commodity less than what it costs to supply it. The objective of the valuation of ecosystem services is to indicate generally the overall economic efficiency of various uses of functions of a particular ecosystem. The underlying assumption is that ecosystem resources should be allocated to those users that yield an overall net gain to the society, as measured through valuation regarding the economic benefit of each use adjusted by its costs (Kumar and Kumar 2008).

In the context of deciding alternative for tembawang ecosystem based on the benefit-cost rule, the decision can be justified if net benefit of ecosystem development is greater than net benefits obtained from conservation. In this case, conservation benefits are measured based on the total economic value of the tembawang ecosystem. Tembawang ecosystem in Ampar Subvillage of West Kalimantan, Indonesia has very important and significant value for the local community, i.e. social, economic and ecology. Tembawang ecosystem has multiple roles for people community and provides other benefits to the nature in maintaining forest structure, diversity, and large carbon stocking in standing biomass (Astiani and Ripin 2016). However, the existence of tembawang ecosystem often became a matter of debate by various parties whether to maintain it in current condition or to convert it into plantation palm area or other uses. The most optimal management option is not known yet as there is a lack of information on the values (benefits and function) of tembawang. Therefore, a study about the total economic value of the tembawang ecosystem is needed. The objective of this study is to determine the total economic value (TEV) of the tembawang ecosystem.

MATERIALS AND METHODS

Study area

The study was done on September 2014 until February 2015. The area of research was in Tembawang Ampar in Ampar Subvillage (*dusun*), Cempedak Village (*desa*), Tayan Hilir Subdistrict, Sanggau District in West Kalimantan, Indonesia which was located in 00°43'06" LU and 110°18,5'39" BT (Figure 1). Ampar Subvillage is situated along the trans-Kalimantan interstate highway and can be reached by car and a motorcycle. Based on the Schmidt and Ferguson classification, climate type of Ampar Subvillage area is classified into the type A (very wet) with mean temperature of 29°C and the average rainfall between 3,000-4,000 mm per year. Ampar Subvillage topography consists of a flat, wavy to hilly with slope level of 0-30% and is located at an altitude of 24-140 m above sea level. The soil type is dominated by red-yellow podzolic. Tembawang Ampar covers ± 6,69 ha (Ripin 2014). Some parts of it are still covered by forest, but most of it has been deforested and converted to community plantation (palm and rubber) and stone quarries.

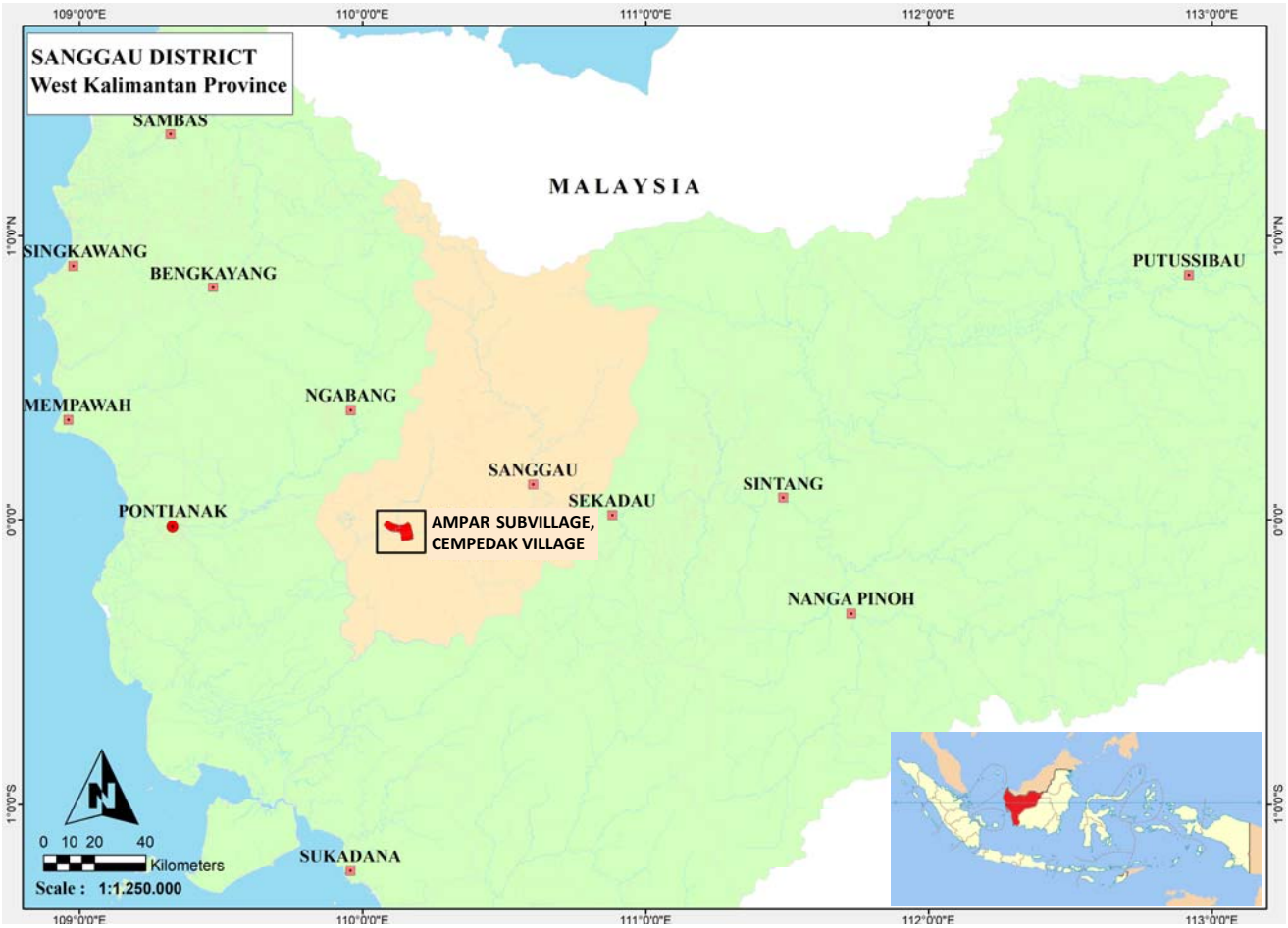


Figure 1. Site location in Ampar Subvillage, Cempedak Village, Tayan Hilir Subdistrict, Sanggau District, West Kalimantan, Indonesia

The dominant ethnic of the community is Dayak Tobak. They still adhere to the customs and culture from their ancient ancestors. The community is led by a tribal chief which plays a very important role. The roles of the chief at Tayan Hilir Sub-district are (i) the *Domong Adat*, a tiered traditional judiciary; and (ii) the Custom Judge, the one who listen to the customary cases at the village level. '*Ope-ope'* is local language of Ampar people for daily internal interaction, and Indonesian language is used to interact with people from outside community. The majority of them are Catholic.

The level of education of people in Ampar Subvillage is relatively low. It can be seen from the percentage of people who did not graduate or only graduated from elementary school, that is 70%. The number of people who are studying in junior high and high school is about 28.4%. And only 1.6% of them have graduated from D3 or S1 (Pemdes Cempedak 2014). Most of the community lives from farming such as rice planting (*berhuma*) on the wet-paddy field (*sawah*) and upland farming (*leading*). Planting and cultivating rubbers is a source of income for them. The rest of community works as traders or as employees of road construction company, PT. Strada Multiperkasa Company, which was operating in those sub-villages at that time.

Data collection

The research was conducted by using survey aiming to collect data from a number of people through interviews, field observation, and literature review. Seventy people were selected by some considerations through census method. Ampar Subvillage is inhabited by 70 households with a total population of 350 people, which consists of 200 men and 150 women (Pemdes Cempedak 2014014). Selection of sample village was done purposively based on tembawang ecosystem existing in the villages. While respondents selected are members of Ampar Subvillage and the people who directly or indirectly got its benefits. Some questions asked to respondents include the kind of products and services they attained, the money they procured and the frequency of the procurement, and the consideration of the importance and the value of the existing tembawang. Secondary data were obtained from the literature, reports and other documents related to the study.

Identify types of tembawang use

Tembawang benefits were analyzed descriptively including type, location, scale, system and model of utilization. Analysis of total economic value of tembawang was conducted using formula from Munasinghe (1993), namely, as follows:

TEV = (DUV +IUV +OV) + (XV)

Where:
TEV = Total economic value
DUV = Direct use value
IUV = Indirect use value
OV = Option value
XV = Existence value

Direct use value (DUV). The immediate benefits are the benefits derived from land use of tembawang based on market prices including timber and firewood, and the benefits from its biological condition and cultivation (fruits, rubber, *apik*, bamboo, medicine, and animal).

Indirect use value (IUV). Indirect use value is the sum of indirect benefits gained from tembawang ecosystem including soil protection, water supply, CO_2 fixation, nutrient cycling, pollutant decomposition and disease and pest control. In this research, indirect benefits are limited to its function as a water supply for domestic use. Estimation of water for domestic use was approached from water consumption costs based on standard needs of average household issued by Ampar Subvillage people.

Option value (OV). Option value is a benefit to preserve the use of goods, services and environmental resources in the future that cannot be used at present. In this research, the value to be used is the benefit of some fruits that are not used at the current time. Option value benefit was approached by market price.

Existence value (XV). Existence value is a benefit perceived by the public from the presence of tembawang ecosystem after other benefits (direct benefits, indirect benefits, and option value) are removed from the analysis. It is benefit to be enjoyed by human from the existence of tembawang ecosystem. In this research, existence value is not being calculated.

The total economic value of this research is derived from direct use value, indirect use value and option value. Direct use value and option value were categorized as intangible products, and indirect use value was categorized as intangible product. The tangible and intangible products of the tembawang were grouped into biomass and hydrological role (see Figure 2).

The economic values were calculated based on market price (biomass), opportunity cost (hydrological role) and contingent valuation method (Munasinghe 1993). Market price method uses the prices of goods and services bought and sold on the commercial market to determine the value of an ecosystem service. This method only measures values and marketed goods or services having an actual price. The following formula is used for calculating the Total Economic Value: TEV = DUV + IUV + OV.

RESULTS AND DISCUSSION

Respondent profile

Seventy respondents were interviewed using census. The respondents' ages are 25-77 years old and reside at Ampar Subvillages (*dusun*). Among the respondents, only five are female. Furthermore, all respondents are Catholic and most of them did not complete the elementary school. This was because the educational facilities in the Ampar Subvillage were still very limited from the past time until now. Most of the respondents are farmers. The respondents' profile can be seen in Table 1.

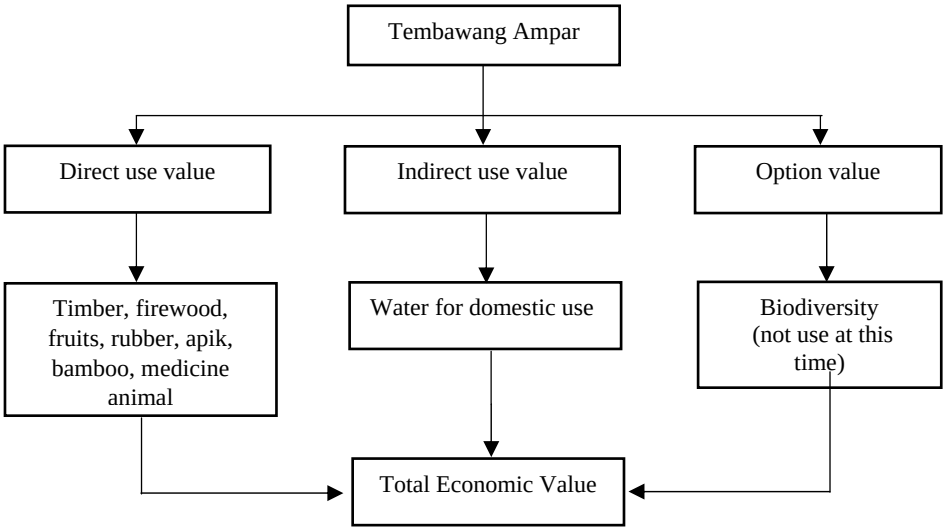


Figure 2. Total Economic Value of Tembawang Ampar in Ampar Subvillage, Cempedak Village, Tayan Hilir, Sanggau District, West Kalimantan, Indonesia

Table 1. Respondent profile

Profile	Category	Number	(%)
Gender	Female	5	7.14
	Male	65	92.86
	Amount	70	100.00
Age	25-35	22	31.42
	36-45	19	27.14
	46-55	19	27.14
	56-65	8	11.42
	66-77	2	2.86
	Amount	70	100.00
Religion	Moslem	4	5.71
	Christian	10	14.28
	Catholic	55	78.57
	Buddhism	1	1.42
	Amount	70	100.00
Education	Elementary School or below	57	81.43
	Junior High School	8	11.43
	Senior High School	5	7.14
	Amount	70	100.00

All respondents stated that their main livelihood is farming, such as rice planting (*berhuma*), both on the wet-paddy field (*sawah*) or on the upland farm (*ladang*). Rice planted on *paya* and *mongo* were harvested once a year. The yields of rice from 1 ha of *paya* and *mongo* are approximately 200 kg and 400 kg, respectively. The rice production is for subsistence.

All respondents have a rubber plantation. Rubber tapping is a second job even though it is economically profitable than farming. This activity is performed routinely in the morning before going to the upland farm, when the trees produce a lot of latex. The latex can be tapped approximately 5 kg per day. The latex is usually collected first and then sold to a middleman at a price of approximately IDR 6,000 per kg^{-1}. The result from rubber tapping is enough to cover the family needs. Besides rubber, oil palm is one of the commercial crops they choose to be planted. Figure 3 shows the agricultural lands of Dayak Tobak community.

Tembawang ecosystem

Tembawang is a system of land use by the customary community of Dayak ethnic in West Kalimantan which is traditionally managed and used communally. Tembawang is often referred to as an agroforestry because it has a variety of plants ranging from trees with a diameter over 100 cm to understory plants. Tembawang is regarded as a unique ecosystem for storing high values in terms of biodiversity, economic, and conservation moral.

Tembawang has a strong relationship with the shifting cultivation which has been practiced since hundreds of years ago. In the past, the Dayak settlements moved to follow the movement of their upland farm location. They usually grew various kinds of fruits, crops, and spices in their settlement location. When they moved, the location of the old settlement will be abandoned and become an agroforestry which is then called tembawang.

Tembawang Ampar has formed hundreds of years ago (Figure 4). Tembawang Ampar has already come into the seventh generations. Based on land ownership (land tenure), Tembawang Ampar is communal property owned by several families that are bound in a big family. They own tembawang as an inheritance from their parents or ancestors.



Figure 3. Source of income for respondents in Ampar Subvillage, West Kalimantan, Indonesia. A. Rice planting (*berhuma*) on the upland farm (*ladang*), B. Planting rice on the wet-paddy field (*sawah*), C. Planting commercial crops, rubber tree, D. Planting oil palm



Figure 4. The figure of Tembawang Ampar of West Kalimantan, Indonesia. A. Fruit trees, B. Apik (*Arenga undulatifolia* Becc.)

In Tembawang Ampar, there are 84 plant species which are utilized by Dayak community. Most of them are fruit tree species. Meanwhile, based on Ripin (2014), there were 97 plant species from 37 families found in the tembawang. Furthermore, Astiani and Ripin (2016) stated that, in Tembawang Ampar, each land cover type was dominated by almost similar species such as karet, mahang, and durian. Tembawang Ampar was dominated with durian (*Durio zibethinus* Murr), rambutan (*Nephelium lappaceum* L.), duku (*Lansium domesticum* Corr var. duku), mahang (*Macaranga pruinosa* Muell. Arg), karet (*Hevea brasilliensis* Will ex A. Juss), asam gandaria (*Bouea macrophylla* Griff.), asam kemantan (*Mangifera torquenda* Kosterm.), jengkol (*Archidendron pauciflorum* (Benth.)

I.C.Nielsen), engkasai (*Pometia glabra* (BI) Teijsm.), and apik (*Arenga undulatifolia* Becc.). The apik was naturally grown plant on the hilly site which was found abundant and grew among the fruit trees. In Tembawang Ampar, the main characteristic of tembawang is durian (*Durio zibethinus*). High economic value is the major reason that made durian as the main crop in tembawang. Durian fruit harvest is performed one to two times a year, the great harvest (*panen raya*) period is in August and the end of harvest (*panen ujung buah*) period is in February. Fruits that are obtained at the end of the fruit harvest period are not as many as in August. Fruit tree species found in Tembawang Ampar can be seen in Table 2 and Figure 3.

Table 2. Fruit tree species found in Tembawang Ampar

Vernacular name	Scientific name	Family
Ambacang	*Mangifera swintoniodes* Kosterm	Anacardiaceae
Asam gandaria	*Bouea macrophylla* Griff.	Anacardiaceae
Asam kemantan	*Mangifera torquenda* Kosterm.	Anacardiaceae
Asam mawang	*Xanthophyllum excelsum* Miq.	Anacardiaceae
Belimbing darah	*Baccaurea angulata* Merr.	Euphorbiaceae
Cempedak	*Artocarpus teysmanni* Miq.	Moraceae
Cempedak air	*Artocarpus champeden* (Lour.) Stokes	Moraceae
Ceriak	*Baccaurea* sp.	Euphorbiaceae
Duku	*Lansium domesticum* Corr var. duku	Meliaceae
Durian	*Durio zibethinus* Murr.	Bombacaceae
Emponat	*Litsea* sp.	Lauraceae
Engkasai	*Pometia* glabra (Bl.) Teijsm.	Sapindaceae
Engkubu	*Nephelium* sp.	Sapindaceae
Kapul	*Baccaurea bracteata* Müll. Arg.	Euphorbiaceae
Kedondong hutan	*Canarium* sp.	Burseraceae
Kelampai tupai	*Blumeodendron tokbrai* Bl.	Euphorbiaceae
Kelampai	*Elateriospermum tapos* Bl.	Euphorbiaceae
Kelawik/teratungan	*Durio oxleyanus* Griff.	Malvaceae
Ketup	*Lansium domesticum* Corr. var. aquaeum	Meliaceae
Kubing	*Artocarpus heterophyllus* Lam.	Moraceae
Kumpang	*Gynnacranthera forbesii* Warb.	Burseraceae
Langsat	*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet	Meliaceae
Manggis	*Garcinia mangostana* L.	Guttiferae
Mentawa	*Artocarpus anisopyllus* Miq.	Moraceae
Nangka	*Artocarpus heterophyllus* Lam	Moraceae
Peluntan	*Artocarpus rigidus* Bl.	Moraceae
Pingan	*Artocarpus odoratissimus* Blanco	Moraceae
Porik	*Nephelium* sp.	Sapindaceae
Rambai	*Baccaurea motleyana* Muell. Arg.	Euphorbiaceae
Rambutan	*Nephelium lappaceum* L.	Sapindaceae
Salak	*Salacca edulis* Reinw.	Arecaceae
Sibau	*Nephelium uncinatum* Radlk. ex Leenh.	Sapindaceae
Sotol/kecapi	*Sandoricum koetjape* (Burm. F.) Merr	Meliaceae
Sukun	*Artocarpus altilis* (Park.) Fosberg	Moraceae
Tampoi	*Baccaurea grifithii* Hookf.	Euphorbiaceae



Figure 4. The figure of: A. Ceriak (*Baccaurea* sp.), B. Belimbing darah (*Baccaurea angulata* Merr.), C. Ketup (*Lansium domesticum* var. aquaeum.), D. Apik (*Arenga undulatifolia* Becc.)

Identification of* tembawang *ecosystem benefits in Ampar Subvillage

By understanding the value, those involving goods and services produced by a natural resource and the environment can be found particularly different. It depends on the view angle used by the scientist. The difference on the concept of value can make difficulty in understanding the importance of an ecosystem. Therefore, it is necessary to have a similar perception of the ecosystem assessment (Harahap 2010). Benefits of tembawang ecosystems in Ampar Subvillage consisted from direct, indirect, and option value.

Total economic value (TEV) of tembawang ecosystem

Direct use value

The direct benefits of tembawang ecosystem come from the activities of producing a log, collecting firewood, collecting some fruits (usually used and have a market price), collecting apik, collecting rubber, collecting bamboo, collecting some medicinal plants and collecting animals. Tembawang Ampar provides some commercial log, i.e. Meranti batu (*Shorea palembanica* Miq.), meranti padi (*Shorea leprosula* Miq.), Tengkawang rambai (*Shorea splendid* (de Vr.) Ashton) Tengkawang tungkul (*Shorea stenioptera* Burck.). They were valued by market price based on Regulation of the Minister of Trade of the Republic of Indonesia Number 92/M-Dag/Per/10/2015 and they have to reach the level of the tree with diameter > 20 cm. In this research, data of wood potency used the method from Ripin research (2014). The total value of wood is IDR 67,821,402 year^{-1}. Table 3 will describe the economic value of wood in Tembawang Ampar.

Although most people in Ampar Subvillage have already used oil and gas stoves, some still uses firewood as additional energy. It was found that the use of firewood from tembawang has several advantages, i.e., faster heating time and money saving. Collecting firewood is done on an area close to their settlement. The economic value of firewood was counted by a willingness to pay. Based on the interview, there were 27 HH who still do the activity of collecting firewood in tembawang area. The value of firewood in Tembawang Ampar is IDR 1,296,000 year^{-1}. Table 4 describes the economic value of firewood.

Fruit trees are the dominant species in Tembawang Ampar. Therefore some researchers called it the garden of mixed fruits. There are many kinds of fruits used by the community and have their own market prices, such as durian, cempedak, langsat, duku, belimbing darah and rambai. The value of collecting some fruits (27 HH) is IDR 108,000,000 year^{-1}. Table 5 describes the economic value of some fruits.

Tembawang provides some food for the community, one of them is apik. Apik is used for the community as a vegetable for their daily food. Apik has a market price. The price of apik based on their size, the price of the larger ones is IDR 15,000 per bunch (*ageh*). Meanwhile, the little one is IDR 5,000 per bunch. The value benefits of collecting apik (27 HH) is IDR 540,000 per year. Table 6 describes the economic value of apik.

Rubber tapping was a common activity in Ampar Subvillage and a source of livelihood to increase the family income. Rubber has a certain market price. Although, the rubber prices continue to decline, this activity is being conducted as an alternative to community work. Collecting the rubber is done on an area close their settlement. Assuming that there are 6 HH who are still tapping rubber in tembawang, The value of rubber tapping (6 HH) is IDR 25,920,000 year^{-1}. Table 7 describes the economic value of rubber tapping.

The high potential of bamboo can be found in Tembawang Ampar since it can be found in considerable amounts. Bamboo was utilized by people to make a pole, *trigai* (used to pick fruits) and rebung (bamboo shoots = can be eaten as a vegetable). The value of bamboo follows market price. Assuming that, there are 27 HH of total respondents collecting the bamboo in tembawang, so the value of bamboo is IDR 9,720,000 per year. Table 8 describes the economic value of bamboo.

The potential of medicinal plants can be found in Tembawang Ampar. Based on Zuhud et al. (2015), the use of medicinal plant species in the tembawang area can be grouped into 13 groups of diseases curement, i.e: wound, stomachache/diarrhea, body stool, toothache, swell, breath shortness, stiff, sprain, dizzy, *malaria*, eyesore, fever, postpartum. So far, the community usually collects medicinal plants only when they are needed. Value of medicine plants is counted by substitution price with assumption that: (i) the price of medicinal plants is assumed to be equal to the price of medicine in the market around the community, except for the price of *akar kuning*, (ii) there are 70 households who utilize the medicinal plants, (iii) medicinal plants are commonly used by community. So the value of medicinal plants is IDR 18.480.000 per year. Table 9 describes the economic value of medicinal plants.

Some animals often found in tembawang area are wild boar, monkeys, rabbits, hedgehogs, squirrels, *munsang*, *pelandok* and deer. The animals which are calculated their direct use values are deer (*Muntiacus muntjak*), pelanduk (*Tragulus* spp.), *musang* (*Paradoxurus hermaphroditus*), and tupai (*Tupaia javanica*). They are caught by hunting. Assuming that there are 5 HH of total respondents who hunted in tembawang, so the value of the animal is IDR 8,075,00 year^{-1}. Table 10 describes the economic value of the animal.

Indirect use value

An indirect benefit of tembawang function has a relation to hydrology. All the value of tembawang relating to hydrology is obtained by using market price. From field observation and interview results, people now are able to utilize water for various domestic needs in better way. The value of hydrology in tembawang is determined by water consumption for domestic use on HH who have to purchase it. Only 38 HH utilized the water for consumption from Tembawang Ampar source, the rest (32 HH) use water from the other source. The value of hydrology is IDR 21,970,080 per year. Table 11 describes the economic value of the hydrological function.

Table 3. Economic value of log

Log species	Volume (m^3)	Price (IDR)	Amount (IDR)
Jabon (*Neolamarckia cadamba* (Roxb.) Bosser)	4.6735	90,000	420,615
Meranti Batu (*Shorea palembanica* Miq.)	17.4176	760,000	52,949,443
Meranti Padi (*Shorea leprosula* Miq.)	1.7097	730,000	1,248,103
Tengkawang Rambai (*Shorea splendida* (de Vriese) P. Ashton)	0.4154	730,000	303,258
Tengkawang Tungkul (*Shorea stenoptera* Burck.)	16.9737	760,000	12,899,984
Total			67,821,402

Note: Price of log based on Regulation of Ministry of Trading Republik Indonesia Nomor 12/M-Dag/Per/3/2014. Assuming the price of *tengkawang tungkul* is same with the price of other shorea. The potency of log was based on Ripin (2014)

Table 4. Economic value of firewood

Item	Price (IDR)	Need	Unit	Benefit value (IDR)
Firewood	5,000	4	Bunch HH^{-1}	20,000
Willingness to pay		4,000	Bunch $HH^{-1}year^{-1}$	16,000
Time needs for collecting firewood		30	Minute $bunch^{-1}$	
Times of cooking		3	Times day^{-1}	
Value of firewood $27HH^{-1}year^{-1}$				1,296,000

Table 5. Economic value of fruits

Species	Price (IDR)	Product/HH	Unit	Benefit value (IDR)
Durian (*Durio zibethinus* Murr.)	10,000	300	Fruit	3,000,000
Cempedak (*Artocarpus teysmanni* Miq)	3,000	200	Fruit	600,000
Langsat (*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet)	5,000	40	kg	200,000
Duku (*Lansium domesticum* Corr var. duku)	5,000	20	kg	100,000
Belimbing darah (*Baccaurea angulata* Merr.)	5,000	10	*Ageh* (bunch)	50,000
Rambai (*Baccaurea motleyana* Mull. Arg).	5,000	10	*Ageh* (bunch)	50,000
Value of fruits/HH/year				4,000,000
Value of fruits for 27HH/year				108,000,000

Table 6. The economic value of apik

Component	Price/bunch (IDR)	Product/year	Unit	Benefit value (IDR)
Apik	10,000	2	Bunch	20,000
Value of apik (27 HH) $year^{-1}$				540,000

Table 7. The economic value of rubber

Item	Price/Kg (IDR)	Product/day	Unit	Benefit value (IDR)
Rubber	6.000	3	kg	18,000
Value of rubber for 6 HH day^{-1}				108,000
Value of rubber for 6 HH $month^{-1}$				2,160,000
Value of rubber for 6 HH $year^{-1}$				25,920,000

Table 8. The economic value of bamboo

Item	Price	Product/month	Unit	Benefit value (IDR)
Pole	2,000	5	Pole	10,000
Rebong	2,000	10	*Ageh*/ikat	20,000
Value of bamboo (/HH/month)				30,000
Value of bamboo (/HH/year)				360,000
Value of bamboo (/27HH/year)				9,720,000

Table 9. The economic value of medicinal plants

Medicinal plants	Price (IDR)	Product/month	Unit	Value (IDR)
Sugi (*Pleomele angustifolia* Medik)	3,000	2	Pieces	6,000
Akar kuning (*Fibraurea tinctoria* Lour)	10,000	1	Stem	10,000
Pakis engkomok (Pterydophyte)	3,000	1	Pieces	3,000
Bakung (*Crinum asiatacum* Linn.)	3,000	1	Fruit/pieces	3,000
Value/HH/month				22,000
Value/70HH/month				1,500,000
Value/70HH/year				18,480,000

Table 10. The economic value of animal

Animal	Price (IDR)	Produts	Unit	Value (IDR)
Kijang (*Muntiacus muntjak* Zimmerman)	50,000	1 = 20	kg	1,000,000
Pelanduk (*Tragulus* spp.)	50,000	2 = 6	kg	300,000
Musang (*Paradoxurus hermaphroditus* Pallas)	35,000	3 = 9	kg	315,000
Tupai (*Tupaia javanica* Horsfield)	35.000	5 = 1	kg	35.000
Value HH year^{-1}				1.615.000
Value 5HH year^{-1}				8.075.000

Table 11. The economic value of hydrology

Variable	Data	Unit	Note
The average of water consumption/person/day	144	Liter person^{-1}day^{-1}	Survey result from Directorate of Drinking Water Development cq. Cipta Karya 2006
The average of water consumption/household/day	720	Liter day^{-1} Household^{-1}	144 x 5 (the average of household member in Ampar village are 5 person
The total water consumption/year	9,986,400	Liter year^{-1} Household^{-1}	38 x 720 x 365
Waterer price	2,186	IDR m^{-3}	Based on PDAM data (2011) distribution of water in Tayan Hilir Sanggau sub with value of water is IDR 135.476.000
	2.2	IDR liter^{-1}	
Value of water	21,970,080	IDR year^{-1}	9,986,400 x 2,2

Table 12. Total economic value of tembawang

Type of value	IDR/year	Percentage
Direct use value	239,852,402	90.91
Indirect use value	21,970,080	8.33
Option value	2,014,600	0.76
Total	263,837,082	100.00

Option value

Option value shows individual's willingness to pay to preserve certain natural resource for future utilization (Harahap 2010; Rachmansyah and Maryono 2010; Napitupulu 2012). Option value of tembawang was estimated by using contingent valuation method (CVM). Through this method, respondents are asked to assess the significance of the presence of tembawang ecosystems. All respondents are asked their willingness to put aside a certain amount of money. The result obtained from people's willingness to pay is IDR 2,014,600 year^{-1}.

Total economic value

From the analysis of potential economic value calculation, the total potential economic value of the tembawang ecosystem is IDR 263,387,082 year^{-1} or IDR 36,900,291 ha^{-1}year^{-1}. Table 12 shows that the highest benefit is from direct value (90.91%), followed by indirect value (8.33%) and the lowest is option value (0.76%). The research result is rather different with another research that indirect value has the highest value (Roslinda 2002; Siregar 2012; Roslinda 2013; Malik et al. 2015). It is because of the limitation of this research. Actually, tembawang ecosystem has ecological benefits. However, since most of the ecological benefits derived from tembawang ecosystems mostly cannot be acquired directly, real and close to the place they exist. Therefore, the existence of tembawang must be preserved along with the increase awareness and understanding of local community on the value and the importance of tembawang ecosystems.

Discussion

Tembawang is one of the land uses in Dayak Toba community. Besides tembawang, in Dayak Toba land use, there were *waqf land*, *rimba*, *mongo/meh*, rubber plantation, oil palm plantation, *paya*, and *jamin*. Every land use has a special function and can support the community needs. This situation is similar to Dayak Desa community in Ensaid Panjang (Roslinda 2016). Tembawang was created by local knowledge and had been practiced by the communities for a very long time, so there was no difficulty to obey management of rule in utilizing tembawang. Utilization in tembawang was regulated by social norms existing in the community. One hesitating thing is that the social capital cannot guarantee the sustainability of tembawang. Tembawang can be categorized as community forestry. With strong social capital, the community forestry can be developed (Roslinda 2017).

Identification of the benefits of tembawang was performed with an analysis of the characteristics of ecosystem function that is translated into a list of goods and services (De Groot et al. 2002). The benefits of tembawang for community were material for energy (firewood), food and feed, resin, construction material, medicinal plants, material for a religious ceremony, wildlife (for hunting), hydrological function, air and climate regulation function, cultural/religious ritual, farming location, and nutrient cycling. Based on Zuhud et al. (2015, tembawang has all of those services, and the ecosystem services could provide benefits to the community because both the knowledge about the ecosystem and their services were used for subsistence of the people in the community. In addition, the Dayak Toba tradition perceives that the forests should be protected and preserved as a source of community livelihood. In some cases such as in the villages of the Mendalam Sub-watershed, Kapuas Hulu (Roslinda 2010), the communities believe that excessive use of forest will cause damage and catastrophic disaster. This social benefit of tembawang is actually the major reason for tembawang existence and sustained management.

The unexplored potency of tembawang is its ability to sequester and sink carbon. Based on Astiani and Ripin (2016), the tembawang sinks a large amount of above ground carbon especially the one found within mixed fruit garden patches reaching ~300 ton/ha. Another potency that has not been explored is the use of tembawang as a natural laboratory for education and research. Many researchers have come to the site to conduct research. Till now, there is no charge at all for entering the site as long as they have permission from the local government. If every activity of research pays a certain amount of money as a fee, then there will be some amounts of fund can be used to preserve the existence of tembawang.

Tembawang ecosystem in Ampar Subvillage has significant role economically, socially, and ecologically. Therefore, any efforts of tembawang management should accommodate its role in avoiding negative externalities. Results of the calculation of the total value of tembawang potential benefits show that utilization of Tembawang Ampar, so far, has been done without disturbing existing tembawang ecosystems. Public awareness of the value and importance of tembawang is the crucial factor for careful maintenance of tembawang ecosystem. There are some issues that should be anticipated for the sustainability of tembawang ecosystem. The need for land conversion of secondary forest (including tembawang) into plantation (palm and rubber plantation) and mining is very high. Based on the interviews with communities and relevant stakeholders, it can be found that total area of palm plantation in Sanggau District tends to increase, because, economically, the plantation gives more promising prosperity than tembawang. This should be a concern of all parties because if economy burden continues to increase, while the alternative sources of income are limited, it is possible that the conversion of tembawang into plantation would be done extensively in the future. If this happens, then all the values of the tembawang ecosystem will be lost.

In conclusion, the benefits of tembawang ecosystem in Tembawang Ampar consist of direct benefits, indirect benefit, and option benefit of biodiversity values. Total potential economic value of Tembawang Ampar is 263,837,082 $year^{-1}$, which is equivalent to IDR 36,900,291$year^{-1}ha^{-1}$. It is IDR 33,545,791 $year^{-1}ha^{-1}$ from direct use value, IDR 3,072,738 $year^{-1}ha^{-1}$ from indirect use value, and IDR 281,762 $year^{-1}ha^{-1}$ from option value. The economic value of tembawang ecosystem was higher than the real benefit felt by the society. It was derived from an ecosystem, such as forest ecosystem. Forests have long been recognized as the main ecological construction and restoration means for their multiple ecosystem services (Deal et al. 2012). To optimize tembawang ecosystem management, there is a potential benefit that can be developed, i.e., benefit as carbon sequestering agent and sinker and a natural laboratory for education and research. In formulating strategies for optimal tembawang ecosystem management, significant consideration on research result on total economic value of tembawang ecosystems along with some issues that need to be anticipated including tembawang benefits (carbon sequestration and sink; natural laboratory) and the need of government's attention should be done to maintain the existence of tembawang as a landscape having rich in biodiversity and benefits.

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The practice of farming, processing and trading of tobacco by Sukasari people of Sumedang District, West Java, Indonesia

JOHAN ISKANDAR[1,2], BUDIAWATI S.ISKANDAR[3] , AZRIL[1], RUHYAT PARTASASMITA[1,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia. Tel.: +62-22-77912. ♥email: ruhyat.partasasmita@unpad.ac.id, rp2010rikkyo@gmail.com

[2]Postgraduate of Environmental Science (PSMIL & DIL) and Institute of Ecology (PPSDAL), Universitas Padjadjaran. Jatinangor, Sumedang 45363, West Java, Indonesia

[3]Department of Anthropology, Faculty of Social and Political Science, Universitas Padjadjaran. Jatinangor, Sumedang 45363, West Java, Indonesia

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Abstract. *Iskandar J, Iskandar BS, Azril, Partasasmita R. 2017. The practice of farming, processing and trading of tobacco by Sukasari people of Sumedang District, West Java, Indonesia. Biodiversitas 18: 1517-1527.* Tobacco (*Nicotiana tabacum* L) is an original crop of Cuba, Latin America, discovered by Christoper Columbus in 1492 and introduced to Europe. Moreover, it was distributed to Asia countries, including Indonesia. Local people of Sukasari village, Sukasari sub-district, Sumedang district, West Java, has cultivated tobacco for a long time, since the Dutch colonial, based on local ecological knowledge transmitted by inter-generations. As a result, local people of Sukasari village have rich knowledge on the tobacco. Nowadays, however, since the agricultural lands as well as tobacco farmers have decreased, the local ecological knowledge of the Sukasari people have eroded. This paper elucidates the local ecological knowledge of Sukasari people, Sumedang District of West Java on landraces, cultivation, process, and local trading of tobacco. The method used in this study was qualitative with descriptive analysis applying the ethnoecological approach. The result of the study showed that the Sukasari people have predominantly cultivated four landraces of the tobacco. The cultivation of tobacco include the selecting of seeds, nursery, preparing land, planting, caring, harvesting and processing of tobacco products, requiring diligent efforts and high skill. Today, the cultivation of tobacco has many constrains, such as climate anomalies, decrease of agricultural lands, and the lack of finance; consequently, the tobacco farmers have less enthusiasm to cultivate the tobacco. As a result of decreaase of tobacco cultivation, the local ecological knowledge of the Sukasari people has eroded and may extinct in the near future.

Keywords: tobacco, local ecological knowledge, Sumedang

INTRODUCTION

Tobacco (*Nicotiana tabaccum* L.) is an original crop of Cub, Latin America, discovered by Christoper Columbus in 1492 (Cotton 1996). It was introduced to Europe and later distributed to Asian countries. According to Arnez (2009), tobacco was introduced to Asia in 1575, when the Spanish brought it to the Philippines from Mexico, and in 1601 it was introduced to Java. Moreover, the tobacco has been farmed in some places of Java, including West, Central, and East Java (Geertz 1963; Elson 1994; Boomgard 2002; Djajadi 2015). In West Java, this crop have been traditionally farmed by village farmers in some district areas of Sumedang, Bandung, Garut, Majalengka, and Cirebon (Igarashi 1985; Elson 1994; Boomgard 2002; Alamsyah 2011; Cakrabawa and Nuryati 2014; Djajadi D 2015).

Farmers of Sukasari Village, Sukasari Sub-district, Sumedang District, West Java, have traditionally farmed tobacco for a long time, since the Dutch colonial. They have cultivated tobacco in the dry land, based on the local ecological knowledge which is transmitted from generation to generation through three main general stages, namely parental, peer, and individual learning (cf. Puri 1997). Tobacco has been planted mostly in the dry land. Furthermore, the production leaf of tobacco has been processed by farmers to be special tobacco called *tembakau rakyat* or *bako mole* (Alamsyah 2011). The *bako mole* has been sold to village middlemen, local traditional market or cigarette factories.

The cultivation of tobacco and tobacco leaf processing to *bako mole* undertaken by each hold of tobacco farmers has been based on the local ecological knowledge which is strongly embedded in local tradition. The local ecological knowledge or traditional ecological knowledge, indigenous knowledge, folk knowledge is different from Western Knowledge, in that it istransmitted by oral, teaching through doing, experiential, diachronic, holistic, and vulnerable to extinction (Ellen 2002; Sillitoe 2002). Although the local ecological knowledge has many terms, but in general it may be defined it as “cumulative body of knowledge, practice, and belief, evolving by adaptive process and handed down through generations by cultural transmission, about the relationships of living being (including humans) with one another and with their environment” (Bereks et al. 2000). The local knowledge is traditionally obtained based on the close interrelationships between people with a sustainable local environment for a long time (cf. Fowler 2000; Ford 2000). As a result, generally the local knowledge on tobacco farming and its production processing are traditionally obtained by tobacco farmers who have been intensively involved in the tobacco

farming for generations. The cultivation of tobacco in the Sukasari Village has been influenced by many factors, particularly the ecological, local ecological knowledge, and socio-economic village people (cf. Mitchell 2006; Karim 2016; Iskandar et al. 2016). The local ecological knowledge of the tobacco farming, particularly the processing of tobacco to *bako mole* has traditionally been owned by the male gender. Moreover, this knowledge is traditionally inherited to their sons. Therefore, if the farmers household does not have any son, the tobacco farming rarely is continued. In addition, as of today, the cultivation of tobacco has many constraints, such as climate anomalies, the decrease of agricultural lands, the lack of finance, also fluctuation of input and output prices; consequently, the tobacco farmers suffer less enthusiasm to cultivate the tobacco. Indeed, the local ecological knowledge of the Sukasari people has eroded and may come to extinction in the near future.

The paper elucidates cultural practices among the Sukasari People of Sukasari Village, Sumedang, West Java, on tobacco farming, particularly regarding the local ecological knowledge on tobacco variations, traditional farming, and tobacco trading.

MATERIALS AND METHODS

Study area

Administratively, Sukasari Village is recorded as one of seven villages of the Sukasari Sub-district (prior to 2001 registered as Tanjungsari sub-district), Sumedang District, West Java. Geographically, Sukasari Village is located at approximately 179^{o} 47'16.5164" E and 6^{o} 52' 35.0261" S (Figure 1).This village is located between 8 masl and 1,040 m asl (above sea level) which has a hilly landscape. The Sukasari village has a total of about 438 hectares. About 157 hectares of the land is devoted to the wet-rice field, on the other hand 212 hectares is devoted to the dry land field, then a further 69 hectares has other land-uses (Pemdes Sukasari 2016). The Sukasari is a village that lies north-east of Bandung city and west of Sumedang District. To the north it is bordered by Kadakajaya Village; to the south it is bordered by Mekarsari Village; to the west it is bordered by Genteng village; and to the east it is bordered by Margaluyu Village.Reaching Sukasari Village by public transportation is considered to be quiet easy. Embark by bus or '*angkot*' from Bandung city to the Grand Mosque (*Masjid Agung*) of Tanjungsari Sub-district with

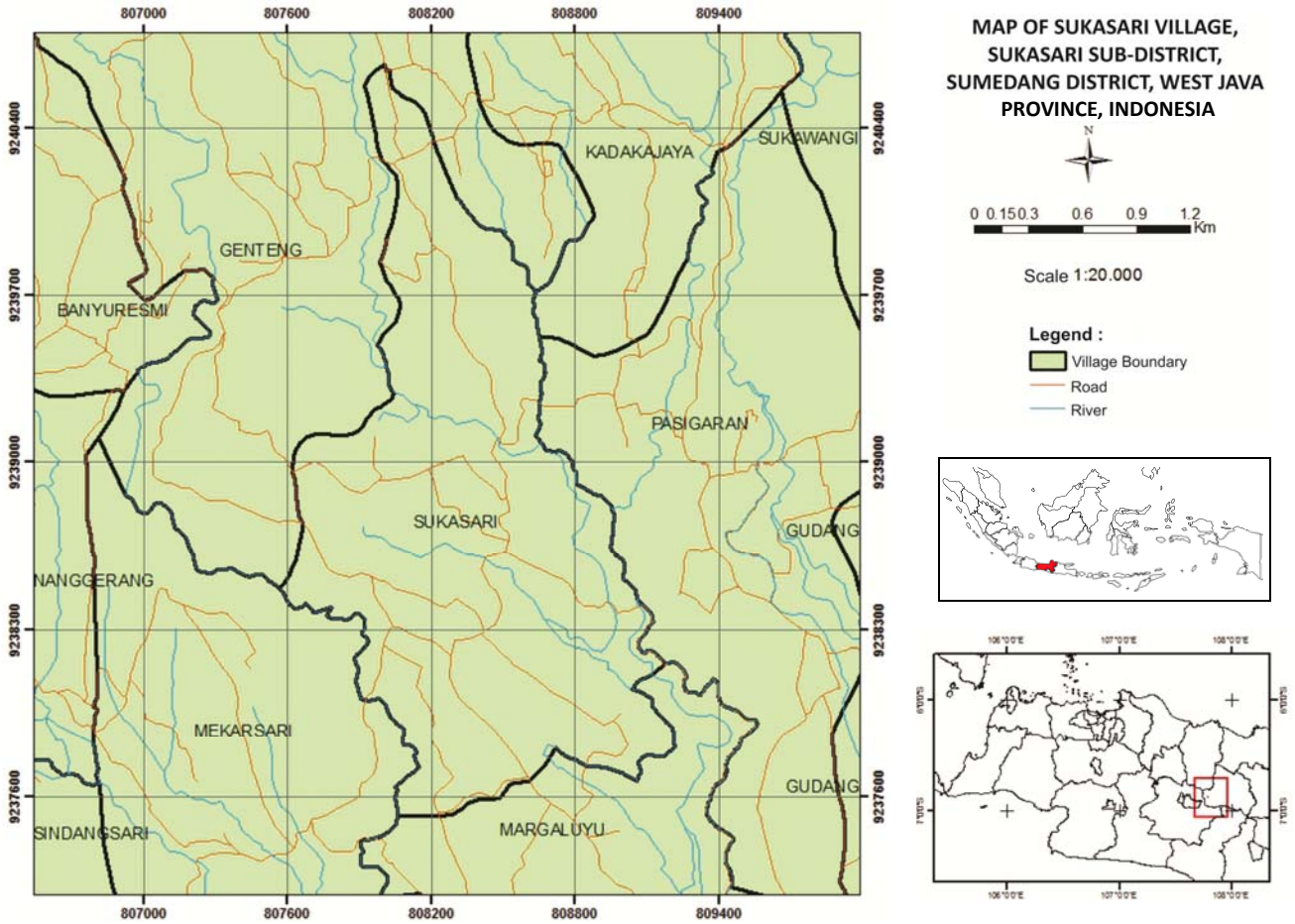


Figure 1. The map of Sukasari Village, Sumedang, West Java, Indonesia

an estimated distance about 30 km, then continue by *'ojeg'* or '*angdes*' for about less than half hour to reach the destination.

The agricultural system of Sukasari Village can be divided into two groups, namely the wet-rice field (*sawah*) and the dry-land field system that consists of homegarden (*pekarangan*), perennial mixed-garden (*kebun campuran* or *talun*), bamboo garden (*kebun bambu*), and annual crop garden (*palawija*). The wet-rice field is predominantly planted by rice crops while alternated by tobacco during the dry season. The tobacco cultivation, however, is predominantly undertaken in the dry field.

The registered population of Sukasari Village in 2006 was 1,711 households, with a total population of 4,937 individuals consisting of 2,477 males and 2,460 females. The population has increased over time which is determined by the death rate, birth rate, in-migration, and out-migration rate. In 2016 the total population of Sukasari village was registered 4,937 individuals consists of 3,145 male individuals and 3,326 female individuals (Table 1). Therefore, between 2006 and 2016, the population of Sukasari Village has increased by 1,804 individuals.

The predominant occupation of people in Sukasari village are registered as farmers (59.88 %) and farmer-labor (31.93 %) (Table 2). The wet-rice field cultivation system is predominantly practiced by people for the subsistence purpose, while the tobacco farming is undertaken for the commercial purpose. It has been historically recognized that some areas of Tanjungsari Sub-district, Sumedang District as a center of both tobacco farming and tobacco trading of West Java (Alamsyah 2011).

Table 1. Number of household and people of Sukasari Village, Sumedang, West Java, Indonesia

Sub-hamlet (RW)	Household	Male	Female	Male and female
01	271	371	375	754
02	217	342	301	643
03	315	504	473	977
04	187	265	235	498
05	189	266	257	523
06	172	212	246	458
07	156	201	217	418
08	147	206	206	412
09	146	194	183	377
10	102	129	134	263
11	100	122	113	235
12	238	321	314	635
13	87	109	111	220
14	128	265	158	323
Total	2458	3145	3326	6741

Note: Pemdes Sukasari (2016)

Table 2. The main occupation of people of Sukasari Village, Sumedang, West Java, Indonesia

Main occupation	People number	Percent of the total (%)
Farmer	1,500	59.88
Farmer-labor	800	31.93
Trader	62	2.47
Worker/Employee	50	1.99
Civil servant/Indonesian National Army	18	0.71
Entrepreneur	75	2.99

Note: Pemkec Sukasari (2016)

Method

The method used in this study was qualitative which is based on the ethnoecological and biological approach (Martin 1995, Cunningham 2001; Newing et al. 2011; Alburquerque et al 2014). Techniques to collect field data, include observation, participant observation, and interview. The observation was undertaken in the field, particular observation of the ecological condition of tobacco gardens, tobacco crops, and activities of farmers in managing of tobacco crop and processing of tobacco productions. The observation participation was carried out by involving researchers in various activities of farmers in managing the tobacco farming, including planting and harvesting of tobacco in the gardens, and processing of tobacco production in the farmer houses. While the interview was applied with deep interview or semi-structured-interview with local experts or competent informants which are purposively selected by the snowball technique (cf. Martin 1995). Some informants were selected in this study, including old tobacco male and female farmers, female and male who are involved in the left tobacco, village middlemen, informal leaders, and formal village leaders.

The collected data was analyzed by crosschecking, summarizing, synthesizing, and built up a narrative with descriptive analysis and evaluative (cf. Newing et al. 2011).

RESULTS AND DISCUSSION

Variations of tobacco

Tobacco crop (*Nicotiana tabaccum* L) of Family *Solanaceae* is traditionally named in vernacular name by people of Sukasari as *bako* in Sundanese or *tembakau* in Indonesian language. On the basis of the local knowledge, it has been known seven variations of tobacco crops, namely *bako bibit nani, bako bibit sano, bako bibit juhana, bako bibit kedu hejo, bako bibit kedu nangka, himar*, and *aromanis*.These tobacco variations are named after the first people who introduced the tobacco seeds to their village. For example, the tobacco variations have locally been named as Juhana, Nani and Sano due to these tobacco seeds which are firstly introduced by Juhana, Nani and Sano.

Although there are seven variations of tobacco, the predominant tobacco variations, *bako bibit sano, babo bibit juhana, bako bibit kedu*, and *bibit nani* are locally cultivated by farmers of Sukasari village. Four tobacco variations are traditionally classified based on phenotype, ecological characteristics, processing, and taste for smoking purposes (Table 3). On the basis of the phenotype, tobacco crops may be divided into morphological characteristics, namely leaf shape, leaf thickness, shape of

leaf base, and leaf color (Figure 2). Based on ecological characteristics, the tobacco variation can be divided into two categories, namely *bako mole beureum* (red tobacco) and *bako mole bodas* (white tobacco). The *bako mole beureum* consists of *bako bibit kedu hejo* and *bako bibit nani* are considered to be appropriately cultivated in the wet season, in a special area of upland, cold temperature, and light sandy loam soil. While the bako *mole bodas* consists of *bibit bako sano* and *bibit bako juhana* that is appropriately cultivated in the wet season with special are of low-land, cool temperature, and stony soil. In terms of cultivation systems, bako *mole beureum* is mainly provided with urea fertilizer, while *bako mole bodas* is mainly applied with NPK fertilizer. While based by the processing and taste, the tobacco can be traditionally divided into two types. The first type is traditionally considered as strong taste with strong aromatic and the second one is considered as light taste accompanied with less or no aroma. The strong taste and aroma is produced from *mole beureum*, while the light taste and non-aroma is produced from the *mole bodas*. The *bako mole beureum* is mainly processed when the tobacco namely leafs are placed in the house and are allowed to dry (*dipeuyeum*) between 3 and 4 days to get yellow leaf.Afterwards, the tobacco leafs are finely sliced, further dried, and then further moistened by dew (*diibun*). As a result, the tobacco has a strong taste of tobacco and aromatic. While the *bako mole bodas* is mainly processed by placing it in the house for 2 or3 days. In addition, the tobacco leafs are roughly sliced, dried shorter and less moistened by dew. Consequently, it results in the light taste and non-aromatic tobacco.

The research has similar results to the report by Djajadi (2015) noting that some tobacco variations are traditionally farmed by tobacco farmers of Sumedang and Garut of West Java. He reported that the tobacco variations namely *bako mole Nani, Kedu Omas, Kedu Hejo, Leuwiliang, Dasep, Adung, Nani Kanceh,* and *Kenceh Darawati* are predominantly cultivated by farmers of Sumedang and Garut, West Java.

The tobacco farming

People of Sukasari Village have cultivated tobacco crop both in their village and neighboring villages at the same sub-district or different sub-district areas, including Jatinngor Sub-District. The tobacco are cultivated in agricultural lands located in their village, also in other villages by renting or share-cropping from other farmers of others villages. Traditionally, they have predominantly farmed tobacco in the dry land (*lahan kering* or *tegal)* although sometimes, cultivated in the wet-rice field *(sawah*), particularly in the rain-fed rice field (*sawah tadah hujan*) strictly in the dry season.

The cultivation of tobacco in the dry land is undertaken in both the wet-season and dry season. In the wet season, the planting tobacco is carried out in between October and November or December depending on the beginning of the rainy season. The tobacco is available for harvest between 3 to 4 months after planting. Therefore, it is usually harvested in December, January or February. While in the dry season, the tobacco is planted around March then harvested by June, but it may shift either earlier or later depending on the rainy season. After harvesting the tobacco, sometimes the land is altered by planting annual crops (*palawija*), including *jagon*g/corn (*Zea mays* L), *kacang tanah*/peanut (*Arachys hypogaea*), *bonteng*/cucumber (*Cucumis satvus* L), *cabe/*chili (*Capsicum annum* L), and *kacang*/beans (*Phaseolus* spp). The planting of *palawija* is usually in July and is harvested in September (Table 4).



Figure 2. Variation of tobacco leafs, *bako bibit juhana, bako bibit kedu hejo*, bako *bibit sano,* and *bako bibit* nani are classified such as based on phenotypes, particularly leaf shape

Table 4. Tobacco cultivation at the dry field/garden (*kebon*) of Sukasari Village, Sumedang, West Java, Indonesia

Crops	Months*)											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Tobacco	**h-xxxx**									**p-xxx**	**xxxx**	**Xxxx**
Palawija							**p-xxx**	**xxxx**	**h-xxx**			
Tobacco			**p-xxx**	**xxxx**	**Xxxx**	**h-xxx**						

Notes: p-xx=planting; h-xxh=harvesting. *) It will change depending on the rainy season

Table 3. Variation of tobacco crops based on tobacco farmers of Sukasari Village, Sumedang, West Java, Indonesia

Variation of tobacco	Phenotype/morphology				Ecology	Processing and taste of tobacco for smoking
	Leaf shape	Leaf thickness	Shape of leaf base	Leaf color		
Mole bibit sano	*Jorong* (oval)	*Kandel* (thick)	*Berombak* (wavy shape)	*Hejo koneng* (green yellowish)	Planted in the dry season of the slight low land with cool weather	Placedtobacco leafs that are allowed to dry in the slightly short between two and three nights, more rough chopped, condensed in a shorter evening day, and less strong taste and less aromatic
Mole bibit juhana	*Jorong (*oval*)*	*Ipis (*thin*)*	*Berombak* (wavy shape)	*Hejo* (green)	Planted in the dry season of the slight low land with cool weather	Placed tobacco leafs that are allowed to dry in the slightly short between two and three nights, more rough chopped, condensed in a shorter evening day, and less strong taste and less aromatic
Mole bibit kedu hejo	*Buleud endog (*ovate*)*	*Kandel (thick)*	*Rata* (flat)	*Hejo koneng (*green yellowish*)*	Planted in the wet season of the upland with cold temperature	Placed tobacco leafs that are allowed dry in the slightly longer between three and four nights, more finely chopped, condensed in the evening dew longer, and strong and aromatic cigarette taste.
Mole bibit nani	*Jorong (*oval*)*	*Kande (thick)*	Berombak (*wavy shape*)	*Hejo koneng (*green yellowish*)*	Planted in the wet season of the upland and with cold temperature	Placed tobacco leafs that are allowed to dry in the slightly longer between three and four nights, more finely chopped, condensed in the evening dew longer, and strong taste and aromatic cigarette.

Table 3.Main stages of tobacco cultivation of Sukasari Village, Sumedang, West Java, Indonesia

Seed selection (*milih binih*)	**Nursery preparation (*ipukan*)**	**Land preparation of tobacco garden (*nyiapkeun kebon*)**	**Cultivation of tobacco (*ngebon bako*)**	**Tobacco processing (*ngolah hasil bako*)**
Selecting individual good flowering tobacco at the garden	**Nursery in the paranggong**	*Nyacar/ngababad* (shrub cutting)	*Ngaseuk jeung melak* (making holes by wood dibbling stick and transplanting)	*Meuyeum* (allowing tobacco leafs to dry)
Harvesting the flowering and selecting tobacco seeds	Making paranggong	*Ngagaringan* (dryingvegetation shrub biomass)	*Nurub* (shading)	*Nyebit* (tearing off of midribs)
Drying the seeds	Put good soil and humus in the paranggong	*Dihuru* (burning)	*Mupuk/ngagemuk* (manuring)	*Ngaleleng* (rolling)
Storing seeds in the bottles or bamboo segments	Sowing the seed tobacco	*Mencug* (hoeing)	*Ngored jeung nyaeur* (weeding and repairing ridges)	*Ngeureu*t (slicing)
Continued by nursery preparation	Giving water in the morning and the afternoon	*Ngalaci* (furrowing)	*Ngepel* (severing the lowest level)	*Ngicis* (laying on the *sasag*)
	Moving to polybag and transplanting in the tobacco garden	Continued by cultivation of tobacco	*Ngahileud jeung nyemprot hama* (killing caterpillars and spraying pests)	*Moe* (sun-drying)
	Nursery in the field		*Naruk (*nipping off inflorescence*)* *Nyirung (*nipping off buds*)*	*Ibun* (moisten with dew in several nights)
	Selection of the fertile soil block		*Ngala* (harvesting)	*Meungkeut (*tying*)*
	Hoeing and putting fertilizer		*Nyabut tangkal* (pulling tobacco trees)	*Jual/ngical bako mole* (selling tobacco of mole)
	Sowing the tobacco seeds		Cultivation of tobacco crops or planting *palawija*	*Jual/ngical bako mole* (selling tobacco mole)
	Giving water in the morning and the afternoon			
	Transplanting in the tobacco garden			

Unlike the dry land, the cultivation of tobacco in the rain-fed rice field is undertaken only in the dry season. The tobacco is usually planted in April or May and harvested in July or August. Following the harvest of the tobacco, the land is cropped by paddy in the rainy season. Like Sukasari Village, the people of Salamungkal hamlet, Cigentur Village, Paseh Sub-district, Bandung District, West Java cultivate tobacco in the dry land. The tobacco is farmed at a particular plot for one to two years while mixed with corn. The tobacco is cultivated in March and harvested four months after harvesting (Igarashi 1985). However, unlike Sukasari Village and Salamungkal hamlet, the people of Pamekasan, Madura cultivate tobacco of local variation called *prancak*, namely in both *tegal* and *sawah* in between April and August (Hasan and Darwanto 2013). In general, the cultivation of tobacco is determined by weather and climate condition. Since the lack of water often causes a drought that hinders the growth of tobacco crops. Conversely, the tobacco cannot be properly processed due to a lot of rain and not enough sunlight (cf. Ihsannidin 2010).

In other words, the tobacco crops is sensitively determined by the local environment, such as weather conditions. In addition, the tobacco cultivation is also affected by the market economy, such as fluctuation of input and output prices (Larsito 2005). The tobacco cultivation, therefore, needs special knowledge and high attention (Mitchell 2016). Although the cultivation of tobacco has high risk, many farmers are involved in the tobacco farming due to the high benefit potentialfor providing the household a high income (Hasan and Darwanto 2013).

Tobacco farming cycle

There are five main stages of in the tobacco farming cycle, namely seed selection, nursery preparation, land preparation, tobacco cultivation, and tobacco processing (Table 3).

Seed selection

The preparation of tobacco seedling is traditionally undertaken by each household of the tobacco farmers. The seeds of tobacco are obtained from the previous harvest. First, some tobacco trees are selected that have very good quality. Second, the tobacco leafs of the selected tobacco trees are not allowed to harvest to keep a good quality of seeds. When the flower of the selected tobacco trees mature, its flowers are selectively harvested with a good quality. Third, the flowers are dried by sunlight for about 5 days, so that the seeds in the dried flower can be easily taken. Fourth, to acquire the seeds, the dried flowers of tobacco are motioned or blown. The tobacco seeds of good quality and uniform size which are not empty are selected. Fifth, the seeds are put in the bottle or *bekong*, a segment of bamboo that is covered by plastic. As a result, the tobacco seeds can be traditionally protected and durable for two planting season.

Nursery preparation

The seedbed preparation can be undertaken in two ways, namely in the homegarden and the dry land field to be planted tobacco. The seedbed preparation in the homegarden is undertaken with more care and will not cultivate to large area. First, to construct *paranggong* which is a kind of table made from bamboo with length about 3 meters and width about 1 meter. Second, the fertile land is put in the *paranggong*. Third, it is covered by humus of plant leafs. Fourth, the top is covered by the remaining residue of burned bamboo leafs (*tai durukan*). Fifth, the tobacco seeds in the *bekong* are sown in the soil at the *ranggong*. Sixth, before the tobacco seeds grow, the seedbed is provided with water in the morning and the evening. The provision of water is continuously undertaken approximately for 60 days. Seventh, the tobacco seedling age 60 days are then moved to a polybag, banana leaf or arenga leaf so that the seed will develop well and strong roots. After two weeks the tobacco seedling are stored in the polybags and transplanted in the dry land or the rainfed field.

The seedbed preparation in a particular plot of the dry land must also be undertaken in several stages. First the particular plot of fertile land is selected. In particular it is safe from livestock disturbances and strong wind. The selected plot of land is finely hoed (*dipencug*). Second, the soil is provided by fertilizers, such as compost. Third, the tobacco seeds are sown in the seedbed and provided water in the morning and evening. Fourth, the tobacco seedling aged 60 days are transplanted in the dry land field or the rainfed field.

Land preparation and tobacco cultivation

At the time waiting for the tobacco seedlings to be readily planted, the land preparation is undertaken before the rainy season. First, the trees are selectively slashed (*dituar)* by machete and undergrowths are cleaned (*ngababad*) by machete and knife. Between two and three weeks after the trees have been felled, all cut organic matter will have dried. The trash is collected into piles and burned (*ngaduruk*) to supplement nutrients in the soil also to kill insects, pests and weed populations (cf. Rambo 1984). Second, the land is hoed and furrowed into some plots with distance between plots about 1 meter. Third, the land of each plot is made holes (*cogekan*) with a distance between holes about 80 cm. Fourth, each hole is provided by various manure (*ngagemuk*), such as dung of chicken sheep, goat, and cow. Fifth, after it is left about between 7 to10 days, tobacco seedlings are transplanted in to the holes after the wrapping areopened. Each hole is inserted with one individual of seedling. This tobacco transplanting work is undertaken in the evening to avoid strong sunlight radiation which may result in wilt.

Managed prepared tobacco garden

At the first week, the tobacco crops are regularly provided with water every day. However, in the next week, they are provided with water only twice each week. After

one week from the transplanting, the tobacco crops are planted with inorganic fertilizers, such as NPK, Urea, or mixture of both. Before providing fertilizers, the land is firstly perforated by a dibble (*aseuk*) with distance about 10 cm to each tobacco tree. Moreover, the liquid fertilizer is inserted into each hole and repeated after two weeks. After the age of tobacco plants are about one month reaching a height about 30 cm, ridges are repaired and the tobacco tree crops are put soil to produce strong growth trees.

After one month repair ridges (*nyaeur*), the number of tobacco leafs of each tree is counted. If the number tobacco leafs of each tree has about between 18 to 20 leafs, the shoots are cut (*ditaruk*) to properly grow and equitable grow. After cutting shoots, the underneath branches are cut. At the same time, the tobacco crops are maintained due to a lot of pest attack. Some tobacco pests, such as grasshopper/*simeut (*Family *Acridae)*, tobacco horn worn/*hileud* (*Manduca secta*), bereng (*Aphis* sp), are predominantly found. The tobacco leafs are usually eaten by caterpillars, grasshoppers, and tobacco horn worm. As a result, the tobacco leafs are damaged. For example, the tobacco leafs have become perforated. To combat the pests are undertaken by farmers, by picking pesta and using pesticides. Like the wet rice cultivation, the pesticides are intensively used in tobacco farming. Consequently, the costs for buying pesticides have increased, but at the same time the pest has also exploded. Intensive use of pesticides often causes alot of natural enemies of pest to be killed and pests have become resistant (cf. Nurinda 2006; Thei et al 2013; Fox 2016; Winarto 2016).

Harvesting

After the cutting shoots are undertaken three times, the tobacco is considered ready to be harvested. Signs of the tobacco tree are ready to be harvested, include that two or three leaves have become yellowish and have white nodules and many stripes. Before starting tobacco leaf harvesting (*diunduh*), some preparations must be carried out. For example, bamboo plates (*sasag*) for drying tobacco leafs are made. Between 50 till 55 day after transplanting or 3 months after tobacco seed nursery are established, the harvest starts, beginning withthe lower leaves between one and three leafs that has bad quality (*obreg*). Then one week later, middle leaves are harvested, and finally upper leafs are harvested, these are considered to be best quality, because the ripening goes from the lower to upper. The upper leafs have been mature longer and the rarely obtain rainfall. As a result, it will produce good taste and aromatic. The harvest of tobacco leafs is usually undertaken in the morning after the dew on the leaves have dried. The young leafs are avoided to be accidentally harvested since it will result in bad quality of tobacco. Conversely, if harvested too old the tobacco leafs that will get bad color.

Processing tobacco production

The tobacco processing is traditionally undertaken in several steps. First, the tobacco leafs are cut and carried to homes. Second, the leaves are put in the sack and tied with a rope or wrapped by banana leafs. Third, tobacco leafs are placed and allowed to dry (*dipeuyeum)* for one to three nights to be yellowish and withered. One sack consist of 50 kilograms of tobacco leafs. Fourth, after one the withered leafs are torn off midribs (*disebit* or *diroeh*) by folding the leaves in half (Figure 3.A). As a result, the midrib of each leave can be discarded.

Fifth, the tobacco leafs are collected and carefully laid, and separated based on the quality. Sixth, the tobacco leafs are rolled (*digulung*) to be some pillow shapes. One pillow consists of 25 leaves. The biggest leafs, intact, and homogenous color are then separated and used as wrapped of leaf roll to be sliced (*diiris* or *dirajang*). After the leaf rolls are placed for one night the next day they are sliced by using knife (*pisau rajang*) at the *rajangan* (Figure 3.B). The slice process is appropriately undertaken at night starting from midnight to 10 o'clock a.m. since the sliced tobacco leafs can be directly dried.

The slice of tobacco leafs must be undertaken as smooth as possible. After the sliced leafs are sorted by women based on length. The short sizes are separated as low quality of tobacco. Moreover, sliced leafs are laid (*diicis*) on bamboo plates with has length 1 m and width 0,5 m that is called *sasag* or *ebeg* and its base is covered by fabric. Normally one roll of tobacco leafs is for *sasag*.The sliced tobacco leafs on *sasag* are carried on *panagan* made of bamboo at the homegarden (Figure 3.C).

After the tobacco is dried by sunshine and moisten with dew (*diibun*) from one night to three nights the tobacco takes a dark color. This work is very hard since if the tobacco gets rainwater, it may grow fungus and must be re-dried by sunshine to become dry.When the tobacco is moistening by dew it may produce the aromatic characteristics of tobacco. Moreover, the tobacco slabs are folded into three parts or wrapped by dried banana midrib and placed in the house for between one to two weeks. Finally, the product that is called *bako mole* is ready to be sold. Normally, each *sasag* plate of tobacco ties 20 *sasag* of tobacco and is made as one package for sale to middlemen (*pengepul*).

Tobacco trading

There are two emic categories by which the people of Sukasari Village divide the households in involving into the tobacco farming system, namely *petani tembakau* (tobacco farmers) and *petani tembakau dan pengepul* (mixed as tobacco farmers and village middlemen). The first category is recognized as farmers who involve in the tobacco farming. They are annually involved in various activities of tobacco cultivation systems, including seed selection, nursery preparation, land preparation, tobacco cultivation, and tobacco processing. While the second category is recognized and acknowledged as tobacco farmer which is similar to that of the first category they are involved in various activities of tobacco cultivation, but they are also involved in buying the tobacco products as middlemen.

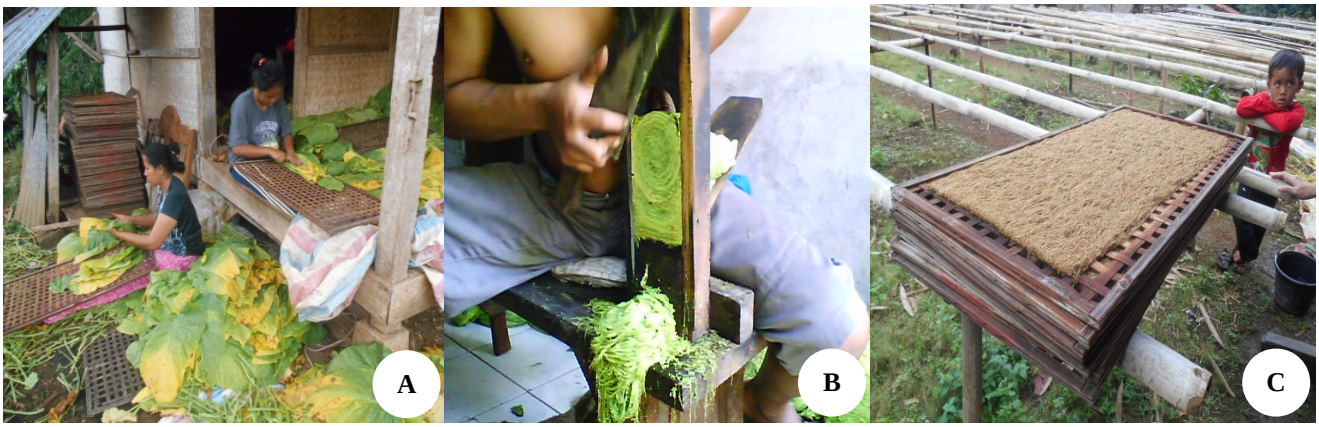


Figure 3.A. The tear off of the tobacco midribs (*nyebit* or *ngaroeh*). B. Slice leaf roll of tobacco (*ngereut* or *ngarajang*), C. Sun dry of the sliced tobacco leafs on *sasag* or *ebeg*

The farmers who are known as the first category, *petani tembakau*, have annually cultivated the tobacco cultivation for generations over time. The harvested tobacco leafs are usually processed by each household. The tobacco leafs processing activities, namely leafs are allowed to dry (*meuyeum*), tear off midribs (*nyebit* or *ngaroeh*), sliced (*ngeureut*), lay on sasag (*ngicis*), sun-dry (*moe*), and moisten with dew (*ngibun*) are undertaken by males and females. For example, *nyebit,ngicis*, *moe*, and *ngibun* are predominantly carried by males. While *ngeureut* (slice) is undertaken by males who own special knowledge and technique obtained by learning from their father. In other words, men know more knowledge and expertise in slicing (*ngeureut*) the tobacco leafs than women do since men intensly participate in their father activities, particularly in *ngeueut* (cf. Lizararralde 2004). Consequently, this knowledge and skill is not continuously inherited, if the nuclear family does not have sons. Indeed, the tobacco processing, particularly in relation with *ngeureut* the family will be seriously disturb.

Unlike the first category, the second category, *petani tembakau dan pengepul*, they are both involved in the tobacco cultivation and processing of production as well as involved in the trading of tobacco from other people, mainly from the first category (*petani tembakau*). Based on information of informants, it has been revealed that about 90 per cent of people Sukasari Village involve in the tobacco farming, and most of them act as the first category, while about only 13 households are recognized as middlemen (*pengepul*) to buy tobacco in their village.

Traditionally, two kinds of the folk tobacco (*tembakau rakyat*) are produced by people of Sukasari, namely *bako mole beureum* and *bako mole bodas*. The *bakomole beureum* is considered strong taste and aromatic, while the *bako mole* putih is considered as light taste and non-aromatic. The people of Sukasari as other tobacco farmers of West Java predominantly produce *bako* of *bako mole* as bako *sambaraan* that need processing namely condiments and readily sold. The low quality tobacco, traditionally named *bako mole bodas*, produced by each household of Sukasari Village is usually sold to local middlemen (*pengepul*). Moreover, the *bako mole* products are carried by *pengepul* to be sold to other middlemen in other districts of West Java, such as Tasikmalaya, Garut, and Cianjur that tobacco *mole bodas* products are applied spice (*disamaraan*), packed, then sold to consumers. The high quality of tobacco, locally named *bako beureum* from each household are sold to local middlemen, then the *bako beureum* that has been collected by some middlemen are sold to the tobacco factories, such as BAT, Sampurna, and Gudang Garam. The selling of *bako mole beureum*, however, is annually sold in between August and November, due to the tobacco products having highly demanded by factories at that time. About 2 tons of *bako mole beureum* from the Sukasari Village are transported for each two weeks to tobacco factories in Central Java and West Java between August and November. In other words, the *bako mole beureum* are traditionally sold by the group of local middlemen to tobacco factories only in certain time, namely when the tobacco factories '*membuka'* (open to) buying the folk tobacco. In addition, the local middlemen usually sell bako *mole beureum* when the price of tobacco has a high price. As a result, if the tobacco price low, the *bako mole beureum* products are stored in the house and it will sell later if the tobacco price has increased.

The tobacco farming system of Sukasari Village has been determined by socio-economic and ecological factors (cf. Rambo and Sajise, 1984; Brush 2000; Futri et al. 2005; Ridwan 2009; Fauziyah 2010; Hasan and Darmanto 2013; Masruroh 2015). The socio-economic factors consist of local knowledge, technology, capital, and market; while the environmental factors, such as soil fertility, water, pest, and climate. Unlike the wet-rice field as subsistence economy, the tobacco farming has been predominantly practiced for the commercial purpose to provide cash income for the household. As a result, the tobacco farming systems have been dynamically influenced by market economy. For example, the cause of increasing various pests of tobacco farming due to the frequent climate anomalies results in an

increase cost of buying pesticides. Conversely, since the pesticide use has increased, the pest has increased due to the pest gaining resistant to the pesticides and a lot of natural enemies of pest are killed (cf. Fox 2016; Winarto 2016). Indeed, predominant of climate anomalies in the last several decades, has seriously influenced the tobacco farming. For example, since the length of the rainy season has frequently increased, the quality of tobacco is low producing low prices of tobacco as well as reduce in the household income. In addition, because the agricultural land areas that have decreased due conversion to other land-uses, the tobacco farming systems as source of income are disturbed. The fluctuation of the selling price of tobacco has also become constraints for the tobacco farming. In terms of local knowledge, the Sukasari peoples tobacco farming systems, including the processing of tobacco leaf slice has eroded and may extinct in the near future since neglected by younger generations.

The tobacco farming systems have been considered a high risk. Since the tobacco farming systems have been influenced by various ecological and socio-economic factors. Many farmers of Sukasari, Sumedang, however, have predominantly involved in the tobacco farming, to provide the household an income. Based on this study, it can be concluded that the Sukasari, Sumedang have various local ecological knowledge in relation with practicing of the tobacco farming system, including various tobacco crops (land races), cultivation and processing of tobacco. There are 4 tobacco variations, namely *bibit kedu hejo*, *bibit ani*, *bibit sano*, and *bibit juhana* that produce the folk tobacco, called *bako mole bodas* and *bako mole beureum* are predominantly planted by the people. These tobacco variations are planted in the dry land and rainfed field that are managed through five main stages, including seed selection, nursery preparation, land preparation, tobacco cultivation, and tobacco processing which are based on the local ecological knowledge. The folk tobacco productions of tobacco are traditionally sold to local middlemen, then sold to outer village middlemen, and tobacco factories.

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Identification of Aves, Herpetofauna and Arthropods in Miangas Island (Indonesia), the borderline of Wallacea ecoregion

ADY SURYAWAN♥, ISDOMO YULIANTORO, ANITA MAYASARI, MARGARETTA CHRISTITA
Environment and Forestry Research and Development Instute of Manado. Jl. Raya Adipura, Kima Atas, Mapanget, Manado 95259, North Sulawesi, Indonesia. Tel./fax.: +62-431-3666683, ♥email: suryawanbioconserv@gmail.com

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Abstract. *Suryawan A, Yuliantoro I, Mayasari A, Christita M. 2016. Identification of Aves, Herpetofauna and Arthropods in Miangas Island (Indonesia), the borderline of Wallacea ecoregion. Biodiversitas 18: 1528-1537.* Miangas Island is located on the northeast of Wallacea and bordered by the Philippines and it belongs to Talaud archipelago. This study aims to identify the species of terrestrial wildlife in Miangas Island. Observations used the direct encounter method starting at 7:00 to 17:00 pm and they were conducted through four times visits in October and December 2015, April and May 2016. Some species were found, such as 15 species of birds, 3 species of lizards, 1 snake and 1 species of Arthropods. *Hirundapus caudacutus* and *Butastur indicus* are migrant bird species. *Lamprolepis smaragdina* is green and classified as tree climber type. It is included to hybrid lizard type of the Pacific and the Philippines. Based on IUCN database, *Eos histrio* is classified as endangered group (E), *Ducula pickeringii* as vulnerable (V) *Todirhamphus enigma enigma* is classified as near threatened group (NT), *Birgus latro* is classified as Data deficient (DD), while *Butastur indicus, Lanius Schach, Nectarinia jugular plateni, Todirhamphus chloris, Hirundo tahitica, Hirundapus caudacutus, Egretta sacra, Ardea alba, Anthus cervinus, Motacilla cinerea, Cuculus saturatus, Passer Montanus, Ducula pickeringii, Eutropis multicarinata multicarinata, Lamprolepis smaragdina, Emoia caeruleocauda and Laticauda laticauda* are classified as low risk (LC).

Keywords: Miangas, species diversity, Wallacea, wildlife

INTRODUCTION

Based on the explanation of Lubis (2014), Miangas has a geostrategic role because of its position as the outer island of Indonesia and based on Sarbidi (2010) it is categorized as a small island with an area of 3.15 km^2. Administratively, the Miangas Island is included to the Talaud Archipelago Regency, North Sulawesi Province. Miangas Island is bounded by the Pacific Ocean on the east and south, Sulawesi Sea on the south and The Philippines on the west and north (BPS 2014).

45 million years ago, tectonic and geological activity caused Sulawesi Island and its surrounding islands, especially Miangas which was derived from the same cluster with a maritime Philippines (Stelbrink 2014 and Stelbrink et al. 2012). Miangas was considered as an isolated island because of its distance to the Mindanao island (Philippines) namely 48 nautical miles and to Nanusa Island (Indonesia) namely 145 nautical miles (BPS 2014).

Miangas Island is included to the Northeast of Wallacea ecoregion based on the original line of Wallacea (Coates and Bishop 2000) and the map of Cept.net (2016). This insulating effect will reduce biodiversity (Purnomo et al. 2015). This statement is in line with Woodruff (2010) which explains that the principle of island biogeography has a positive correlation between the wide of the island and the number of biodiversities. Longer distance of an island from the main island will reduce species diversity but will increase endemic. Saryanti (2013) explains that 80% of the forest area of Wallacea is currently in degraded condition and at least 535 species are endangered. This condition becomes a serious note for conservationists. Miangas Island as a small habitat will affect genetic flow, genetic drift, and fitness and become a factor against extinction rate especially for endangered species (Mbora and McPeek 2010).

The Talaud Island is the northernmost part of Wallacea Ecoregion where two endemic bird species, namely Talaud Bush-hen (*Amaurornis magnirostris*), Taluad Rail (*Gymnocrex taluadensis*), have small population in primary forest. Talaud island also as the habitat of Endangered-Vulnerable species i.e. *Eos histrio, Tanygnathus lucionensis, Halcyon enigma, Ducula concinna and Ducula pickeringii* (Riley 2003). While Burung.org (2010) explains that there are 10 species of endemic birds in the Sangihe-Talaud islands, namely, *Ducula pickeringii, Eos histrio, Loriculus catamene, Ceyx fallax, Cittura cyanotis, Todiramphus enigma, Coracina bicolor, Eutrichomyias rowleyi, Aethopyga duyvenbodei, and Ducula concinna.*

Based on Koch et al. (2009) there are two types of herpetofauna in Miangas Island, i.e. *Lamprolepis smaragdina* and *Eutropis multifasciata*. *L. smaragdina* is a type of the Philippines and Pacific taxa hybrid. Koch et al. (2013) have described a new cryptic member of the mangrove monitor (*Varanus indicus*) from Talaud Island, based on morphological and molecular evidence. However, from such references that have been obtained, there is no specific wildlife biodiversity of Miangas Island. Based on preliminary research, it is known that Miangas Island is

dominated by three taxa, they are herpetofauna, aves, and arthropods, while mammals are less. This study aims to provide information on wildlife biodiversity on a remote island in the transitional region of two Asian bioecoregions and Wallacea which has not been previously reported.

MATERIALS AND METHODS

Study area

The research was conducted in Miangas Island of North Sulawesi, Indonesia in 2 to 4 November 2015, in 14 to 16 December 2015, in 20 to 22 April 2016 and 25 to 27 May 2016. Observations were conducted at 07-10 and 16.30-17.00. The location was along the coastal of Miangas, mangrove, coconut plantation area and prairie, with a width of 3.5 km at an elevation of 0-98 meters above sea level.

Materials

The research material was Miangas ecosystem. The equipments were DSLR camera, GPS, and book: Field Guide introduction of bird species (Coates and Bishop 2000).

Procedures

The study was conducted through direct observation referring to operational standards by Fachrul (2008) using transect point on bird, Bismark (2011) using visual encounter survey (VES) on herpetofauna and arthropods. These methods were done by walking along thetransects determined. The observations were done during the day. Observations resulted photographic documentations and then the species identification was done using the field guides (Coates and Bishop 2008; Koch et al. 2009 and Motoh and Kuronuma 1980) and information from IUCN Redlist.org, Birdlife.org and some scientific journals of each species about conservation status, distribution and characteristic.

Data analysis

Data were analyzed and presented descriptively including the habitat, distribution, the wild population, and conservation status by the International Union for Conservation Nature and Natural Resources (IUCN 2016), Birdlife International (Birdlife.org 2016).

RESULTS AND DISCUSSION

Results

The study was conducted at the hand of the east, southwest and center of the island. This is done because in the north and southwest, the areas have been opened for the use of airport construction, so the animals encounter relatively low. The study found 15 species of birds, 4 species of reptiles and one species of crustaceans.



Figure 1. Location of Miangas Island, North Sulawesi, Indonesia (5º33'50"N, 5º32'10"N and 126º34'05"E, 126º35'25"E) in the Wallacea ecoregion. Note: ~ = transect line

Identification of birds

Grey Faced Buzzard, Butastur indicus *(Family: Accipitridae)*

Grey faced buzzard (Figure 2.A) was found in a coconut and tall trees, i.e., banyan, but it often flew to stalk the prey. The Solo Hill is an ideal location for raptor observation because it is an ideal place to observe the whole landscape of Miangas. It had distribution area of 1.830.00 km^2, including Japan, South Korea, and North Korea and migrated to Southeast Asia over Thailand, Malaysia, Sumatra, Java, Kalimantan (Borneo), Sulawesi and North Moluccas (BirdLife 2016). According to Coates and Bishop (2000), it can be found at Karakelong Islands, Salibabu Island, Sangihe Islands, Siau Island, Sula Island, Morotai Island, Halmahera Island and Ternate Island in September to March. BirdLife (2016) released the information that its populationwas more than 100,000 birds in 2001. It is considered as low risk (LC) species based on IUCN Redlist.

Long tail shrike, Lanius schach *(Family: Laniidae)*

Long tail shrike (Figure 2.B) was found in an open area bordered by mangrove ecosystems at 4 meters above sea level. This area provides various types of insects as food. The bird has 11.200.00 km^2 of distribution area covering Afghanistan, Pakistan, Southeast Asia, to Papua New Guinea (BirdLife 2016). Whilst, according to Caotes and Bishop (2000), the distribution has a limited area to Nusa Tenggara covering the islands of Lombok, Sumbawa, Alor, Timor, Wetar and Kisar. According to BirdLife (2016), the birds' populations reached 10000-100000 pairs, then, by the IUCN, it is categorized as low risk (LC) to extinction.

Olive Backed Sunbird, Nectarinia jugularis *plateni (Family: Nectariniidae)*

Olive-backed sunbird (Figure 2.C) spread evenly from coconut plantations on the coast to the hills at an altitude of 75 meters above sea level. It reaches 5,200,000 km^2 spreading area covering the whole of Southeast Asia, Papua New Guinea, Northern Australia (BirdLife 2016). Coates and Bishop (2000) split it into two groups based on the color of the abdomen. One sub-type is N.j. platen, a yellow-bellied group. Its habitat includes coastal areas and small islands such as Talaud, Sangihe, Siau, Bangka Island, Manado Tua, Togian Islands and Selayar. According to BirdLife (2016), its population has not been studied, but this species is common and has a stable population trend, so it is categorized as low risk of extinction (LC).

Collared Kingfisher, Todirhamphus chloris *(Family: Alcedinidae)*

Collared kingfisher (Figure 2.D) was found in coastal areas ranging from beaches, mangrove forests until the coconut grove which was far from the sea. BirdLife (2016) mentions that it is a wide distribution species, reaching 3,940,000 km^2 covering an area of northern Australia, Southeast Asia, the Pacific island countries, and parts of India, Saudi Arabia, Ethiopia and Yemen. There is a conclusion that the population is declining, but it has not been calculated yet exactly, and it is categorized as low risk (LC) by IUCN.

Talaud kingfisher, Todirhamphus enigma enigma *(Family: Alcedinidae)*

Talaud kingfisher (Figure 2.E) was found in the coastal areas covering beaches, mangrove forests to the coconut plantations near the beaches. BirdLife (2016) mentioned that it has a distribution of 1,000 km^2 as an endemic species on the island of Karakelong, Salebabu and Kabaruang. This endemicity has been declared by Coates and Bishop (2000) that Talaud kingfisher is endemic to Islands of Talaud, which includes Karakelong Talaud Island, Salebabu and some areas of North Sulawesi subregions. Riley (2003) has estimated the population Talaud Kingfisher in Karakelang was 5290-8690 individuals with low density, and it should be considered as threatened (VU). Based on IUCN (2016), the status of this species has approached Near Threatened (NT).

Pacific swallow, Hirundo tahitica *(Family: Hirundinidae)*

Pacific swallow (Figure 2.F) was easily found flying along the coast of Miangas. BirdLife (2016) said that it has a distribution of 3.36 million km^2 covering Indonesia, the Philippines, Malaysia, Singapore, southern Thailand, and the Pacific island countries. It was considered as increasing population trend, and categorized as low risk conservation status according to IUCN (2016).

White-throated needletail, Hirundapus caudacutus *(Family: Apodidae)*

White-throated needletail (Figure 2.G) was found flying across the upperside of the Solo hill or a prairie. According to Coates and Bishop (2000), white-throated needle tail was a migratory bird which stopped in this area on September until early April. It can be found at Wallacea ranging from Sangihe archipelago to the islands of Lombok and East Banda. BirdLife (2016) has concluded that its distribution area reaches 3.72 million km^2, and has presented a map of non-native breeding area covering eastern parts of Australia such as Sydney, Victoria, New South Wales, Queensland, Papua New Guinea, and the native breeding area in East Asia including Japan, North Korea, South Korea, Mongolia and China. Based IUCN (2016), these species are categorized into low risk of extinction (LC).

Pacific reef-egret, Egretta sacra *(Family: Ardeidae)*

Pacific reef-egret (Figure 2.H) was found in the afternoon and flying over the beach of east Miangas. Coates and Bishop (2000) explained that the Pacific reef-egret is also a natural resident of beach with coral reefs. It is widespread in Wallacea ranging from the island of Talaud-Sangihe-Siau, Talise, North Halmahera, Seram, Ambon, Nusa Tenggara to islands of Tanimbar. BirdLife (2016) explained that the Pacific reef egrets have an extensive distribution i.e. 4.1 million km^2 covering the coast of Asia, Australia, Papua New Guinea and the Pacific island countries. It had a stable population trend and based on the IUCN, it is classified as low risk of extinction

White Egret, Ardea alba *(Family: Ardeidae)*

The white egret (Figure 2.I) was easily found around the mangrove forest on Miangas Island. According to Coates and Bishop (2000), this species was found in a variety of wetland ecosystems such as mangrove, swamp, lakes at an altitude of Sub-tropical countries with widespread reach 57.2 million km^2. BirdLife (2016) stated that the data of growth population and its trend was unknown. Currently, the white egret is classified as low risk of extinction (LC) by IUCN (2016).

Red-throated pipit, Anthus cervinus *(Family: Motacillidae)*

Red throated pipit (Figur 2.J) was found roosting in branches around the Solo Hill. According to Coates and Bishop (2000), it is a kind of nomad bird in Wallacea and often flew over the coastal areas to the highlands and other specific area near the water. Based on BirdLife (2016), this species has a distribution of 3,790,000 km^2. In Asia, the population spread in north of the equator area, covering Kalimantan, Sangihe, Talaud, the Philippines, Singapore, Malaysia, Thailand, Myanmar, into Northern China. This species can also be found in African continent including Kenya, Somalia, Ethiopia, Nigeria, the coast of Egypt, Libya, Tunisia to Southern Europe such as Turkey and Greece. The population was relatively stable and categorized low-risk of extinction.

Grey Wagtail, Motacilla cinerea *(Family: Motacillidae)*

The Grey Wagtail (Figure 2.K) was usually found around the mangroves. The observations showed that the Grey Wagtail moved by running. According to Coates and Bishop (2000), it is a common visitor in August to the beginning of May. It usually flew over the open areas, riverbanks at altitudes of 0 to 2,000 meters above sea level. It was found in various locations in the Wallacea region. The distribution area reached 16.6 million km^2 covering Southeast Asia, Eastern Asia, Northern Africa and Southern Europe. BirdLife (2016) mentions that it has a stable population trend and is classified as low risk of extinction (LC)

Himalayan Cuckoo Cuculus saturatus (Family: Cuculidae)

Himalayan Cuckoo (Figur 2.L) was usually found around the coast and perching on power lines. According to Coates and Bishop (2000) this bird is a migratory bird in September to early of June. Himalayan Cuckoo visits the area of cultivation, forest edges, savanna. It is migratory birds from Asia that has sub type of C. s. saturatus spreading in Talaud Islands, Sangihe, Ternate, Morotai to Nusa Tenggara archipelagos. The other sub-type is C.s. lepidus. IUCN (2016) concluded that it has a stable population growth trend, although there is no definitive data, it is classified as a low risk of extinction species.

Grey Imperial Pigeon, Ducula pickeringii *(Family: Columbidae)*

Grey Imperial Pigeon (Figur 2.M) was rarely found on a small island. Coates and Bishop (2000) explained that it inhabited the primary and secondary forest, in the coastal areas, in Karakelang, Salebabu and Kabaruang Island. BirdLife (2016) released data on bird populations globally and it mentioned that the number of Eurasian tree sparrows ranged from 159 million to 576 million individuals. Based on IUCN (2016), this species is categorized as low risk of extinction (LC).

Eurasian Tree Sparrow, Passer montanus (Family: Passeridae)

Eurasian Tree Sparrow (Figure 2.N) was found around the housing of residents at the villages. Based on Fitzsimon et al. (2011) this bird belonged to the type of introduction and was first reported to be located in the northern part of central Sulawesi then spread to North Sulawesi. Coates and Bishop (2000) explain that it has spread over the whole of Wallacea. Based on the memoirs, it has spread to the Islands of Siau, Sangihe, Talaud, Sulawesi, Sula, Halmahera, Buru, and Lombok. The distribution reached 38.4 million km^2 covering the whole of Europe and most of Asia. BirdLife (2016) released data on bird populations globally which stated that the populations of Eurasian tree sparrow ranged from 159 million to 576 million individuals and based on IUCN (2016) this species was categorized as low risk of extinction (LC).

Red and Blue Lory Eos histrio talautensis *(Family: Psittacidae)*

Coates and Bishop (2000) mentioned that Red and Blue Lory (Figure 2.O) are inhabitats of the primary forest and hill, but they also visit coconut plantations in the coastal areas. Distribution: Red and Blue Lory is endemic to Talaud Islands, such as in Miangas (E.h challengeri), Karekelong Island, Salebabu and Kaburuang (E.h talautensis) and Sangihe Island, Siau, Ruang (E.h histrio). Arini (2014) estimated that its population was only 2229.33 individuals which were only found on the Karakelang Island. However, this study encountered an individual of this type around the houses. Based on confirmation, this type was imported from the island Karakelang and taken by boat, so it was believed that this type of Eos was actually histrio talautensis not E.h. chalengeri. IUCN (2016) categorized this species as endangered, and Appendix 1 of CITES.

Identification of Herpetofaunas

Eutrophic lizard, Eutropis multicarinata *(Family: Scincidae)*

Eutropis multicarinata (Figur 3.A) is one of twenty-eighth species belonging to family of Scincidae (Datta-roy et al. 2015), this species can be differentiated from the phonetically similar species, i.e. E. rudis and E. multifasciata, by the absence of postnasal scales and five or seven strong keels on nuchals, dorsals and laterals (Koch et al. 2009). It was common and easy to find this species in the garden. It was usually found among piles of litters. Its life has adapted to a semi-arboreal life style, because it would climb tree trunks when it was being chased. Datta-roy et al. (2015) said that this Scindae Genus is the most widespread lizard groups in tropical Asia. Koch et al. (2009) suggested that this species has distribution until the east southernmost in the Salibabu Island. The island is

located about 145 nautical miles from Miangas. Beukema (2011) mentioned that the lizard is not classified in the red list of IUCN Redlist data base.

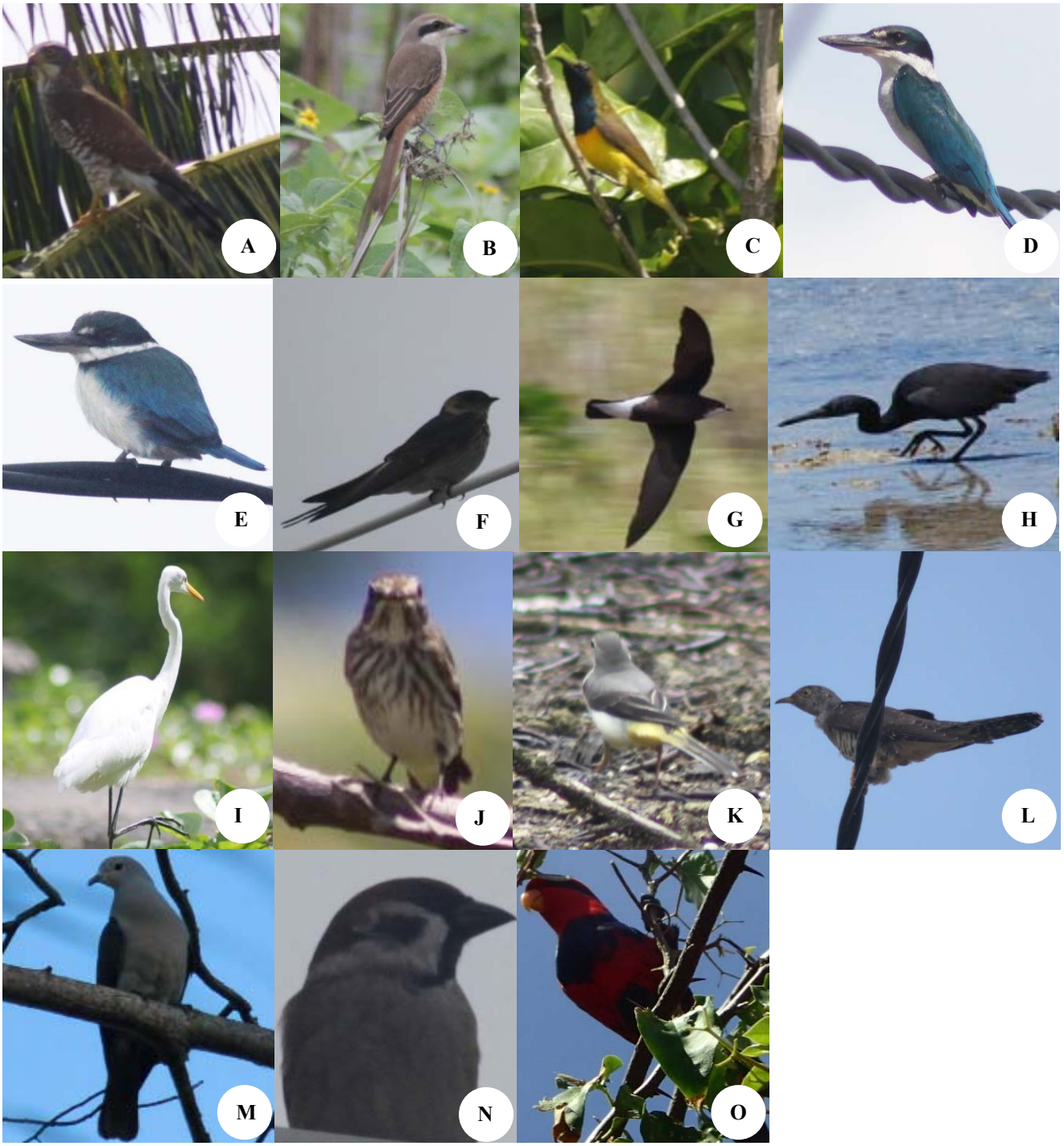


Figure 2. Various species of birds in Miangas Island, Indonesia. A. *Butastur indicus*, B. *Lanius schach*, C. *Nectarinia jugularis*, D. *Todirhamphus chloris*,E. *Todirhamphus enigma enigma*, F. *Hirundo tahitica*, G. *Hirundapus caudacutus*, H. *Egretta sacra*, I. *Ardea alba*, J. *Anthus cervinus*, K. *Motacilla cinerea*, L. *Cuculus saturatus*, M. *Ducula pickeringii*, N. *Passer montanus*, O. *Eos histrio talautensis*

Pacific bluetail skink, Emoia caeruleocauda *(Family: Scincidae)*

The Pacific bluetail skink (Figure 3.B) is common and easy to find in the garden, usually among piles of litter. Based on IUCN (2016), It is often found in open forest are, community gardens and orchards with a maximum altitude of 400 meters above sea level. Its spreading includes the Pacific islands such as Solomon, Marshal, Bismark, Fiji, and Indonesian islands such as Papua, Maluku, Kalimantan, and Miangas Island. This species is also found in Malaysia and the Philippines. The populations are relatively stable and are not the threatened habitat. According to IUCN (2016), this species is categorized as low risk of extinction.

The Emerald Tree Skink, Lamprolepis smaragdina *(Family: Scincidae)*

The emerald tree skink (Figure 3.C) is easily found in gardens, it usually climbs a coconut tree and other trees. Koch et. al. (2009) mentioned that it is a hybrid of Pacific and Philippine taxa. It is an arboreal lizard in lowlands with light wood or thick forest(Kaiser et al. 2011). Based on Phylogenetic analysis, this species is divided into more than 40 sub-species and spreads in Wallacea and the Pacific Islands (Linkem et al. 2013). According to Hamidy and Mulyadi (2007), this species is not protected and belongs to non-CITES Appendix. It was categorized as low-risk to extinction (LC) in 2011 by category of IUCN (2016).

Columbrine Sea Krait, Laticauda laticauda *(Family: Elapidae)*

The natural habitat of Columbrine Sea Krait (Figure 3.D) is in the sea but it can be found on the embankment of abrasion in the island of Miangas. According to IUCN (2016) these snakes live in an area which ismaximal of 50 meters below sea level, and it can be found in the eastern Indian Ocean, the Pacific Ocean to the north, central and western area of it. Its populations are stable and unfragmented habitat, then they are categorized as low risk (LC).



Figure 3. Various types of Herpetofaunas in Miangas Island, Indonesia. A. *Eutropis multicarinata*, B. *Emoia caeruleocauda*, C. *Lamprolepis smaragdina*, D. *Laticauda laticauda*



Figur 4. One of Arthropods in Miangas Island, Indonesia

Identification of Arthropods

Coconut Crab, Birgus latro *(Family Coenobitidae)*

Coconut crab (Figure 4) will be easy to find at night among the fallen coconuts. According to IUCN (2016), this species is solitary and lives alone by making a hole under the ground or rock to avoid predators and water loss. Sulistiono et al. (2009a) described that this species spreads out in the Western Pacific Ocean to the eastern Indian Ocean, usually is found at the rocky island but not at atoll because of the inadequate availability of food. Drew and Hansson (2014) explained that it is widely distributed on remote tropical islands in the Pacific and Indian Oceans. Christmas Island (Indian Ocean) is currently thought to be one of the places with the largest population reaching 962 individual. According to Anagnostou and Schubart (2014), in 1981, Birgus lastro has been grouped on vulnerable species. It has been reassessed as "Data Deficient (DD)" since 1996.

Discussion

Biodiversity

This research has found 15 species of birds, 3 species of lizards, 1 species of snake and 1 species of arthropods. Based on IUCN (2016), there are 1 species included in the endangered group (E), 1 species as Vulnerable (V), 1 species as in the near threatened group (NT), 1 species as data deficient (DD), 17 species classified as low risk (LC).

The diversity of bird species in Miangas is lower than that in 90 hectares of Pulau Rambut (Kepulauan Seribu National Park) which is found 24 species of birds (Alamsyah and Marhento 2016), that on Ternate Island which is found 21 species of birds (Ahmad et al. 2017), but higher than that in Pasoso Wildlife Reserve (64 hectares) which is found 14 species (Karar et al. 2016).

Butastur indicus (Grey Faced Buzzard) and *H. caudacutus* (white-throated needletail) are migrant bird species. One species identified as an endemic species is *E. histrio. E. histrio* (Red and Blue Lory) which found in Miangas Island is an introduction species from Karakelang Island. Arini et al. (2016) explained that *E. histrio* consists of three sub-species of *E. histrio challengeri*, *Eos histrio histrio* and *Eos histrio talautensis. E.h. talautensis is* currently only found in 6 big trees in SM Karakelang, Talaud. Indrawan (2005) explains that this species is often caught for sale outside of Karakelang Island. Research on breeding has been done by Mayasari and Suryawan (2013), concluding that acclimatization of Red and Blue Lory takes a long time, the information about ecology and the appropriate can cage greatly determines the success of reproduction.

The diversity of herpetofauna species is affected by the land covers; it is more common in the forest area than the garden (Subasli 2015). All Herpetofauna and Arthropods at this research is found in coastal ecosystems, such as in the piles of nekromass, tree and beaches. The *E. caeruleocauda* has been distributed in the Pacific Islands, i.e., the small island of Federated State of Micronesia which is poorly known biologically (Buden 2013). Linkem et al. (2013) concludes the *L. smaragdina* is the species with excellent dispersal capabilities and it invades the Sangir-Talaud, Banggai Island, New Guinea, Solomon Island and West Pacific. *E. multicarinata* is often found in the Philippines (Barley et al. 2013). *L. laticaudata* (Blue-banded sea kraits) is one of third of Laticauda groups (sea kraits) and the widest distribution species with relatively intermediate dehydration rates in seawater (Brischoux et al. 2013; Gherghel et al. 2016).

Based on Buden (2007) *E. caeruleocauda* is one of the fifth species of skinks sympatric of the genus Emoia. Based on studies of Barley et al. (2013) in several species of herpetofauna, the phylogenetic analysis needs to be done, because it is genetically unrelated but morphologically similar to each other. DNA and morphological analysis results have made it to be divided into sub species of *E. multicarinata multicarinata*, *E. multicarinata borealis*, *Eutropis* sp. and *E. bontocensis*.

Birgus lastro is the largest terrestrial crab in the world and distributed on Indo-Pacific island, but it is declined rapidly (Drew et al. 2010), because it has high economic value for local community and can be sold at high prices (Widiyanti et al. 2016). Jahidin (2010) has estimated that the population of it in Siompu Island (near Buton Island) is only 44 individuals. Christmas Island has the highest population of B lastro reaching 962 individuals, but today the population is declining (Drew and Manson 2014). Based on Sulistiono et al. (2009b) to breed *B. latro* was quite difficult, and the survival rate in captivity was only 12.5%. Sulistiono et al. (2009a) explains that the survival rate can increase to 82% through the addition of freshwater and seawater in the breeding pond.

Habitat ecology

Based on the observations, the highest diversity of wildlife species is found in coastal ecosystems, even though the area is dominated by coconut trees. It is suspected due to the presence of mangrove forests around

coastal ecosystems. Mangrove forest is dominated by *Lumnitzera littorea* and *Xylocarpus mekongensis* (MMAF 2015). Mangrove forest is a habitat for birds and other wildlife. It is in accordance with Oostra et al. (2008) that the existence of the forest gives very positive effect on the abundance of bird species. According to Kuswanda (2010), plant species composition of the forests is the most influential factor on the index of diversity, abundance, and density of birds.

The existence of forests is the best assurance of the long-term survival of avifauna sustainability (Oostra et al. 2008). Miangas Island only has 2.4% area of primary forest, consisting of mangrove and lowland forest ecosystems on the hill which has been sacred by the people. The other existing ecosystems are coastal forest which has been dominated by coconut plant, mangrove forest, lowland forest, plantation, and settlements. On the other hand, Buden (2007) says that *E. caeruleocauda* prefers the shady forest. The existence of bird species has a close relationship with its habitat, Sukandar et al. (2015) explains that kingfisher species has high dependence on forest and aquatic ecosystem, so it is often used as a health indicator of ecosystem.

On coastal ecosystems, we can find 10 species of birds such as Long tail shrike, The Collared kingfisher, Talaud kingfisher, Pacific reef-egret, White Egret, Himalayan Cuckoo, Eurasian Tree Sparrow, Pacific Swallow, Olive Backed Sunbird, Red and Blue Lory and The Grey Wagtail. In the lowland forests which is located in Bukit Keramat and dominated by grassland and some trees, we can find five species of birds, such as: Grey Faced Buzzard, Olive Backed Sunbird, The white-throated needletail, Red-throated sparrow and Grey Imperial Pigeon. This proves that the diversity of wild animal is higher in thick forests than in thin forests.

A seawall has been built along the coastal islands, it affects the life cycle of *L. laticaudata.* Based on Gherghel et al. (2016) the life cycle of *L. laticaudata* is 19,75% in land and 80,25% in seawater. The seawall causes *L. laticaudata* having difficulty to reach the mainland. Rasmussen et al. (2011) concludes that sea snake population has decline.Some populations of species which used to go up the rivers or main land are already extinct and others are endangered.

This research needs to be completed by calculating the population density, the diversity index, the importance value index of species in each habitat, so that the ecological can be monitored. The time and duration influence the number of species (Marshal 2015).This study is only carried out for 3 days in each visit. This research also needs a support from the nocturnal wildlife diversity information, so the data can be used as the basis for Miangas Island development.

Fox (2008) explains that animal identification using acoustic individual recognition is a noninvasive method which has several benefits namely: time saving, relatively low cost, and easy to apply. In addition, the need for monitoring genetic diversity, particularly endangered species (*Eos histrio*) in small habitats is urgent. Charpentier et al. (2007) explains that a decrease in genetic variability is likely to reduce the capability for adaptation to change and adaptation to unpredictable environments, and this capability is proportional to the genetic variance. Meanwhile, according to Waltert et al. (2004) endemic species is the most vulnerable to the slightest changes in forest functions. Even if the changes occur in the secondary forest that is located very close to the primary forest. The species that can adapt to the change is the nectarivorous frugivorous bird. The coastal ecosystem can provide fruits and flowers all year round both from coconut trees, mangroves, and coastal vegetation.

Challange and recommendation

Mangrove ecosystems and lowland forest in Bukit Keramat are the last primary forest in the Miangas Island. But unfortunately, the mangrove ecosystems hardly ever get the tidal supplies. In the rainy season, the mangrove vegetation will always submerge by fresh water, whereas, there is no water flow passing through the vegetation in the dry season, unless there is a big wave coming. This condition will gradually decrease the salinity, which in turn can lead to the death of mangrove vegetation. The loss of tidal supplies is caused by the construction of seawall encircling the island since 2014.

According to Nurse et al. (2014) small island biodiversity will experience high pressure due to climate change that is 1) ecosystem and species horizontal shifts and range decline; 2) exotic and pest species range increase and invasions mainly due to temperature increase in high latitude islands; 3) altitudinal species range shifts and declines mainly due to temperature increase on high island. Larrue et al. (2014) describes how the invasive species has become a serious problem in tropical islands especially on the island of Hawaii. This will certainly have an impact on the carrying capacity of the habitat to wildlife and human life. Habitat is very important for the survival of living beings. Based on the analysis of Heriyanto et al. (2008), habitat quality and quantity give more impact to the preservation of wildlife than a hunting does. So it takes the effort to conserve habitat and wildlife as well as enriching the vegetation species to support the preservation of ecosystems and life on the island of Miangas.

Other challenges faced by Miangas Island, which the intrusion of sea water has reached 500 meters from the beach, are abrasion and soil degradation (Suryawan et al. 2015, pers. obs.) and it is estimated that the reserves of fresh water will not be able to meet the need in 2030 (Sarbidi 2010). Nurse et al. (2014) also explain that the porosity of the limestone and the coral causes the rapid surface flow to enter the substrate so that the vegetation will experience the lack of fresh water. Tanaguchi (2016) predicts that future climate conditions will be dominated by rain with low intensity, thus it will reduce the water infiltration. The forest is the only hope to preserve water resources in Miangas Island which will also serve as a wildlife habitat. Yuniati et al. (2013) suggest the existence of customary forests or village forests is the best management system to be implemented.

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Land cover changes in the Air Telang Protected Forest, South Sumatra, Indonesia (1989-2013)

SYAIFUL EDDY[1,♥], ISKHAQ ISKANDAR[2], MOH. RASYID RIDHO[2], ANDY MULYANA[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas PGRI Palembang, Jl. Jend. A. Yani, 9/10 Ulu, Palembang 30251, South Sumatra, Indonesia. Tel. +62-711-510043, Fax. +62-711-514782, ♥email: syaifuleddy@gmail.com
[2]Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Indralaya, Ogan Ilir 30862, South Sumatra, Indonesia
[3]Faculty of Agriculture, Universitas Sriwijaya. Indralaya, Ogan Ilir 30862, South Sumatra, Indonesia.

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Abstract. *Eddy S, Iskandar I, Ridho MR, Mulyana A. 2017. Land cover changes in the Air Telang Protected Forest, South Sumatra, Indonesia (1989-2013). Biodiversitas 18: 1538-1545.* The Air Telang Protected Forest (ATPF) is a mangrove forest in the Banyuasin District, South Sumatra, Indonesia. It has an area of about 12,660.87 ha. In fact, that the ATPF area has been converted into aquacultures, plantations, agricultural lands, settlements and ports during recent decades. The objective of this study is to identify the land cover changes in the ATPF from 1989 through 2013 using satellite remote sensing data. Three Landsat satellite imageries for 1989, 2001 and 2013 have been used to build maps and to predict the land cover changes in the study area. A ground-truthing verification was done to increase the accuracy of image classification in each region. The results showed that the anthropogenic forcing had caused significant degradation of primary mangrove forest in the ATPF from 1989 to 2013. This forcing was categorized as mangrove conversion into coconut plantations, oil palm plantations, aquacultures, farms, ports, and settlements. Of these six conversions, the coconut plantations, oil palm plantations and aquacultures have potential tendencies to increase construction that could threaten the existence of mangrove forest in ATPF. It was found that during 2013, the coconut plantations, oil palm plantations, and aquacultures accounted for about 18.0% (2,278.62 ha), 4.7% (591.87 ha) and 3.1% (386.18 ha) of mangrove forest changes, respectively.

Keywords: Air Telang Protected Forest, degradation, land cover changes, mangrove forest

INTRODUCTION

Mangrove forests have grown in the tidal zones such as swamps, lagoons, rivers and coastal estuaries in the tropical and subtropical regions and they are tolerant to salt water (Piou et al. 2006; Fatoyinbo et al. 2008; Strauch et al. 2012; Motamedi et al. 2014). They have several important functions, such as in carbon transport, primary productivity, nitrogen, and phosphorus transport, creating a niche as well as protecting coastal regions from disturbances (Lee et al. 2006; Fatoyinbo et al. 2008; Mukherjee et al. 2014).

Mangrove forests in Indonesia are among the largest ones in the world which consist of an area of around 3.2 million ha or 22.6% of the total mangrove forests in the world (DasGupta and Shaw 2013; Kusmana 2014). In addition, around 60% of the total mangrove forests in Southeast Asia are located in Indonesia (Giesen et al. 2007). However, the condition of mangrove forests in Indonesia continues to degenerate both quantitatively and qualitatively. It is mostly due to their conversion into infrastructures, farms, aquacultures, plantations, settlements, and mines, as well as extraction of their timber for industries, fuels and charcoals (Thu and Populus 2007; Giri et al. 2014; Jones et al. 2014; Komiyama 2014; Mukherjee et al. 2014).

According to Soepraptohardjo et al. (1979), there was about 230,260 ha of mangrove forests in Southern Sumatra. However, recent data from the National Coordinating Agency for Survey and Mapping, Republic of Indonesia (2009) showed that the remaining mangrove forests in South Sumatra were only around 149,707 ha. This data indicates a continuous degradation in which South Sumatra region lost about 35% of its mangrove forests within those about 30 years.

One of the mangrove forest areas in South Sumatra is Air Telang Protected Forest (ATPF). It is located in the Banyuasin District covering an area of about 12,660.87 ha. A study by Department of Forestry and Plantation of Banyuasin District (2010) has shown that about 4,272.63 ha of the ATPF areas have been converted into plantations, aquacultures, farms, and settlements. In addition, passenger and cargo ports were recently built in Tanjung Api-api located close to the ATPF. Moreover, since 2014, the area in the vicinity of the ATPF has been designated by the Central Government of the Republic of Indonesia as a Special Economic Zone (SEZ) in South Sumatra. Consequently, all economic activities in this area will influence the quality and quantity of the mangrove forests due to more abrasion, increased seawater intrusion and decreased self-maintenance as coastal processes (Berger et al. 2008; Onrizal and Kusmana 2008). In addition, overlapping interests in the mangrove forest will create ambiguous ownership leading to a complex management of the mangrove forests (Walters et al. 2008).

In this paper, three Landsat satellite imageries taken in 1989, 2001 and 2013 have been used to build the ATPF map. A ground-truthing verification was carried out to

evaluate the classification of each region. This research is expected to contribute to the formulation of management strategy and restoration program of the mangrove forest. Note that the formulation of effective strategies, monitoring of environmental changes and management plans of mangrove forest require spatial information which is related to the fragmentation of mangrove forests through mapping of mangrove forest entities accurately, reliably and efficiently (Li et al. 2013; Jayanth et al. 2016). In addition, mangrove forest restoration requires data of site conditions and monitoring of ecosystem level as integrated components of restoration projects (Bosire et al. 2008).

MATERIALS AND METHODS

Study area

This study was conducted in the mangrove forest of the ATPF area in Banyuasin District, South Sumatra, Indonesia with an area of about 12,660.87 ha (Figure 1). The study area is directly adjacent to the Bangka Strait and the Banyuasin River in the North, and to Muara Telang District in the South. To the East, it is bordered by the Muara Telang District and the Banyuasin II District, while the Banyuasin River is the boundary to the West. According to Department of Forestry and Plantation of Banyuasin District (2010), ATPF region has a flat coastal area, which is a tidal marsh area with slopes between 0-8% and a height of 0-7 m above sea level. Various types of mangrove forest flora are commonly found in this area, such as *Rhizophora* sp, *Avicennia* sp, *Sonneratia alba* and *Bruguiera gymnorhiza*. Species of fauna that exist in this area include, among others, *Hilobatus sybactilus*, *Crocodylus prosus*, *Sus barbatus*, *Eugretta* sp and *Maccaca fascicularis*. The study area has been partially converted into plantations, agricultural lands, settlements, and aquacultures. Recently, there are two ports, marine and ferry terminals that had been built near the study area. These ports are located in Tanjung Api-Api, which are situated on the western coast of the study area. The Tanjung Api-Api region has been declared as the SEZ, which consists of two zones located in the District of Banyuasin II and District of Tanjung Carat.

Data collection

In this study, three Landsat satellite imageries from 1989, 2001 and 2013 were collected. Specifications of the satellite data acquired for land cover change analysis are given in Table 1. The map coordinate system used UTM coordinate system (zone 48 S). Some 22 locations were physically visited for ground-truthing purpose using the Geographical Positioning System (Table 2). These sites were used to support the image classification and to improve the accuracy of the classification results. In addition, we also used forestry thematic maps and land cover maps that were obtained from several related agencies in Indonesia such as Forestry Agency and Agency for Geospatial Information.

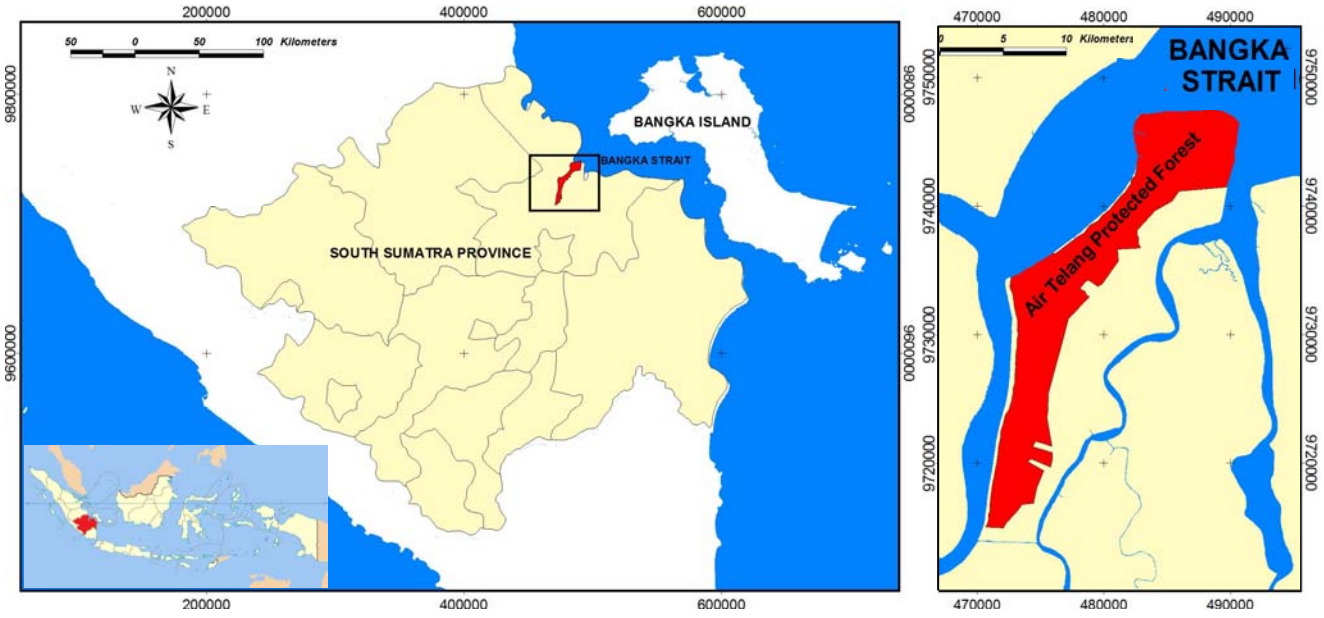


Figure 1. Location of the study area in the Air Telang Protected Forest, South Sumatra, Indonesia (red color). Map coordinate system: UTM projection, zone 48 S, datum WGS 1984

Table 1. Specifications of satellite data

Data	Date of acquisition	Bands	Resolution (m)	Source
Landsat 5 TM	17 May 1989	Multi-spectral	250	USGS glovis
Landsat 7 ETM+	06 June 2001	Multi-spectral	250	USGS glovis
Landsat 8 ETM+	17 January 2013	Multi-spectral	250	USGS glovis

Table 2. Locations of the ground-truth sites in the study area

UTM coordinate		Existing condition
474170	9719249	Secondary forest
480305	9737692	Secondary forest
482109	9737782	Secondary forest
482552	9738829	Secondary forest
475365	9734232	Secondary forest
478382	9737886	Primary forest
481564	9740503	Primary forest
482001	9741427	Primary forest
477885	9736030	Primary forest
474740	9720742	Oil palm plantation
475256	9722749	Oil palm plantation
474822	9723408	Coconut plantation
475045	9723893	Coconut plantation
475347	9726851	Coconut plantation
475537	9727279	Coconut plantation
476038	9728477	Coconut plantation
479467	9734060	Coconut plantation
478422	9735845	Aquaculture
482334	9738404	Aquaculture
480534	9737243	Shrubs
478438	9737733	Port
488874	9748356	Water body

Table 3. Land use classification in the Air Telang Protected Forest, South Sumatra, Indonesia

Class name	Description
Primary forest	Dominated by stands of true mangrove, which consist of *Rhizophora apiculata, Avicennia alba, Bruguiera cylindrica, Excoecaria agallocha, Nypa fruticans* and *Xylocarpus granatum*.
Secondary forest	A mixture of mangroves and shrubs.
Coconut plantation	Dominated by stands of coconuts.
Oil palm plantation	Dominated by stands of oil palms.
Shrubs	Dominated by cover plants.
Aquaculture	Fish and shrimp farming.
Port	The anchored place of the large ship to transport passengers and goods.
Water body	The land is still inundated due to tides.

Data analysis

The analysis and classification of land cover changes in the ATPF were conducted by using Geographic Information System (GIS) analysis with ArcView 3.3. The classification was based on the type of land use and the density of the canopy. The land use types were classified into (1) primary forest, (2) secondary forest, (3) coconut plantation, (4) oil palm plantation, (5) shrubs, (6) aquaculture, (7) port, and (8) water body (Table 3).

The GIS analysis was divided into four steps, namely *pre-processing*, *processing*, *image classification* and *field survey*. In the *pre-processing* step, Landsat imagery at a scale of 1: 250,000 is rectified using spatial map. This rectification process included a rotation, translation and geometric correction into the UTM coordinate system (zone 48 S). In addition, an atmospheric correction was also performed in this step to minimize the variation of the spectral object due to the influence of atmospheric particles and solar radiation.

In the *processing* step, an image sharpening was conducted to increase the amount of information that can be interpreted visually from the images. In this study, the False Color Composite (FCC) was used to define the mangrove forest with the RGB (Red, Green, Blue) composite with band combination of 4, 5 and 3. Further, the land use and land cover mapping were performed through an on-screen digitization. The next step was *image classification*. The images were classified using supervised classification method (visual interpretation). This method requires the knowledge and experience of researchers in the study area. Visual interpretation of satellite imagery is an effective method for documenting changes in land use and land cover (Butt et al. 2015). The supervised classification method was also employed by Boori et al. (2015) to analyze land cover disturbance caused by tourism activities. Meanwhile, Ghebrezgabher et al. (2016) used this method to analyze the forest cover change in Eritrea, Africa. Finally, a *field survey* was conducted to verify the information on a land cover as well as to check the boundaries of each land cover types. The results of field verification were used to perform re-classification in order to obtain the final land cover map.

RESULTS AND DISCUSSION

Qualitative changes in land cover

Qualitative changes in land cover in the ATPF during the period of 1989-2013 are shown in Figure 2. It was found that there has been a continuous reduction in the primary forest area. Nevertheless, there has been an increase of primary forest area in the northern and western parts of the study area due to enhanced sedimentation in the Banyuasin River. The siltation in the tidal zone for a long time has formed new lands in the coastal area of Banyuasin River (Department of Forestry and Plantation of the

Banyuasin District 2010). Although the primary forest area has increased due to the sedimentation, yet it is still not significant compared to the conversion rate of the primary forest into the coconut plantations, oil palm plantations, and aquacultures as shown in Figure 2. Furthermore, the increase of the secondary forests and shrubs were mainly caused by the degradation of primary forest that was neglected by the community for a long time.

The oil palm plantation did not exist in 1989, but it had been detected by 2001. In 2013, plantations were significantly found in the southern part of the ATPF in 2013. Similarly, the ports have been identified by 2013. The aquaculture areas have been identified by 2001 and by the year 2013, the number has increased. The decrease of water body areas (refer to Figure 2) occurred continuously due to an intensive sedimentation in the tidal zone both in the northern and western parts of the study area, which in turn could lead to the formation of new lands.

Quantitative changes in land cover

The quantitative changes in land cover in the ATPF area are listed in Table 4. The area of primary forests had decreased during the period 1989-2013. The area of primary forests identified in 1989 was about 9,219.82 ha (72.8%), declined to 8,131.49 ha (64.2%) in 2001, and then to 6,585.28 ha (52.0%) in 2013. Furthermore, the total area of secondary forests identified in 2001 was about 2,589.31 ha (20.5%), and it slightly decreased in 2013 to 2,389.52 ha (18.9%).

The total area of coconut plantations had doubled from 1,167.80 ha (9.2%) in 2001 to 2,278.62 ha (18.0%) in 2013. The oil palm plantations had been identified only by 2013 with a total area of about 591.87 ha (4.7%). In 1989, the total area of shrubs was identified as large as 318.11 ha (2.5%). However, by 2013, it had been continuously decreased to less than 1% of the total area of the ATPF. Similarly, the water bodies have continuously decreased from 1989 to 2013. In 1989, the total area of the water bodies was about 718.01 ha (5.7%) and then decreased to 354.63 ha (2.8%) in 2013. Meanwhile, the total area of aquacultures has increased from 238.03 ha (1.9%) in 2001 to 386.18 ha (3.1%) in 2013. The ports were identified only in 2013 with an area of about 37.72 ha (0.3%).

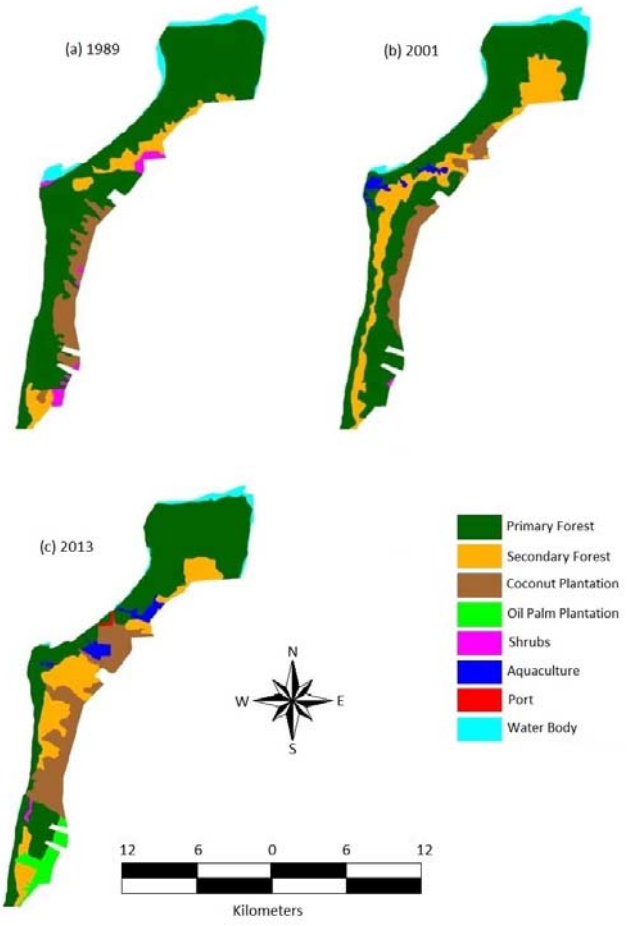


Figure 2. The land cover changes in the Air Telang Protected Forest region, South Sumatra, Indonesia in 1989, 2001 and 2013

Table 4. Total area of each type of land cover in the Air Telang Protected Forest, South Sumatra, Indonesia in 1989, 2001 and 2013, and changes in land cover over the period of 1989 to 2001 and 2001 to 2013

Land cover	Area at 1989		Area at 2001		Area at 2013		Changes (1989-2001)			Changes (2001-2013)		
Type	(ha)	(%)	(ha)	(%)	(ha)	(%)	(ha)	(%)	Size	(ha)	(%)	Size
Primary forest	9,219.82	72.8	8,131.49	64.2	6,585.28	52.0	1,088.33	11.8	dec	1,546.21	19.0	dec
Secondary forest	1,063.13	8.4	2,589.31	20.5	2,389.52	18.9	1,526.18	143.6	inc	199.79	7.7	dec
Coconut plantation	1,341.80	10.6	1,167.80	9.2	2,278.62	18.0	174.00	13.0	dec	1,110.82	95.1	inc
Oil palm plantation	-	-	-	-	591.87	4.7	-	-	-	591.87	100.0	inc
Shrubs	318.11	2.5	15.25	0.1	37.05	0.3	302.86	95.2	dec	21.80	143.0	inc
Aquaculture	-	-	238.03	1.9	386.18	3.1	238.03	100.0	inc	148.15	62.2	inc
Port	-	-	-	-	37.72	0.3	-	-	-	37.72	100.0	inc
Water body	718.01	5.7	518.99	4.1	354.63	2.8	199.02	27.7	dec	164.36	31.7	dec
Total	12,660.87	100.0	12,660.87	100.0	12,660.87	100.0						

Note: dec = decrease, inc = increase.

Degradation of mangrove cover

Protected forest is an area that supports an ecosystem by controlling the water system, preventing flooding, controlling erosion, preventing seawater intrusion and maintaining soil fertility. The ATPF is one of the protected forests that experienced significant degradation. In this study, we found that the ATPF area has been affected by the anthropogenic activities. These anthropogenic activities include coconut and oil palm plantations, aquacultures and ports (Figure 3). In addition, the forest area continued to be converted into settlements and area for agricultural activities. In the ATPF area, the settlements have existed since the 1970s. In fact, the construction of highways has contributed to the increased in the number of the population in this area. The people in this study area have initiated construction of coconut plantations since 1972. The aquaculture activity has existed since 1987, and in 1997 the total area of the aquacultures rapidly increased due to an intensive construction of aquacultures by a private company. Meanwhile, the oil palm plantations have started since 2008 after the construction of the highway. The physical infrastructure developments and other anthropogenic activities have several negative impacts on the environment, such as decrease of water quality and dynamic shifts of mangrove vegetation that reduced fish production (Satyanarayana et al. 2013).

Primary forest cover (Figure 2, Figure 4a and Table 4) has decreased for the last 24 years, *i.e.,* between 1989-2013. In the first period of 1989-2001, the primary forest cover decreased by about 1,088.33 ha (11,8%) or 90.69 ha per year. Meanwhile, in the second period (2001-2013), the decrease occurred in a larger quantity, by 1,546.21 ha (19.0%) or 128.85 ha per year. The decline in the primary forest cover was mainly due to the conversion of the mangrove forests into oil palm plantations, coconut plantations and aquacultures with the percentage area in 2013 being 4.7%, 18.0%, and 3.1%, respectively.

There have been transformations of some areas in the ATPF into new primary forest area (Figure 2 and Table 4). These transformations were contributed by two succession mechanisms, the re-vegetation in the tidal zones (new land) and the succession of secondary forests into primary forest. The first succession mechanism, the primary succession, has taken place due to sedimentation that resulted in the formation of new land in the tidal zone of the Banyuasin river. According to Fromard et al. (2004) and Setyawan et al. (2005), the presence of mud bank is an early stage of mangrove colonization in a new land. Mangrove colonization has begun to occur as the sediment becomes stable due to the tidal cycle. Mangrove propagules were carried out by tidal currents into the new land area and adapted to grow therein. This process would result in a new cycle in the ecosystem of mangrove forest. However, anthropogenic pressures would eliminate the aerial root masses which in turn, disturb the distribution of mangrove propagules (Di Nitto et al. 2008). Furthermore, seaward mangroves could migrate landward as a result of the rise of sea level, increase in salinity, and increases in the frequency, period and depth of inundation (Gilman et al. 2007).

Secondly, the secondary succession of the secondary forest into primary forest happened in the northern area of the ATPF between 2001-2013. Vegetation dynamics could be influenced by anthropogenic effect, changes in the hydro-edaphic condition and species interaction (Iftekhar and Saenger 2008). Additionally, the structure and composition of mangrove vegetation were influenced by geomorphological and environmental settings (Cunha-Lignon et al. 2009).

The secondary forest cover (Figure 2, Figure 4b and Table 4) increased significantly by 1,526.18 ha (143.6%) or 127.18 ha per year in the first period. This increase was due to land clearance of primary forests for agricultural lands and plantations, as well as timber utilization. During the second period, there was a slight decrease by 199.79 ha (7.7%) or 16.65 ha per year in the secondary forest cover. This decline was due to the conversion of secondary forests into coconut plantations, oil palm plantations, and aquacultures. Furthermore, some secondary forest areas, such as those in the north region, has been transformed into primary forests through natural succession.

The land area used for coconut plantations and aquaculture have increased significantly by 1,110.82 ha (95.1%) and 148.15 ha (62.2%), respectively, during the period of 2001 to 2013. (Table 4). Meanwhile, the oil palm plantations which have been initiated by 2013 span a total area of 591.87 ha or 4.7% of the ATPF area. From 2001 to 2013, degradation in the ATPF area caused by the construction of the coconut plantations, aquacultures, and oil palm plantations reached 92.57 ha, 12.35 ha and 49.32 ha per year, respectively. According to our analysis, the oil palm plantations are expected to accelerate the reduction of mangrove forest cover in the ATPF area.

According to data from Department of Forestry and Plantation of Banyuasin District, disturbances occurred in the ATPF area in 2010 were contributed by the forest conversion into coconut plantations (3,289.73 ha), oil palm plantations (521.98 ha), aquacultures (377.81 ha), agricultural lands (26.11 ha), and settlements (57.00 ha). Coconut plantations, oil palm plantations, and aquacultures contributed significantly to the degradation of mangrove forests in ATPF. According to Laulikitnont (2014), the existence of aquacultures, plantations and agricultural land in mangrove forests area are the main cause of the forests in Indonesia. Similarly, several previous studies showed that the decrease in mangrove forest number in several countries in the world are caused by aquacultures, plantations and agricultures (Thu and Populus 2007; Donders et al. 2008; Ilman et al. 2011; Bryan et al. 2013; Li et al. 2013; Nfotabong-Atheull et al. 2013; Jones et al. 2014; Komiyama 2014).

Some studies showed significant vegetation changes due to anthropogenic activities. According to Hansen et al. (2013), Indonesia has the highest rate of forest loss, which is about 1,021 km^2/year during 2000 to 2012. Rahman et al. (2013) have shown a rapid land use change in the Delta Mahakam, in which the total mangrove loss in that area reached 21,000 ha during 2000 to 2010. Using modeling study, Elz et al. (2015) have shown a significant land cover change in the protected forest in Berbak, Jambi Province. They argued that by the year 2040, land cover in this

protected forest will be reduced by about 25%. Similar rapid land use and land cover changes were also found in the neighboring country of Malaysia. The vegetation cover change in swamp forests of Peninsular Malaysia, Sumatra and Borneo due to land conversion into palm plantations spanned an area of 3.1 Mha in 2015 (Miettinen et al. 2016).



Figure 3. Conversion of mangrove forests in the Air Telang Protected Forest, South Sumatra, Indonesia by coconut plantations (A), oil palm plantations (B), aquacultures (C), and ports (D)

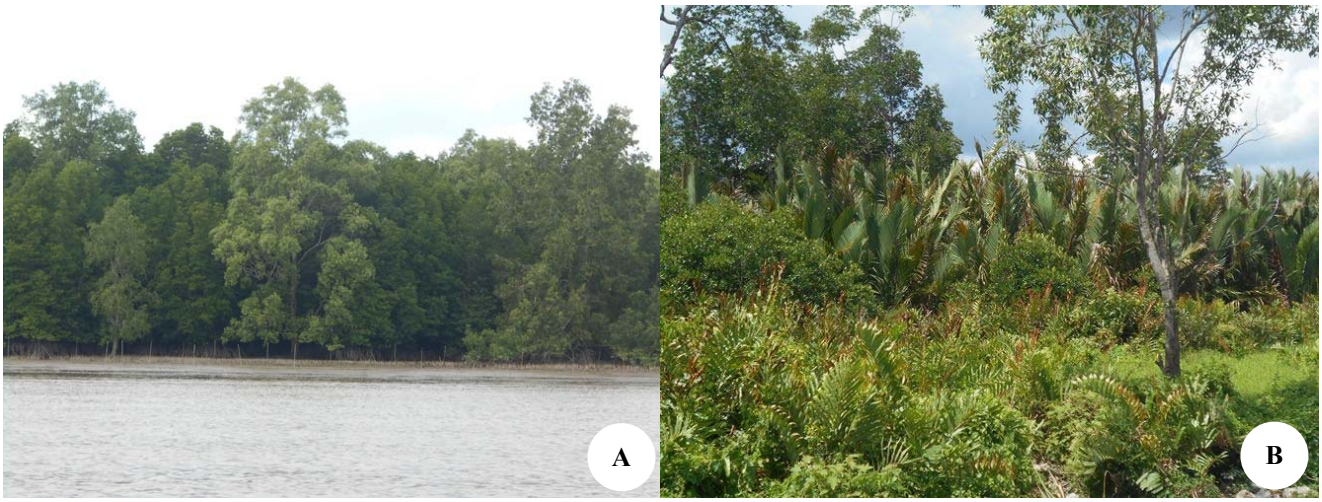


Figure 4. Primary forest (A) and secondary forest (B) conditions in the Air Telang Protected Forest, South Sumatra, Indonesia

Anthropogenic activities in ATPF has become more complicated because the Government designated the area as a SEZ. The SEZ consisted of two zones that included Banyuasin II District and Tanjung Carat District. Therefore, it is imperative that the Government takes a stand to implement restoration of the area to ensure the sustainability of the mangrove forests. Mangrove forest restoration has a significant potential to increase the forest resources, protect the tropical coastlines, and enhance fisheries biodiversity and productivity (Kairo et al. 2001).

This study used a supervised classification method to analyze the land cover changes in the ATPF area using time series of satellite remote sensing data. We demonstrated that anthropogenic forcing in the forms of coconut plantations, oil palm plantations, aquacultures, ports, settlements and agricultural lands was the primary cause of degradation of the primary mangrove forest in the ATPF during the period of 1989-2013. Furthermore, the anthropogenic forcing that shows an increasing trend includes coconut plantations, oil palm plantations, and aquacultures, which reached 18.0% (2,278.62 ha), 4.7% (591.87 ha) and 3.1% (386.18 ha) of the total of the land cover changes in 2013, respectively. In addition, our study identifies the formation of new primary forest in several regions of the ATPF, which are contributed by the re-vegetation in the tidal zones and the secondary forest succession into the primary forest. Furthermore, the area of the newly formed primary forest was much smaller compared to the area that was lost as a result of primary forests conversion due to anthropogenic activities. This study could be used as a reference for other researchers to investigate issues related to anthropogenic land cover changes of mangrove forests.

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The distribution and variability of chlorophyll-*a* bloom in the southeastern tropical Indian Ocean using Empirical Orthogonal Function analysis

ISKHAQ ISKANDAR[1,♥], QURNIA WULAN SARI[2], DEDI SETIABUDIDAYA[1], INDRA YUSTIAN[3], BRUCE MONGER[4]

[1]Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Jl. Palembang - Prabumulih, Km. 32, Inderalaya, OI, Sumatra Selatan, 30662, Indonesia. Tel./Fax. +62-711-580056, ♥email: iskhaq@mipa.unsri.ac.id; iskhaq.iskandar@gmail.com

[2]Graduate School of Environmental Science, Universitas Sriwijaya. Jl. Padang Selasa, Bukit Besar, Palembang, 30139, Indonesia.

[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Jl. Palembang - Prabumulih, Km. 32, Inderalaya, Ogan Komering 30662, Sumatra Selatan, Indonesia

[4]Department of Earth and Atmospheric Sciences, Cornell University, Ithaca, NY 14853, USA

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Abstract. *Iskandar I, Sari QW, Setiabudidaya D, Yustian I, Monger B. 2017. The distribution and variability of chlorophyll-a bloom in the southeastern tropical Indian Ocean using Empirical Orthogonal Function analysis. Biodiversitas 18: 1546-1555.* The Indian Ocean Dipole (IOD) events cause anomalously strong upwelling along the sourthen coast of Sumatra-Java leading to the bloom of chlorophyll-*a*. An empirical orthogonal function (EOF) analysis was applied to the time series of the satellite-observed chlorophyll-*a*, sea surface temperature (SST) and surface winds. Spatial eigen functions of the first EOF mode revealed the broad areas of coherent temporal variation in chlorophyll-*a*, SST and Ekman pumping, which was observed in the southeastern tropical Indian Ocean (SETIO) region. The corresponding time series of principal component of the first EOF mode revealed a robust seasonal variation and relativley weak inter-annual variation. The second EOF mode exhibited a distinct inter-annual variation with the high surface chlorophyll-*a* concentration was observed along the southern coast of Sumatra-Java. This high chlorophyll-*a* concentration is co-located with the low SST, the positive Ekman pumping, and the positive wind-induced mixing. An EOF analysis applied on the seasonal time series showed interesting patterns. The leading EOF mode during the peak IOD season from September to November (SON) showed the high concentration of chlorophyll-*a* was restricted to the southern coast of Java and was co-located with low SST region. The corresponding time series of principal component of the leading EOF mode showed a significant correlation with the Dipole Mode Index (DMI), however it had no correlation with the Ekman pumping. It could be concluded that the chlorophyll-*a* bloom during the peak phase of the IOD event was generated by the alongshore upwelling-favorable winds in the preceding season.

Keywords: Coastal upwelling, EOF analysis, Indian Ocean Dipole, sea surface chlorophyll-*a*, southeastern tropical Indian Ocean

INTRODUCTION

The eastern boundary coastal upwelling system in the Indian Ocean has become the main research worldwide due to its strong coupled atmosphere-ocean dynamics, especially its biogeochemical cycling (McCreary et al. 2013; Wiggert et al. 2013). This has an implication on the marine productivity in the eastern boundary upwelling region, which significantly increases due to the upward motion of nutrient-rich water from deep layer to the upper layer. Previous studies have shown that the eastern boundary coastal upwelling systems in the Atlantic and Pacific Oceans are one of the most productive marine environments in the world, which account for about 5% of the global marine productivity (Pauly and Christensen 1995; Carr 2002).

Unlike the eastern boundaries of the Pacific and Atlantic Oceans, however, the eastern boundary of the Indian Ocean undergoes dramatic seasonal changes (Wyrtki 1961; Qu et al. 2005). The Asian-*a*ustralian monsoon winds cause the variability of SST and surface circulation in the eastern boundary of the Indian Ocean, particularly the SETIO (Susanto et al. 2001; Sprintall et al. 1999). In addition, the SETIO region is located at crossroads of water masses from the Pacific Ocean that are modified through the Indonesian Throughflow (ITF), the Indonesian Seas, and the equatorial Indian Ocean (Figure 1) (Feng and Wijffels 2002; Wijffels and Meyers 2004; Qu and Meyers 2005; Iskandar et al. 2006, Yu and Potemra 2006). Therefore, the SETIO represents a complex region, displaying biophysical variability from seasonal to inter-annual time scale.

Within the seasonal time scale, the reversal of monsoon winds plays a dominant role in generating seasonal modulation of the SST in the SETIO region as well as seasonal reversal of the South Java Coastal Currents (Quadfasel and Cresswell 1992; Susanto et al. 2001). During the southeast monsoon from June to September, south-east winds prevail over the SETIO region, generating upwelling along the southern coast of the Lesser Sunda Islands leading to cool condition of SST. On the other hand, the north-west winds are observed over the SETIO region during the northwest monsoon season from December to March. These north-west winds cause onshore Ekman transport and subsequent downward vertical motion along the coast and warm condition of SST.

The SETIO region is also known as a center for the development of an eastern pole of the Indian Ocean Dipole (IOD) phenomenon (Saji et al. 1999; Webster et al. 1999; Murtugudde et al. 2000). During the positive IOD events, local upwelling along the southern coast of Sumatra-Java raises due to strengthening of south-east winds along the coast. As a consequent, the concentration of chlorophyll-*a* in this region also increases significantly leading to chlorophyll-*a* bloom (Susanto and Mara 2005; Iskandar et al. 2009). On the other hand, during the negative IOD events, north-west winds along the coast of Sumatra and Java is very strong leading to a strong down welling and low surface chlorophyll concentration (Susanto and Mara 2005; Horii et al. 2013).

In this study, we investigated the seasonal phase-locking and inter-annual occurrence of chlorophyll-*a* bloom in the SETIO region. In particular, using a statistical analysis, we examined the main mode of spatial-temporal variability in the concentration of chlorophyll-*a* in the SETIO region and evaluated its relationship with physical forcing patterns.

MATERIALS AND METHODS

Study area

The study area was located in the SETIO region covering an area of 90º-150ºE and 16ºS-4ºN (Figure 1). The SETIO region is a place where the water masses from the Pacific Ocean, South China Sea, Indonesian Seas, and Indian Ocean are meet.

Datasets

Chlorophyll-*a* concentration for the SETIO region was obtained from sublicenses of monthly global mapped imagery derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard the Aqua spacecraft. The data were provided by the NASA Goddard Distributed Active Archive Center for a period from July 2002 to February 2014 at 9 km horizontal resolution. The surface wind data were obtained from the ERA-Interim of the European Center for Medium Range Weather Forecasts (ECMWF). The monthly gridded data at 0.75° × 0.75° resolutions from January 2002 to December 2013 were used in this study. As a proxy for upwelling, the vertical transport was computed using the formula $(1/\rho f)\nabla\times\vec{\tau}$, where ρ is the density of seawater and $\vec{\tau}$ is wind stress. This parameter is then called as the Ekman pumping. Note that, the wind stress was calculated using monthly surface wind data using a constant drag coefficient (1.43×10^{-3}) and air density (1.225 kg m^{-3}) following Weisberg and Wang (1997).

Monthly SST imagery was obtained from the National Oceanic and Atmospheric Administration (NOAA) Optimum Interpolation (OI) (Reynold et al. 2007). This SST product combined the Advanced Very High-Resolution Radiometer (AVHRR) satellite data and direct measurements from ships and buoys collected under the International Comprehensive Ocean-*a*tmosphere Data Set (INCOAD) (Worley et al. 2005). The dataset from January 2002 to December 2013 on 0.25° × 0.25° grids was used in this study.

Data analysis

EOF technique was used to examine the spatial and temporal variations of all parameters (chlorophyll-*a*, ocean wind speed, and SST). EOF analysis extracts multivariate datasets into a series of orthogonal functions or modes based on the covariance matrix of the data (Emery and Thomson 2004). Every mode consists of the spatial eigen functions, representing the spatial patterns of variability in each time, and the principal components where a time series showed on how the spatial patterns was varied with time. Each subsequent mode was being orthogonal, which is consistently the same as the previous one. Typically, the leading mode explains the highest amount of the total variance in which the variations of its spatial and temporal patterns are strongly coupled.

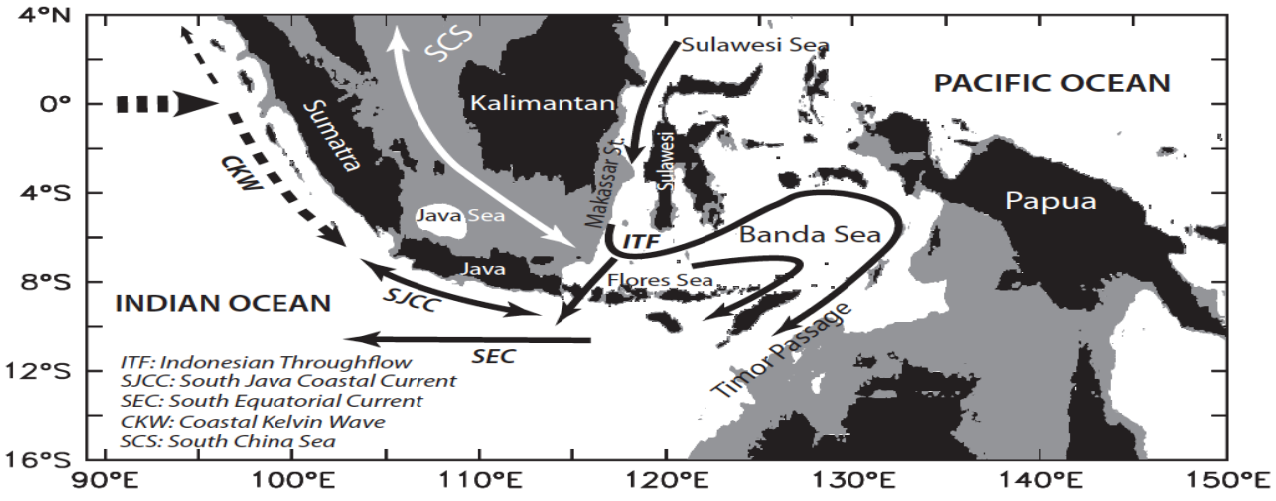


Figure 1. Map of the southeastern tropical Indian Ocean

To reduce the amount of missing chlorophyll values due to the cloud cover and to generally remove high spatial frequency noise due to the inherent limitation of measurement capabilities of the satellite instrument, an area-weighted re-gridding into 0.4° × 0.4° spatial resolutions was applied to the chlorophyll-*a* data. The average of seasonal composite was then applied to the 0.4° × 0.4° chlorophyll-*a* data to reduce missing values and high-frequency noise, The result was a set of 3-month composite-*a*verage of chlorophyll-*a* image (e.g. DJF, MAM, JJA, and SON) spanning from 2002 to 2013. Chlorophyll-*a* data at shallow water locations, for a depth less than 100 m, were then removed. Finally, temporal anomalies were calculated by subtracting the long-term mean at each grid point. A similar procedure was applied to the SST and surface wind data, with an exception that the data do not contain any missing values.

RESULTS AND DISCUSSION

Spatial characteristics

Figure 2 shows the spatial patterns of the temporal standard deviation for chlorophyll-*a*, SST, Ekman pumping and a proxy index for the wind mixing (U^3). U^3 represents the wind-induced surface ocean mixing that is proportional to the cube of the wind fields (Ueyama and Monger 2005). The standard deviation for chlorophyll-*a* showed that the area of high variability was confined along the southern coast of Indonesia, extending from the Nusa Tenggara Islands in the east to the southern coast of Sumatra in the west (Figure 2.A). This suggests that the presence of strong coastal upwelling was forced by the alongshore wind stress. In addition, the map also exhibited the relative-high variability of chlorophyll-*a* concentration in the eastern Banda Sea. Similar features were also observed for the SST, though with an offshore extension (Figure 2.B). This SST feature was not surprising; because a previous study has shown that high SST variations associated with the upwelling off Java progressed westward from the southern coast of the Nusa Tenggara Islands to the southern coast of Sumatra (Susanto et al. 2001). The Ekman pumping also showed the high variability along the southern coast of Java and Sumatra suggesting the presence of coastal upwelling (Figure 2.C). High variability in the Ekman pumping was also found in the eastern Banda Sea, which showed the high variability of the chlorophyll-*a* concentration. Wind-induced mixing showed two areas with high variability (Figure 2.D). The first area was in the offshore west of about 105ºE and the second area was in the Timor Sea. The former one corresponded to the location of high SST and strong winds during the negative IOD event.

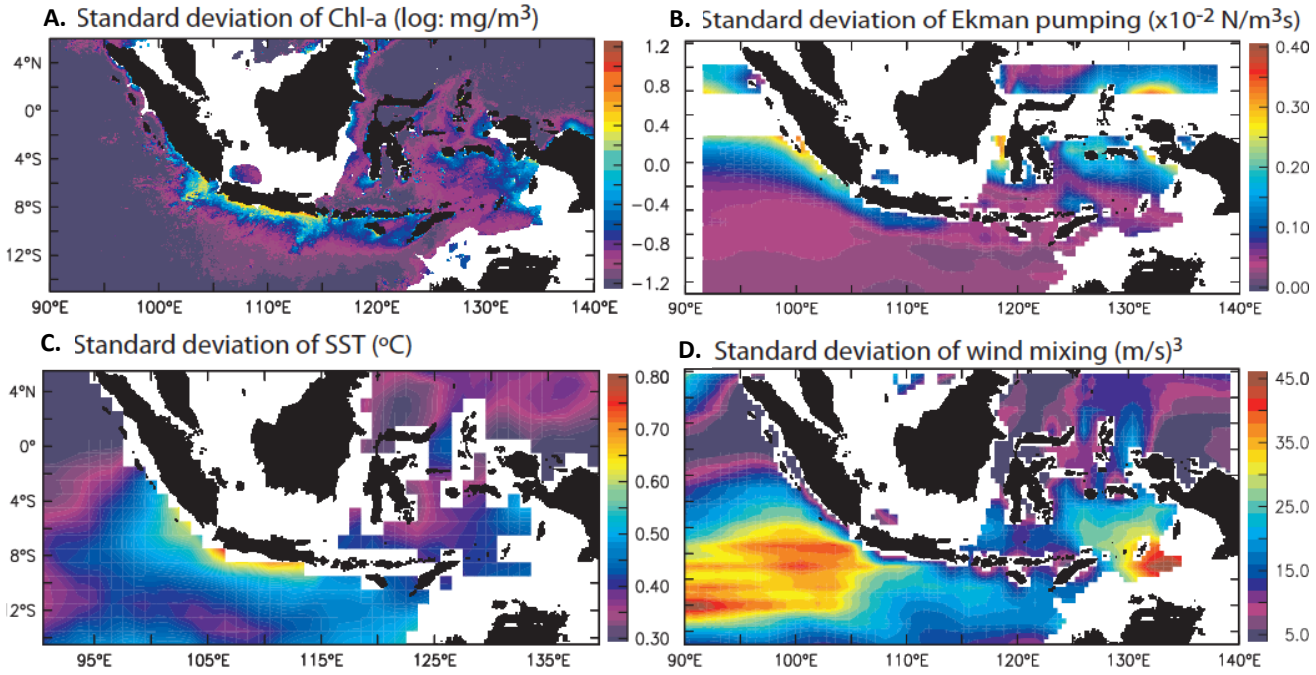


Figure 2. Standard deviations of (A) chlorophyll-*a* from the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard the Aqua spacecraft from July 2002 to February 2014 (B) SST of the Advanced Very High-Resolution Radiometer (AVHRR) satellite data from January 2002 to December 2013, (C) Ekman pumping, and (D) wind-induced mixing (u^3) derived from the ERA-Interim of the European Center for Medium Range Weather Forecasts (ECMWF) from January 2002 to December 2013. The vertical axes indicate the latitude, while the horizontal axes represent the longitude

Seasonal variations

The spatial functions of the leading EOF mode for monthly chlorophyll-*a* concentration, SST, Ekman pumping and wind-induced mixing is presented in Figure 3. The explanation of variances for the first two EOF modes is summarized in Table 1. The chlorophyll-*a* pattern of the first EOF mode, which accounts for 47.8% of the total variance, revealed the presence of the high-relative variability along the southern coasts of Java and Nusa Tenggara Island as well as in the eastern Banda Sea (Figure 3a). The pattern also revealed a distinct offshore extension of high variability of chlorophyll-*a* concentration.

The variability of spatial pattern of SST in each month from the first EOF mode obtained 72.8% of the total variance (Figure 3.B). The pattern showed that the temporal SST variability was relatively strong and broadly covering the SETIO region and the Banda Sea. It means that 180° out of phase with the chlorophyll-*a* variability for these same regions (Figure 3.A). Based on the data of leading EOF mode of the Ekman pumping, it showed that the variability of Ekman pumping covered up to 83.0% of the total variance. Most of them depicted the relatively strong temporal variability especially in the restricted region i.e the southern coast Sumatra and Java. This strong variability also occured in the northern side of the Banda Sea, which was in the roughly phase of chlorophyll-*a* variability (Figure 3.C). Although the stronger phase of variability occurred near the south Sumatra coast; however, the variability based on Ekman pumping data was relatively weak. The same condition was also observed in the south of Nusa Tenggara Islands. This indicated that the presence of coastal upwelling caused two-contrast condition i.e the high concentration of chlorophyll-*a* and the low data of SST in this region. The variability of wind-induced mixing based on the first EOF mode analysis accounted for 57.7% of the total variance (Figure 3.D). The most striking spatial pattern wit a strong variability was located in Sumatra-Java, which was also the area of the highest wind-induced mixing variability as shown in Figure 2.D.

The time series of the principal component analyzed from the leading EOF mode for chlorophyll-*a*, SST, Ekman pumping and wind-induced mixing is presented in Figure 4. It was not surprising that the most apparent feature of all time series was a seasonal cycle, which was associated with the seasonal change in the monsoon winds. The EOF time series of chlorophyll-*a* was highly correlated to those of SST, Ekman pumping, and wind-induced mixing with coefficient correlation exceeding 0.9 (Table 1). In addition to seasonal feature, the time series of the leading EOF modes also indicated inter-annual signal. For example, the chlorophyll-*a* time series showed the relatively high weight between 2006 and 2008, which were defined as the IOD years (Iskandar et al. 2013). Similar signals were also observed in other time series indicating the SETIO region also experienced inter-annual variation. Statistical analysis showed that all time series were significantly correlated to the Dipole Mode Index (DMI) time series (Table 2).

Table 1. Percentage of variance of the first-two EOF modes from each spatial and temporal variations parameters

Variables	EOF-1	EOF-2
Chlorophyll-*a*	47.8%	9.1%
SST	72.8%	15.1%
Ekman pumping	83.0%	6.2%
Wind-induced mixing	57.7%	15.4%

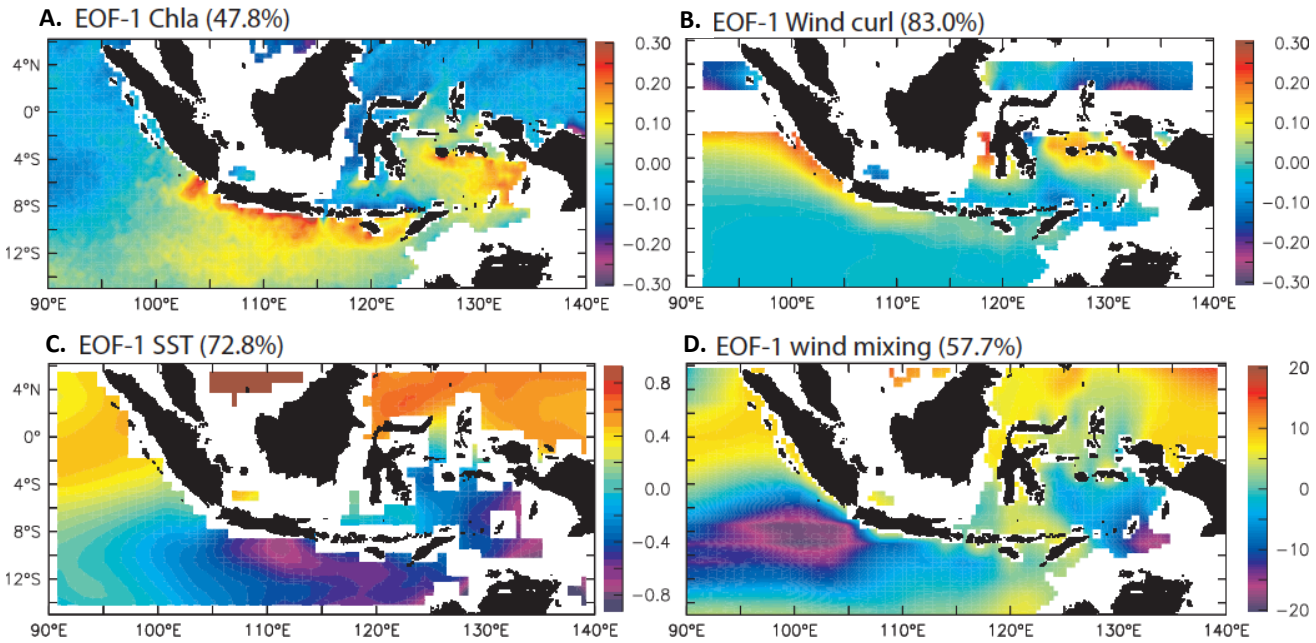


Figure 3. Spatial pattern of eigen functions observed from the first EOF mode (A) chlorophyll-*a*, (B) SST, (C) Ekman pumping, and (D) wind-induced mixing

Table 2. Correlation coefficients between the IOD and first two EOF modes of chlorophyll-*a*, SST, Ekman pumping, and wind-induced mixing.

	IOD		Chlorophyll	
	EOF-1	**EOF-2**	**EOF-1**	**EOF-2**
Chlorophyll	0.41*	0.54*	-	-
SST	- 0.38*	- 0.20	- 0.92*	- 0.82*
Ekman pumping	0.36*	0.41*	0.94*	0.75*
Wind-induced mixing	- 0.33*	- 0.20	- 0.92*	- 0.02

Note: * Significant at 90% confidence level

The spatial patterns of the second EOF mode of the chlorophyll-*a*, SST, Ekman pumping, and wind-induced mixing is presented in Figure 5. In contrast to the first EOF mode spatial pattern, the second mode pattern of chlorophyll-*a* accounting for 9.1% of the total variance. It revealed that the variability of chlorophyll-*a* in the western part of the study area was different with the eastern part of the study area (Figure 5.A). The second EOF mode of the SST showed 15.1% of the total variance and also showed an east-west asymmetry, but the variability is 180° out of phase with the second mode variability for chlorophyll-*a* (Figure 5.B). The variability of Ekman pumping analyzed from second EOF mode had 6.2% of the total variance (Figure 5.C). Its spatial pattern showed the positive weights in Sumatra, but it had, negative weights in the south Java and Nusa Tenggara as well as in the Banda Sea. This indicated that the variability in Sumatra was significantly difference with the other regions. The positive weights of the Ekman pumping were in line with the positive weights of chlorophyll-*a* and negative weights of the SST. On the other hand, the negative weights of the Ekman pumping appeared in the area of negative weights of chlorophyll-*a* and positive weights of the SST (Figs. 5.A-C). Spatial pattern of the second EOF mode of the wind-induced mixing, which accounts for 15.4% of the total variance, revealed that there were two distinct areas in the SETIO region (Figure 5.D). The first area, located southwest of (10ºS, 100ºE), was characterized by prominent negative weights. The second area was characterized by positive weights located near the coast extending westward from the south of Nusa Tenggara Islands to the west of Sumatra with maximum weights near the coast of Sumatra. The strongest positive weights for wind-induced mixing were located in the positive weights of chlorophyll-*a* and negative weights of SST. This observation was consistent with the limit nutrient in the regions, which happened due to the impact of wind-induced mixing bringing nutrient-rich and also the effect of low SST water moving from subsurface to the mixed layer resulting in the promotion of positive distribution of chlorophyll-*a* and negative SST.

The time series of the principal component for the second EOF mode is presented in Figure 6. Apparently, interannual variation dominated the variability of the time series with two distinct signals in 2006 and 2010, which correspond to the positive and negative IOD events, respectively (Horii et al. 2013). The principal time series of chlorophyll-*a* and Ekman pumping was highly correlated to the DMI time series with coefficient correlation of 0.54 and 0.41, respectively. However, the principal time series of the SST and wind-induced mixing exhibit low correlation with the DMI, where the coefficient correlation for both time series is 0.2 and only significant at 80% confidence level.

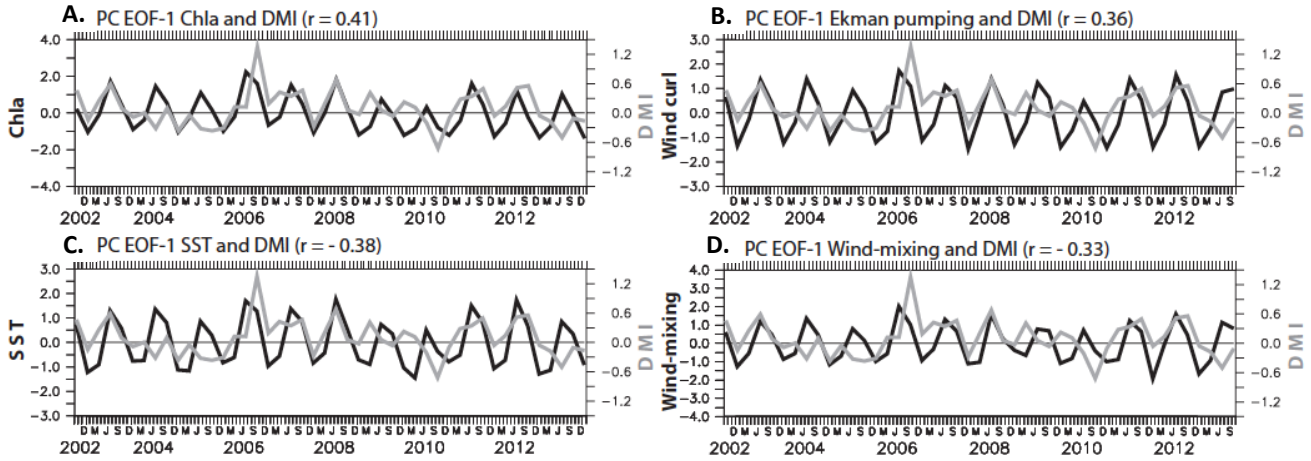


Figure 4. Principal components (*black*) for the first EOF mode of (A) chlorophyll-*a*, (B) SST, (C) Ekman pumping, and (D) wind-induced mixing. Time series of the DMI (*gray*) was shown in each panel. The DMI was calculated as a gradient of sea surface temperature anomaly averaged over the western (10°S−10°N, 50°E−70°E) and the eastern (10°S−Equator, 90°E−110°E) regions (Saji et al. 1999). The time series of SST and wind-induced mixing were flipped.

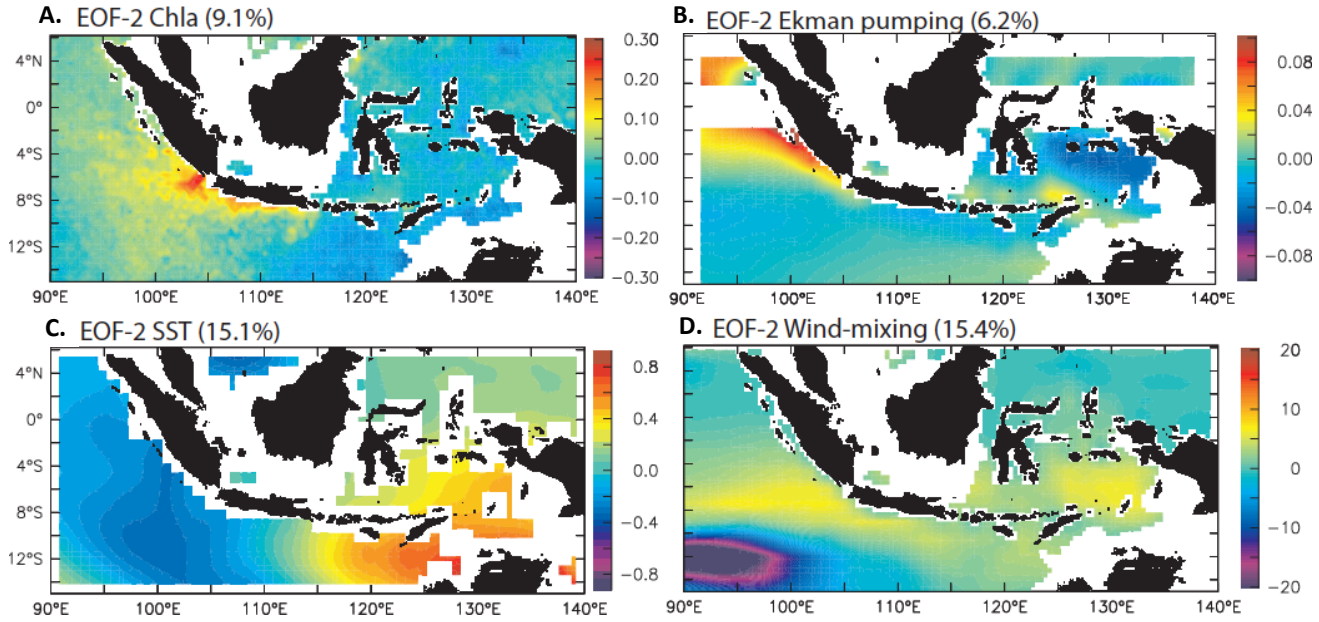


Figure 5. Same as in Figure 3 except for the second EOF mode

Interannual variations

In order to evaluate the inter-annual variation of chlorophyll-*a* blooms in the Sumatra and Java associated with the occurrence of the IOD events, we performed an EOF analysis on the seasonal averages. Here, the EOF analysis was applied to the individual season, specifically from June to August (JJA) and separately from the September to November (SON) seasons. Previous study has shown that the IOD event was developed in summer (JJA), which was correlated with the upwelling season in the SETIO region, and reaches its peak in boreal fall (SON) (*Saji et al.* 1999).

Figure 7 presents the spatial pattern of the first EOF mode for JJA season. Variance of the first EOF for each variable was summarized in Table 3. It was shown that the first EOF mode of all variables explained most of the total variance and their spatial patterns resemble those of the first EOF mode for the full season (*see* Figure 3). The first EOF mode of the chlorophyll-*a* covered 98.2% of the total variance. Its spatial pattern was characterized by strongly positive weights along the southern coast of Java and Nusa Tenggara Islands as well as in the southern tip of Sumatra (Figure 7.A). Strong positive weights were also found in the Banda Sea, which was mostly concentrated in the eastern Banda Sea. In addition, there was an offshore extension of positive weights in south Java, although it was relatively weaker compared to that of the first EOF mode for the full season.

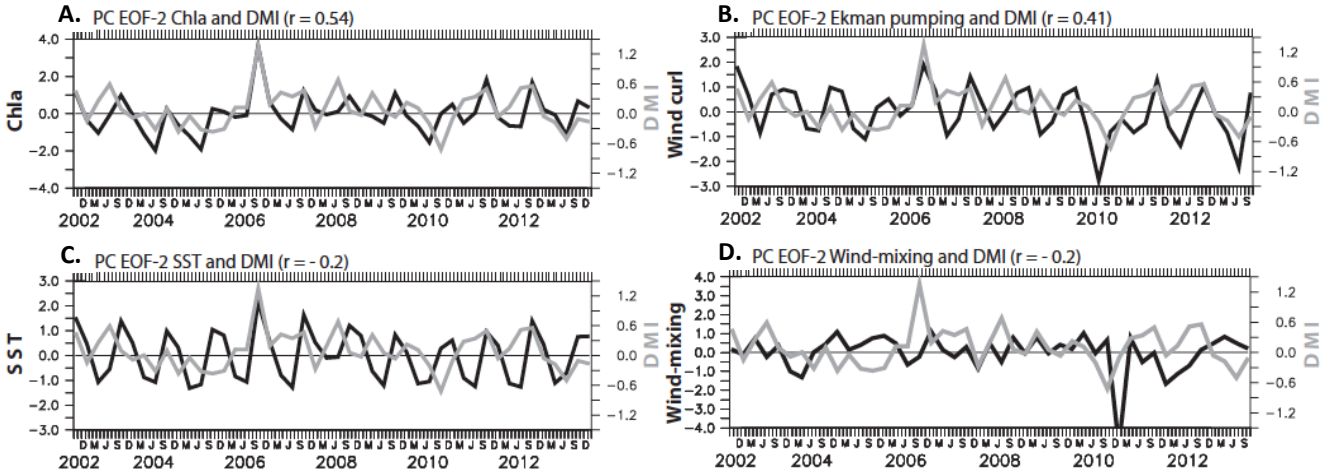


Figure 6. Same as in Figure 4 except for the second EOF mode

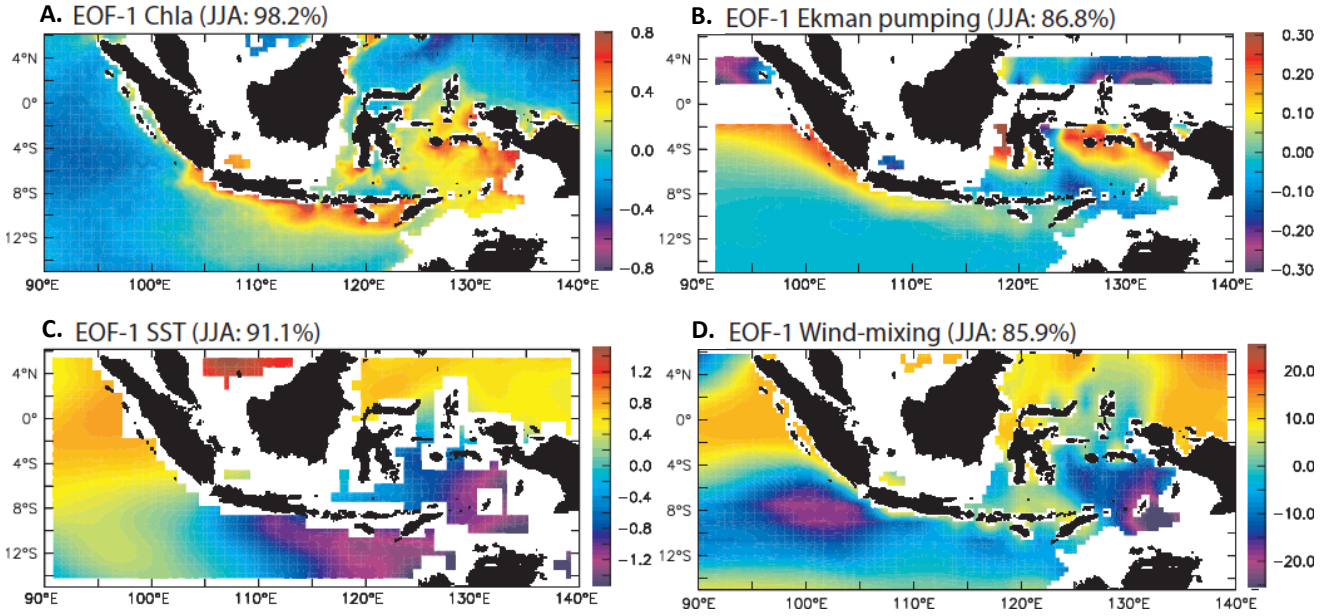


Figure 7. Same as in Figure 3 except for the first EOF mode for JJA season

The similarity between the first EOF spatial patterns of the full season and that of the JJA season was also found in the data analysis of the SST, Ekman pumping, and wind-induced mixing (Figs. 7.B-D). The first EOF mode of the SST accounting for 91.1% of the total variance revealed negative weights that was 180° out of phase with the chlorophyll-*a* weights (Figure 7.B). In addition, the first EOF mode of the Ekman pumping, which explained 86.8% of the total variance, showed positive weights in regions with positive weights of the chlorophyll-*a* suggesting the presence of upwelling (Figure 7.C). Meanwhile, the first EOF spatial pattern of the wind-induced mixing accounts for 85.9% of the total variance. Its spatial pattern showed a prominent negative weight for wind-induced mixing in the west of Sumatra-Java and the east of Banda Sea (Figure 7.D) similar to that of the first EOF mode for the full season (Figure 3.D).

The time series of the principal component of the first EOF mode for the JJA season is presented in Figure 8. Interestingly, only principal time series of SST and Ekman pumping showed significant correlation with the time series of DMI with coefficient correlation of -0.66 and 0.6, respectively. Meanwhile, the principal time series of chlorophyll-*a* and wind-induced mixing indicated the low correlation with the times series of DMI. The coefficient correlations for the chlorophyll-*a* and wind-induced mixing were 0.34 and -0.42, respectively. However, the principal time series of SST, Ekman pumping, and wind-induced mixing was significantly correlated to that of the chlorophyll-*a*, with coefficient correlation >0.6 (Table 4).

Table 3. Same as in Table 1 except for the first EOF mode of JJA and SON seasons.

Variables	JJA	SON
Chlorophyll	98.2%	98.1%
SST	91.1%	82.2%
Ekman pumping	86.8%	78.3%
Wind-induced mixing	85.9%	65.9%

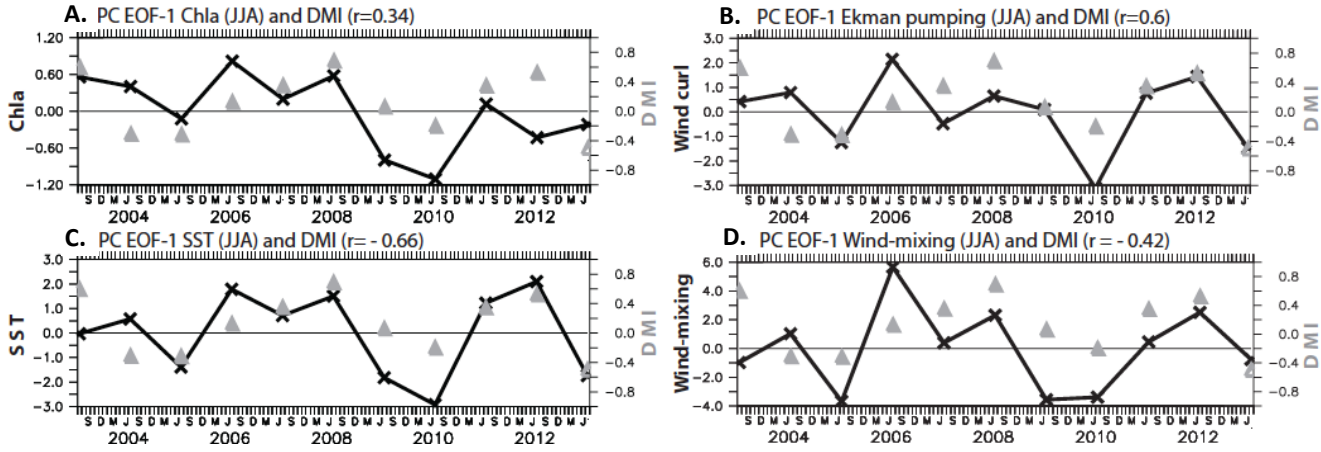


Figure 8. Same as in Figure 4 except for the first EOF mode for JJA season

The spatial patterns of the first EOF mode of SON season are shown in Figure 9 and the explaination of variances are summarized in Table 3. Of the chlorophyll-*a*, the leading EOF mode accounts for 98.1% of the total variance and primarily revealed the changes of chlorophyll-*a* variation along the southern coast of Java and Nusa Tenggara Islands (Figure 9.A). Although the positive weights of the chlorophyll-*a* variability was located in the negative weights of the SST, the greatest SST variability was further located in the west of Sumatra-Java as shown in the spatial pattern of the first EOF mode (Figure 9.B). Notably, the first EOF mode of the SST explained 82.2% of the total variance and its spatial pattern resembled the second EOF mode pattern of the full season (Figure 5.B). The spatial pattern of the first EOF mode for the Ekman pumping, accounting for 78.3% of the total variance, showed the positive weights which were confined to the west coast of Sumatra and only the weak positive weights were found in south Java (Figure 9.C). The first EOF mode of the wind-induced mixing explained 65.9% of the total variance and its spatial pattern showed two distinct areas (Figure 9.D). The first area with strong negative wind-induced mixing weights was found in the west of Sumatra-Java, while the second area characterized by strong positive wind-induced mixing weights was located in the east of Timor Passage and so the temporal variability of these two regions was different from each other.

The principal component time series of the chlorophyll-*a* and SST was significantly correlated to the time series of DMI, with coefficient correlations of 0.62 and -0.94, respectively (Figs. 10a-b). The principal component time series of the chlorophyll-*a* and SST also demonstrated a significant correlation with a coefficient correlation of -0.75 (Table 4). On the other hand, the principal component time series of the Ekman pumping and wind-induced mixing did not show significant correlations with the time series of DMI (Figs. 10.C-D). In addition, the principal component time series of the Ekman pumping and wind-induced mixing also did not show significant correlation with the chlorophyll-*a* (Table 4). During the IOD year, weak surface winds were observed in all over the Indonesian regions. This result indicated that during the peak IOD season (SON season), the bloom of the chlorophyll-*a* and the low SST in the SETIO region were generated by the strong upwelling favorable winds in the preceding season.

Table 4. Same as in Table 2 except for the JJA and SON seasons

	IOD		Chlorophyll	
	JJA	**SON**	**JJA**	**SON**
Chlorophyll	0.34*	0.62*	-	-
SST	- 0.66*	- 0.94*	- 0.69*	- 0.75*
Ekman Pumping	0.60*	0.20	0.68*	0.20
Wind-induced mixing	- 0.42*	- 0.10	- 0.66*	- 0.03

Note: * Significant at 90% confidence level

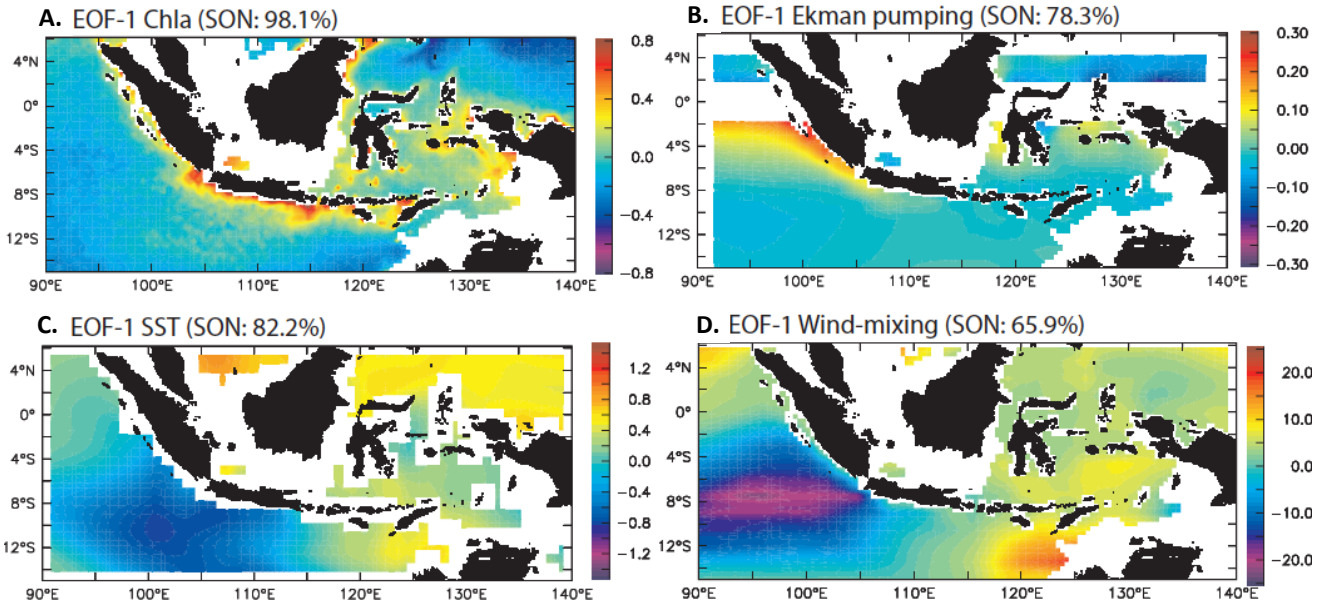


Figure 9. Same as in Figure 3 except for the first EOF mode for SON season

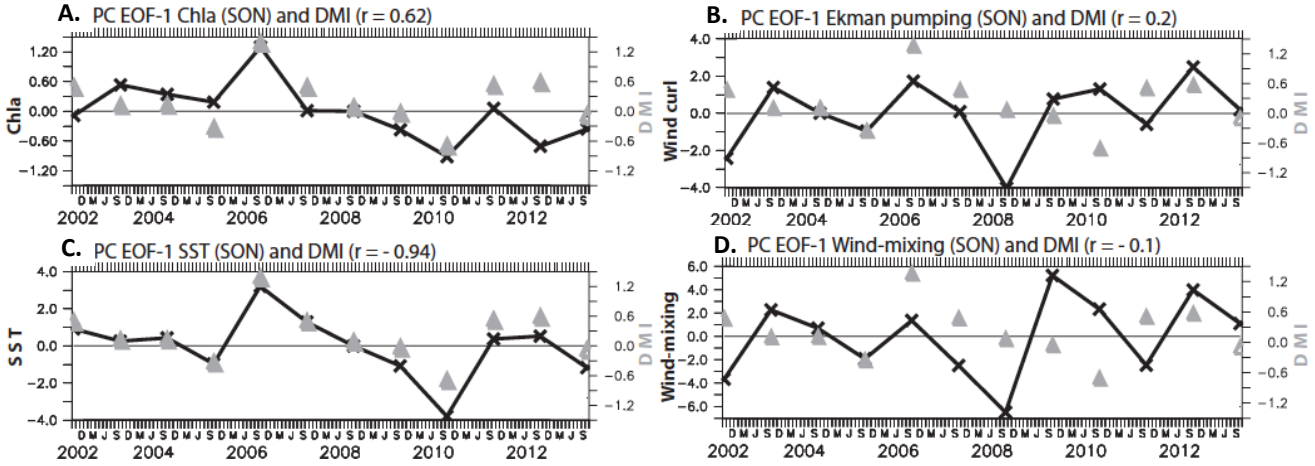


Figure 10. Same as in Figure 4 except for the first EOF mode for SON season

The SETIO region is strongly influenced by the Asian-*a*ustralian monsoon winds. Seasonal reversal of this monsoon winds causes seasonal coastal upwelling in the SETIO region, which occurs during the southeast monsoon. In some years, there was an anomalously strong coastal upwelling due to the enhancement of southeasterly winds along the southern coast of Sumatra and Java. This enhancement is associated with the occurrence of the IOD events in the tropical Indian Ocean.

In order to evaluate the interannual variation in the magnitude of seasonal chlorophyll-*a* increase and its possible relationship with IOD events, we performed an EOF analysis on a multi-annual time series of seasonal average conditions for chlorophyll-*a*, SST, Ekman pumping, and wind-induced mixing. We choose the JJA and SON seasons because they represent the development and the peak phases of the IOD event, respectively. The results show that the chlorophyll-*a* bloom during JJA season is mostly related to the seasonal upwelling generated by southeasterly winds during the southeast monsoon. Meanwhile,.the EOF analysis on the SON season suggests that the anomalously strong upwelling-favorable winds during the development phase of the IOD event in JJA season provided a favorable condition for the chlorophyll-*a* bloom during the peak phase of the IOD event in SON season. Although previous study has suggested that wind-induced mixing could bring subsurface nutrient-rich water to the mixed layer and lead to an increase in the chlorophyll-*a* concentration (Ueyama and Monger 2005), our results suggest there is no correlation between chlorophyll-*a* bloom and wind-induced mixing in the SETIO region.

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Anti-inflammatory activities of ethnomedicinal plants from Dayak Abai in North Kalimantan, Indonesia

SWANDARI PARAMITA[1,♥], KHEMASILI KOSALA[1], DZULKIFLI DZULKIFLI[2], DEBY INDAH SAPUTRI[1], ENGGAR WIJAYANTI[3]

[1]Faculty of Medicine, Universitas Mulawarman. Jl. Kerayan Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia. Tel./Fax. +62-541-748581. ♥email: swandariparamita@gmail.com

[2]Faculty of Forestry, Universitas Mulawarman. Jl. Ki Hajar Dewantara Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia

[3]Center for Research and Development of Medicinal Plants and Traditional Medicine. Jl. Raya Lawu No.11 Tawangmangu, Karanganyar 57792, Central Java, Indonesia

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Abstract. *Paramita S, Kosala K, Dzulkifli D, Saputri DI, Wijayanti E. 2017. Anti-inflammatory activities of ethnomedicinal plants from Dayak Abai in North Kalimantan, Indonesia. Biodiversitas 18: 1556-1561.* Inflammation is a normal process in the human body as a response to injury from the healing process. Meanwhile, chronic inflammation will cause new health problems to patients. Anti-inflammatory drugs generally used for those conditions, have several side effects to patients. The objective of this research was to find alternative anti-inflammatory drugs, especially from natural sources. Three medicinal plants recorded from Dayak Abai in North Kalimantan, Indonesia for health problems caused by the inflammation process i.e. *Amomum xanthophlebium* Baker, *Clerodendrum buchananii* (Roxb.) Walp., and *Donax canniformis* (G.Forst.) K.Schum. were used as material in this research. The experimental method using carrageenan-induced rat paw edema was used followed by the resulting measurement using plethysmometer. The results showed that significant differences of AUC (area under the curve) with $p = 0.001$ ($p < 0.05$) were achieved between negative control, positive control, and treatment group with plant medicinal extracts. AUC of leaves ethanol extract of *C. buchananii* showed the strongest anti-inflammatory activities. It could be concluded that the medicinal plants recorded from ethnomedicinal data from Dayak Abai in North Kalimantan, have anti-inflammatory activities, with *C. buchananii* as the most potential ones which could be further developed as a new source of the anti-inflammatory drug.

Keywords: Anti-inflammatory, *Amomum xanthophlebium*, *Clerodendrum buchananii*, *Donax canniformis*, carrageenan-induced rat paw edema

INTRODUCTION

Inflammation is a physiological process in the body in response to a lesion on the body. Acute inflammation can be triggered by various stimuli which could be seen from the rapid response at the site of infected or damaged tissues such as leukocytes carriers and protein plasm (antibodies) to the site of inflammation. Chronic inflammation can occur due to the subsequent process of acute inflammatory processes from several weeks to months and even years. During the course of acute and chronic inflammation, a number of chemical mediators will be released. A large number of inflammatory mediators are released via arachidonic acid pathways, including prostaglandins, as a result of the breakdown of arachidonic acid by cyclooxygenase enzymes. Although inflammation is a physiological process in the body, it can cause severe impacts for patients, including the emergence of pain, swelling, fever and other symptoms (Souza et al. 2012).

To overcome these effects, most types of anti-inflammatory drugs are applied. There are two classes of anti-inflammatory drugs i.e. NSAIDs (non-steroidal anti-inflammatory drugs) and corticosteroids. NSAIDs work by inhibiting cyclooxygenase enzyme action. While corticosteroids will inhibit the expression of cyclooxygenase enzyme. The inhibition of inflammation process may help patients by: (i) Reducing inflammation process, especially for patients with the muscle injury and other conditions with swelling of the joints. (ii) Reducing pain, especially for patients with osteoarthritis, rheumatism, gout, surgery effects (Pountos et al. 2011). (iii) Reducing fever, mainly due to inhibition of prostaglandin production in the hypothalamus as the main role in the process of fever. (iv) Protecting the heart by inhibiting the production of thromboxane A2 enzymes in the clot formation process, which potentially cause blockages in coronary artery heart (Goodman et al. 2010).

In spite of many advantages, the use of anti-inflammatory drugs also has indisputable side effects. Some prominent side effects of anti-inflammatory drugs are the effects on the gastrointestinal system. This is mainly because the inhibition of cyclooxygenase enzymes, especially COX-1, will trigger the inhibition of prostacyclin and PGE2. In the stomach, prostacyclin and PGE2 are known as the protector of gastric wall mucosa from the effects of stomach acid. As a result, the side effects of those drugs appear such as nausea, severe vomit, and the hardest effects i.e. peptic ulcer with bleeding complications as the major cause of death. The use of high or long-term doses of NSAIDs, along with corticosteroids

and/or anticoagulants, smoking and/or alcohol, increases the risk of these side effects. In addition, some studies have reported that some NSAIDs also have direct destructive effects on gastric mucosal cells, causing these side effects (Siew and Francis 2010). Another side effect is on the cardiovascular system. Prostaglandins produced by the cyclooxygenase process regulate the complex interactions between platelets and blood vessel walls. In the administration of selective anti-inflammatory drugs COX-2, the imbalance production of prostacyclin and TXA2 occurs, leading to an imbalance of a prothrombotic in the blood, which further increases the risk of thrombotic blood events (Antman et al. 2007). Of these reasons, incessant efforts to find alternative anti-inflammatory drugs, especially those derived from natural materials are very important.

Indonesia is known as one of the mega-biodiversity countries with the abundance of medicinal plants. Each ethnic communities has a diverse culture and a local wisdom, including the use of plants for traditional medicine. Knowledge of the use of medicinal plants by indigenous communities is essential for the development of traditional medicine and the development of modern medicine. This is because most plant extracts used in modern medicine are found through local knowledge approach. The use of medicinal plant data from ethnobotany research is an effective way to discover new potential chemicals for treatment. Research on Local Knowledge Exploration of Ethnomedicine and Community-Based Drugs in Indonesia is also known as the Research of Medicinal and Herbs (*Riset Tumbuhan Obat dan Jamu* or RISTOJA) (MoH RI 2016).

The RISTOJA program of the Indonesian Ministry of Health undertaken in North Kalimantan in 2015 produced a number of identified-medicinal plant information up to the species level. This program also successfully recorded the indications/complaints of the disease, including the health problems caused by the inflammatory process. Around 25 medicinal plants with ethnomedicinal data showing anti-inflammatory effects had been recorded. Based on published papers, the comprehensive study of the anti-inflammatory activity of three medicinal plants i.e. *Clerodendrum buchananii* (Roxb.) Walp., *Donax canniformis* (G.Forst.) K. Schum., and *Amomum xanthophlebium* Baker is still limited (Ismail et al. 2012; Ismail et al. 2015; RIMU 2015). These were supported by ethnomedicinal data from Dayak Abai ethnic group in North Kalimantan. Therefore, the objective of this study was to test the anti-inflammatory activity of the three medicinal plants i.e. *Clerodendrum buchananii* (Roxb.) Walp., *Donax canniformis* (G.Forst.) K. Schum., and *Amomum xanthophlebium* Baker originated from Dayak Abai ethnic group in North Kalimantan.

MATERIALS AND METHODS

Sampling and sample identification

The sampling of medicinal plants was conducted at the location of RISTOJA 2015 research, i.e. Dayak Abai settlement in Sentaban Village, Malinau Barat Sub-district, Malinau District, North Kalimantan (MoH RI 2016). The plant sampling was located at about 900 km from Samarinda, the capital of East Kalimantan province. Plants were then be identified in the Laboratory of Ecology and Dendrology of the Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia to certify the plant legality. Based on the plant identification, the medicinal plants used in this study were *Amomum xanthophlebium* Baker., *Clerodendrum buchananii* (Roxb.) Walp., and *Donax canniformis* (G.Forst.) K. Schum. While, experimental research was conducted in This study was conducted in the Research Laboratory of the Faculty of Medicine, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

Medicinal plant extractions

The extraction of medicinal plant followed the guidelines from the Indonesian Herbal Pharmacopoeia (MoH RI 2008). Medicinal plants were dried and then crushed into simplicia. The simplicia were then macerated using absolute ethanol solvent in a ratio of 1 part of simplicia to 10 parts of solvent. The mixture was soaked for 6 hours followed by stirring occasionally with an orbital shaker at room temperature in each interval hours, which were then stood for 18 hours. The mixture was then separated using filter paper, then evaporated with a rotary evaporator at 50°C. The obtained viscous extract was dried to obtain a dry extract. The dried extracts were then stored in a refrigerator of -20°C for further study.

Anti-inflammatory test activity

Anti-inflammatory activity of medicinal plant extracts was tested by carrageenan-induced rat paw edema method and measured using a plethysmometer. There were 5 groups of experimental animals in this study: (i) Group 1 was negative control; (ii) Group 2 was a positive control by giving indomethacin dose 10 mg/kg orally; (iii) Groups 3, 4 and 5 were treated with medicinal plant extracts in different doses. Each group consisted of 5 individuals.

The dosage of medicinal plants was determined using calculation table conversion for various species of animal species (Bacharach and Laurence 1964). Doses for *C. buchananii* was as follows: dosage I 0.91 mg/kg, dose II 1.81 mg/kg, and dosage III 3.63 mg/kg. Doses for *D. canniformis* was as follows: dosage I 0.54 mg/kg, dosage II 1.08 mg/kg, and dosage III: 2.16 mg/kg. Doses for *A. xanthophlebium* was as follows: dosage I 0.25 mg/kg, dosage II 0.5 mg/kg, and dosage III 1 mg/kg.

Carrageenan (0.1 ml of 1% in 0.9% NaCl) was injected in subcutaneous in left paw substrate of rat and waited the work reaction for an hour. After the injection, the volume of rat paw edema was measured with the plethysmometer. The measurement was further done at the 1st, 2nd, 3rd, 4th, 5th, and 6th hours after the injection. Paw edema is defined as the changes of the rat paw edema after the carrageenan injection on the left paw sub-plant measured by plethysmometer in a certain time range. Percentage of edema inhibition changes were calculated using the formula as follows: (V1-V0) x 100%, where V1 is the left

paw volume of the treated rats within a certain time range, V0 is the left paw volume of the rat before carrageenan injection (Eddouks et al. 2012).

Data analysis

The obtained data were calculated for each mean and SE (standard error) for the results of paw edema test. Obtained data was then statistically analyzed by ANOVA followed by Tukey post hoc, with the level of confidence at 95% ($p < 0.05$) using PSPPIRE 0.8.4. software. To compare anti-inflammatory activity among the three medicinal plants, the AUC (under curve area) was calculated from all the obtained results. All data were presented in tables and graphs.

RESULTS AND DISCUSSION

Anti-inflammatory activity of each medicinal plant

The results of the oral administration of the medicinal plants extract for the inhibition of rat paw edema induced by carrageenan are shown in Table 1. The effectivity of those medicinal plant extracts relative to the negative control and indomethacin used as positive control was also observed. It can be observed that the plant medicinal extract showed a significant antiedematogenic activity, within the first hour after the extracts administration; with ANOVA results indicated statistically significant differences between treatment ($p < 0.001$). The Tukey post hoc test showed a decrease in paw edema volume in the first hour after carrageenan injection, compared to the control group. The administration of *C. buchananii* extract with the dosage I produced a significant reduction in the paw edema after 1 h of extract application ($p < 0.05$). The same results were also shown in the application of *D. canniformis* extract with the dosage III ($p < 0.001$), and *A. xanthophlebium* extract with the dosage III ($p < 0.001$), which reduced the paw edema compared to the controls after 1 h of those extract administration. As expected, the reference drug, indomethacin (10 mg/kg), caused a significant inhibition of post-carrageenan edema.

Comparison of anti-inflammatory activity of three medicinal plants

Comparison of anti-inflammatory activity among three medicinal plants was observed based on the calculation of AUC values (area under the curve) from all research results. Table 2 shows the results of paw edema AUC with the administration of *C. buchananii*, *D. canniformis*, and *A. xanthophlebium*. There were significant differences of AUC results amongst three medicinal plants and controls with $p < 0.01$. The lower AUC score indicated that the prevalence of paw edema was less. The Tukey post hoc test showed significant differences in AUC score ($p < 0.05$) for *C. buchananii* dosage I, *D. canniformis* dosage III, and *A. xanthophlebium* dosage III when compared to the controls. Since the edema indicates an inflammatory process, when the prevalence of edema was less after it was administered by the extract of plant medicinal, it means that the activity of anti-inflammatory of the medicinal plant extract was very strong. The lowest AUC results of the three medicinal plants could be seen in the first dose of *C. buchananii* extract.

Table 1. Average of carrageenan-induced paw edema inhibition after the administration of plant extracts

Plants	Group	Average inhibition (mm3) ± SE per-hour						
		0	1	2	3	4	5	6
CB	Control	41.7 ± 3.8	36.6 ± 3.7	34.1 ± 4.4	31.8 ± 4.0	30.6 ± 4.1	29.0 ± 4.2	24.8 ± 3.8
	Indomethacin	76.9 ± 3.3*	71.0 ± 3.1	68.4 ± 3.8	62.8 ± 2.9	60.3 ± 2.5	54.4 ± 3.1	47.3 ± 2.1
	Dosage I	79.3 ± 8.3*	74.2 ± 2.3*	67.8 ± 2.3	61.7 ± 1.9	58.8 ± 1.5	54.0 ± 2.2	46.7 ± 1.8
	Dosage II	66.6 ± 3.6	55.4 ± 4.9	50.3 ± 3.4	44.9 ± 2.9	40.5 ± 3.2	36.6 ± 2.8	29.7 ± 2.2
	Dosage III	72.4 ± 10.3	48.5 ± 10.3	45.7 ± 7.9	43.2 ± 4.9	41.2 ± 5.0	38.8 ± 4.9	37.3 ± 3.9
DC	Control	41.7 ± 3.8	36.6 ± 3.7	34.1 ± 4.4	31.8 ± 4.0	30.6 ± 4.1	29.0 ± 4.2	24.8 ± 3.8
	Indomethacin	76.9 ± 3.3***	71.0 ± 3.1***	68.4 ± 3.8***	62.8 ± 2.9**	60.3 ± 2.5**	54.4 ± 3.1*	47.3 ± 2.1
	Dosage I	78.6 ± 8.3***	47.4 ± 2.3	41.2 ± 2.3	38.0 ± 1.9	34.6 ± 1.5	30.9 ± 2.2	27.8 ± 1.8
	Dosage II	74.2 ± 8.0***	57.1 ± 10.9	44.9 ± 7.6	39.6 ± 6.4	37.6 ± 7.0	34.4 ± 6.3	30.1 ± 4.8
	Dosage III	74.2 ± 9.1***	69.2 ± 10.2***	56.2 ± 7.6	47.0 ± 4.7	44.5 ± 5.1	41.7 ± 4.4	32.4 ± 3.5
AX	Control	41.7 ± 3.8	36.6 ± 3.7	34.1 ± 4.4	31.8 ± 4.0	30.6 ± 4.1	29.0 ± 4.2	24.8 ± 3.8
	Indomethacin	76.9 ± 3.3***	71.0 ± 3.1***	68.4 ± 3.8**	62.8 ± 2.9**	60.3 ± 2.5**	54.4 ± 3.1	47.3 ± 2.1
	Dosage I	85.2 ± 4.2***	72.1 ± 5.0***	61.1 ± 3.5*	56.8 ± 3.5	51.7 ± 3.8	44.4 ± 3.0	37.2 ± 3.0
	Dosage II	77.6 ± 8.2***	67.6 ± 7.9**	61.8 ± 7.3*	58.2 ± 6.9*	53.5 ± 6.0	47.7 ± 5.2	37.8 ± 4.8
	Dosage III	84.3 ± 3.8***	66.0 ± 5.6**	59.3 ± 4.3	53.9 ± 4.8	49.9 ± 6.4	45.3 ± 4.9	37.4 ± 5.1

Note: ANOVA (CB: *C. buchananii* $p<0.001$; DC: *D. canniformis* $p<0.001$; AX: *A. xanthophlebium* $p<0.001$), followed by Tukey post hoc test, with $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to control

Table 2. The average AUC of carrageenan-induced paw edema inhibition after the administration of plant extracts

Group	AUC ± SE
Control	406.69 ± 23.50
Indomethacin	224.57 ± 14.63*
C. buchananii dosage I	224.16 ± 20.50*
C. buchananii dosage II	327.67 ± 25.85
C. buchananii dosage III	328.47 ± 66.46
D. canniformis dosage I	356.22 ± 9.61
D. canniformis dosage II	336.42 ± 14.66
D. canniformis dosage III	292.77 ± 30.66
A. xanthophlebium dosage I	256.20 ± 18.77*
A. xanthophlebium dosage II	258.41 ± 38.69*
A. xanthophlebium dosage III	268.69 ± 26.62

Note: ANOVA ($p<0.01$), followed by Tukey post hoc test, with *$p<0.05$ compared to control

Discussion

Our findings firstly highlight the potency of ethnomedicinal plants from Dayak Abai for anti-inflammatory properties, providing a scientific basis for the alternative uses of ethnomedicinal plants from Dayak Abai in the treatment of inflammatory disorders. The results of this study indicate that all three medicinal plants i.e. *C. buchananii*, *D. canniformis*, and *A. xanthophlebium* had anti-inflammatory effects, with *C. buchananii* leaf extract showed the strongest anti-inflammatory activity of all two other medicinal plants, based on carrageenan-induced paw edema test results.

Specifically, *C. buchananii* used in this study belongs to the *Lamiaceae* family. Dayak Abai ethnic group in Malinau District, North Kalimantan named it as "tengger asam". The leaves are mostly used as traditional medicine. *C. buchananii* is also used in some other places as a medicinal plant. As an example, *C. buchananii* in Serampas, Jambi, known also as "bungo panggil", was used for expelling the certain disease due to the superstitious practices (Hariyadi and Ticktin 2012). *C. buchananii* in Pekurehua, Central Sulawesi which is locally called as "lelimbanua", was used as a medication for shortness of breath, swelling or rheumatism (Susiarti et al. 2009). *C. buchananii* in Tobelo, North Maluku, also locally known as "tatabako", was used to treat sick people by boiling the leaves which were then drunk or rubbing the leaves applied to the body (Susiarti et al. 2015). *C. buchananii* in Kaulong, Papua New Guinea is called "cocoyat", its leaves are used to treat fungus infections and skin lesions (Prescott et al. 2012). *C. buchananii* in Roviana, Solomon Islands called "titimunuhaha", is also used as a traditional medicine there (Furosawa et al. 2014). Based on this many local information regarding with the use of *C. buchananii* in traditional medicine practices, we are interested to study this medicinal plant and; fortunately, we successfully confirmed that this medicinal plants had a high potency as anti-inflammatory medicines.

Several other species of *Clerodendrum* including *Clerodendrum infortunatum* L.; *Clerodendrum paniculatum* L.; *Clerodendrum phlomidis* L.f.; and *Clerodendrum volubile* P.Beauv have been studied their anti-inflammatory activities. *C. infortunatum*, for example, is used as a traditional medicine in India, Bangladesh, Pakistan and surrounding areas. *C. infortunatum* leaves were used as bronchitis and asthma drug in Bangladesh (Apu et al. 2012). One published study found the anti-inflammatory activity *in vitro* on ethanol extract of *C. infortunatum* leaf using the writhing test (peripheral analgesic effect) and the tail flick method (central analgesia effect) (Kale and Maniyar 2015; Chandrashekar and Rao 2013). Another study also reported that ethanol extract of *C. infortunatum* root using writhing method possessed a valuable anti-inflammatory activity (Sumi et al. 2015). The same founding of anti-inflammatory activity also reported on ethanol extract of *C. infortunatum* leaf using carrageenan-induced rat paw edema (Chandrashekar and Rao 2014). Interestingly, other natural compounds such as saponins were successfully isolated from *C. infortunatum* leaf showing analgesic activity with writhing test and hot plate test (Das et al. 2014).

Clerodendrum species which are also frequently studied their anti-inflammatory activity is *C. phlomidis*. This plant is also used as a traditional medicine in India, Bangladesh, Pakistan and surrounding areas, especially for joint pain medication. One study found that *C. phlomidis* leaf extract had anti-inflammatory and anti-arthritic activity using carrageenan-induced paw edema and arthritis induced with FCA (Freund complete adjuvant) in trial rats (Prakash et al. 2014). Other studies also reported the same founding in the root extract of *C. phlomidis* using granuloma cotton pellet method (Parekar et al. 2012). Interestingly, other published papers also found that *C. phlomidis* extracts had analgesic, antiasthma, and antiarthritic effects (Raja and Mishra 2010). Other *Clerodendrum* species, i.e. *C. volubile* have been also studied its anti-inflammatory activity which is mostly used as a traditional medicine in Senegal and surrounding areas, especially for joint pain medication. The presence of anti-inflammatory activity of *C. volubile* leaf extract was successfully found using rat paw edema induction method with fresh albumin (Adediwura and Yewande 2012). From all studies of anti-inflammatory activity of *Clerodendrum* species; however, the published research of *Clerodendrum* species in Indonesia associated with that activity was still limited. A study of *C. paniculatum* in North Sumatra found that anti-inflammatory activity could be obtained using granuloma cotton pellet method and the induction of rat paw edema with carrageenan (Hafiz et al. 2016). Therefore, we used the local *Clerodendrum* species originated from Dayak Abai ethnic group in Malinau District, North Kalimantan as our focus study and successfully confirm their potency as anti-inflammatory medicine which could be further developed.

We also used carrageenan-induced paw edema model in this study because it is a frequently used as the method for testing non-steroidal anti-inflammatory agents. Carrageenan is a linear sulfate polysaccharide derived from an edible seaweed species. Carrageenan is commonly used

in food and pharmaceutical industries because of its ability to form gels, as hardener and preservative. Carrageenan is widely used as a phlogistic agent to induce acute inflammation, and it is a classic model applied in research on NSAIDs. Besides being non-antigenic and having no systemic effect, carrageenan is known to produce a pro-inflammatory response. Edema caused by carrageenan injection is characterized by swelling of localized areas with fluid and leukocytes, resulting in swelling of the legs (Tasleem et al. 2014; Archera et al. 2015).

The formation of carrageenan-induced edema in rat paws is a biphasic event over a period of 1 to 5 hours. The initial phase (1 to 1.5 hours) is dominated by nonphagocytic edema, followed by a second phase with increased edema formation which occurred in the rest five hour. There are differences of mediators in various phases in carrageenan-induced edema. The initial phase (up to the first 1.5 hours) is characterized by the release of histamine, 5-hydroxytryptamine, PAF (platelet activating factor) and serotonin. Kinin is released from 1.5 to 2.5 hours in the last phase, the inflammation will continue until the fifth hour by lipid-derived eicosanoid (prostaglandin, leukotriene 5-hydroperoxyeicosatetraenoic acid, and others) (Ray et al. 2015; Cheng et al. 2016).

In carrageenan-induced paw edema, the following events occur (i) Induction of COX-2 and mPGES-1 during the ongoing production of PGE2 on swollen paws. (ii) Carrageenan-induced paw edema causes COX-2 upregulation and general improvement of prostanoids in the central nervous system during the early phases of inflammation. (iii) Peripheral inflammation causes a significant increase in PGE2 and selective induction of mPGES-1 in the central nervous system. (iv) Prostacyclin levels increase in the central nervous system during the early phase of carrageenan-induced paw edema without significant PGIS upregulation (Zhang et al. 2013; Erasalo et al. 2015). The action mechanism of medicinal plant extracts as the anti-inflammatory agent is predicted due to the flavonoid content of those plant extracts. Therefore, the three medicinal plants used in our study, might contain high flavonoid contents especially in *C. buchananii* leaf extract, which show the strongest anti-inflammatory activities. Although, the further secondary metabolite analysis may need to be intensively observed to confirm this flavonoid concentration. An important mechanism of anti-inflammatory activity is the inhibition of enzymes that produce eicosanoid, including A2 phospholipase, cyclooxygenase, and lipoxygenase, leading to the decrease concentration of prostanoid and leukotrienes. Other mechanisms include inhibition of histamine release, phosphodiesterase, protein kinase and transcriptase activation (Rathee et al. 2009).

In summary, our findings are the first scientific report to show the potency of ethnomedicinal plants from Dayak Abai as anti-inflammatory medicine using the carrageenan-induced paw edema model. Our study finds that there were significant differences in anti-inflammatory activity of amongst treated medicinal plants (*C. buchananii*, *D. canniformis* and *A. xanthophlebium* extracts) compared to the negative control and positive control using carrageenan-induced paw edema method with $p < 0.001$. The extract of *C. buchananii* had the ability to inhibit the paw edema stronger than that of other two medicinal plants. Based on the findings of this study, we suggest that more research in this area especially on the secondary metabolite analysis should be further developed due to the high potency of this medicinal plants as a new anti-inflammatory agent based on natural materials.

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Indigenous *Bacillus* spp. ability to growth promoting activities and control bacterial wilt disease (*Ralstonia solanacearum*)

YULMIRA YANTI[1,♥], TRIMURTI HABAZAR[1], REFLINALDON[1], CHAINUR RAHMAN NASUTION[1], SRIMANO FELIA[2]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Andalas. Jl. Unand, Limau Manih, Padang 25163, West Sumatra, Indonesia. Tel. +62-751-72773, Fax.: +62-751-72702, ♥email: yy.anthie79@gmail.com

[2]Department of Agroecotechnology, Faculty of Agriculture, Universitas Andalas. Jl. Unand, Limau Manih, Padang 25163, West Sumatra, Indonesia

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Abstract. *Yanti Y, Habazar T, Reflinaldon, Nasution CR, Felia S. 2017. Indigenous* Bacillus *spp. ability to growth promoting activities and control bacterial wilt disease (*Ralstonia solanacearum*). Biodiversitas 18: 1562-1567.* Among the species of Plant Growth Promoting Rhizobacteria, *Bacillus* spp. has been found to provide beneficial effects to different plant species. Based on our previous research from in planta screening's method, we found six indigenous strains of *Bacillus* spp., which had the ability to control bacterial wilt and increased growth and yields of chili. Those were *Bacillus subtilis* BSn5,q *Bacillus* sp. RD-AZPVI-03, *Bacillus* sp. Y81-1, *B.cereus* strain C38/15, *B.cereus* strain APSB-03, and *B. subtilis* strain CIFT-MFB-4158A. This research aimed to characterize the mechanism of selected *Bacillus* spp. as biocontrol agents of *R. solanacearum* and as biofertilizer of chili *in vitro*. We have observed the biocontrol characters (production of HCN, siderophore, biosurfactant, protease, root colonization, and antibiotic), and biofertilizer's characters (production of Indole Acetic Acid (IAA), ammonia and phosphate solubilization) of *Bacillus* spp. All strains had ability to produce variable concentrations of IAA, *B.subtilis* strain CIFT-MFB-4158A can produce siderophore, all isolates cannot produce HCN and biosurfactant, 4 isolates can produce protease. Based on the ability to colonize roots, *B.subtilis* strain CIFT-MFB-4158A had the best ability to colonize root surface (4.17 x 10^5 CFU/g root), while *B. subtilis* BSn5 had the best ability to colonize internal roots (2.08 x 10^5 CFU/g), and 2 *Bacillus* strains can suppress *R. solanacearum in vitro*.

Keywords: *Bacillus*, in vitro, Indole Acetic Acid, siderophore, protease

Abbreviations: IAA = Indole Acetic Acid, IRB = Indigenous Rhizobacteria, ISR = Induce systemic resistance, NA = Nutrient agar, PGPR = Plant Growth Promoting Rhizobacteria, R = Ralstonia, B= *Bacillus*

INTRODUCTION

Ralstonia solanacearum, the causal agent of bacterial wilt disease, is one of the important serious vascular diseases of chili crop which cause losses between 15% to 55% around the world (Basu 2014; El-Argawy and Adss 2016). Control is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and its extremely wide host range. Controls using biological agents are more desirable because of the availability of variable results from another methods (Nguyen and Ranamukhaarachchi 2010). Since biological control is a key component of integrated disease management, it is important to search for plant growth promoting rhizobacteria (PGPR) active against specific pathogens and evaluates the antagonists for wider application (Nakkeeran et al. 2006).

Between the species of PGPR, *Bacillus* spp. is well-known as powerful genera. Several *Bacillus* species known to promote the health and control diseases by plant pathogens suppressions or by nutrients competitions like iron and phosphate or indirectly fixing nitrogen (McSpaden Gardener 2004). Many *Bacillus* has the ability to serve as biofertilizers and biopesticides but they are not recognized as pathogenic which makes them easier to handle. *Bacillus* spp. has ability to move freely, and a good competence on rhizosphere and facultative anaerobes, and those adds its survival ability in soil at various environmental conditions. These bacteria can sporulate in unfavorable conditions and make them more resistant to harsh conditions. This ability is very useful for commercial applications because it will provide long shelf life (Niazi et al. 2014). *Bacillus*-based biocontrol agents are quite important to control bacterial plant pathogens, such as *Pseudomonas syringae* on *Arabidopsis* (Bais et al. 2004), *Xanthomonas campestris* pv. *campestris* on Brassica (Wulff et al. 2002), *X. axonopodis* pv. *glycines* on soybean (Salerno and Sagardoy 2003), *Xanthomonas euvesicatoria* and *Xanthomonas perforans* on tomato (Roberts et al. 2008), *Ralstonia solanacearum* on mulberry (Ji et al. 2008) tobacco (Maketon et al. 2008) and chili (Yanti et al. 2017).

Our previous research, based from in planta technique we have found six selected isolates of *Bacillus* spp. to control *R. solanacearum* and increase growth rate of chili plants (Yanti et al. 2017). From previous 16S rRNA sequencing and identification using BLAST with genebank (http://ncbi.nlih.gov), all six isolates were identified that RZ.2.1.AP3 as *Bacillus subtilis* BSn5, RZ.2.1.AP1 as *Bacillus* sp. RD-AZPVI-03, RZ.2.2.AG2 as *Bacillus* sp. Y81-1, RZ.2.1.AG.1 as *Bacillus cereus* strain C38/15,

RZ.1.2.AP1 as *Bacillus cereus* strain APSB-03, and RZ.1.1.AP1 as *Bacillus subtilis* strain CIFT MFB 4158A (Yanti et al. 2017 unpublished). In this research, we want to characterize *in vitro* the selected *Bacillus* spp. as biocontrol agents of *R. solanacearum* (production of HCN, siderophore, biosurfactant, antibiotic) and as biofertilizer (production of Indole Acetic Acid (IAA), ammonia, protease, phosphate solubilization) and in planta (root colonization).

Table 1. List of bacterial strains used in this experiment

Bacterial strain	Species
RZ.2.1.AP3	*Bacillus subtilis* BSn5
RZ2.1.AP1	*Bacillus* sp. RD-AZPVI-03
RZ2.2.AG2	*Bacillus* sp. Y81-1
RZ2.1.AG1	*Bacillus cereus* strain C38/15
RZ1.2.AP1	*Bacillus cereus* strain APSB-03
RZ1.1.AP1	*Bacillus subtilis* strain CIFT MFB 4158A

MATERIALS AND METHODS

Study area

This research has been done as an experiment at Laboratory of Microbiology, Department of Plant Protection, and greenhouse, Faculty of Agriculture, University of Andalas, Padang, Indonesia from April to August 2016.

Bacterial strains and culture conditions

Pathogenic bacteria *R. solanacearum* and six-selected *Bacillus* spp. (Table 1) from our culture collections in 1.5 mL microtube contain sterilized aquadest stored in room temperature (27^{o}C) were used for this study (Yanti et al. 2017). All *Bacillus* isolates were streaked on Nutrient Agar (NA) (Hymedia® composition per litre, peptone 5g, NaCl, 5g, beef extract 1.5g, yeast extract 1.5g, agar 15 g, pH 7.4$\pm$ 0.2) culture medium and incubated at room temperature for 48 h. *R. solanacearum* isolate was streaked on Tetrazolium Triphenyl Tetrachloride (TZC) culture medium (composition per litre dextrose 10g, peptone 10g, casamino acid 1g, agar 18g, TZC 0.005%) and incubated with the same methods. These inoculants were prepared in order for them to be used *in vitro* tests of the *Bacillus* isolates and also their colonization on chili roots in planta.

***In vitro* characterization of selected *Bacillus* spp. mechanisms**

Biological agents

Hydrogen cyanide production. Cyanide (HCN) production was determined with cyanide detection agar containing TSA (Hymedia®) amended with 0.44% of glycine and detected using 1cm^2 of filter paper dipped in CDS solutions (2g picric acid, 8g of Na2CO3 in 200mL of aquadest) based on method of Lorck (1948). Color shifting from yellow to brownish orange on filter paper was sign of HCN productions.

Siderophore production. Siderophore production was determined with a method by putting spotted strains into the center of a Chrome Azurol S agar ((compositions are 60.5 mg of Chrome azurol S dissolved in 50mL of aquadest, and mixed with 10mL of FeCl3.6H2O solutions (1mM of FeCl3.6H2O+10mM of HCl) then added by 72.9mg of HDTMA dissolved in 40mL of aquadest, the solutions then was added to 900mL King's B agar (Hymedia®, per litre contains of protease peptone 20g, K2HPO4 1.5g, MgSO4 1.5g, agar 15g, glycerol 10mL pH 7.2)) and incubated at 28°C for 5 days. The medium color shifting from blue to orange was a sign of siderophore activity (Alexander and Zuberer 1991).

Hemolytic assay Hemolytic activity was determined using agar diffusion technique by Monteiro et al. (2005) namely by using Blood Agar (TSA (Hymedia®) enriched with 5% of sheep blood pH of 7.3) where halo zone (hemolysis) around the colony observed as hemolytic activity.

Biosurfactant production. Biosurfactant production was assayed using Nutrient Broth (NB) Medium (Hymedia®, which was per litre contains of peptone 5g, NaCl 5g, beef extract 1.5g, yeast extract 1.5g pH 7.2). *Bacillus* spp. was cultured in culture bottle containing 20 mL of NB and was incubated for 48h without shaking. Biofilm formations in the surface of the medium was sign of biosurfactant production.

Production of antibiotic against *R. solanacearum.* *R. solanacearum* suspension (10^8 CFU/ml adjusted with McFarland solution scale 8) was spread on NA plates and on three holes of 5 mm diameter punched into the agar. In these holes, 20 µl of the culture filtrate of each *Bacillus* was added and the plates were incubated at 28^{o}C for 48 h. Inhibition of *R. solanacearum* growth with halo clear zone is the indicator.

Biofertilizer

Indole Acetic Acid (IAA) production. *Bacillus* spp. was inoculated in Nutrient Broth (HiMedia®) containing 500mg L^{-1} L-tryptophan and incubated for for 48h on shaker (110rpm). After incubation, the culture was centrifuged and added by 1 ml of supernatant mixed with 4ml of salkowsky reagent (1ml of FeCl3 0.5M, 50ml of HClO4 35%), and incubated for 20 minutes in temperature room. IAA Production quantitatively was analyzed with methods used in Patten and Glick (2002) using spectrophotometer (Thermo Spectronic, Merck, SA), absorbance at 535 nm.

Ammonia production. Isolates assayed separately in 1% peptone was dissolved in sterilized water, *Bacillus* spp. was inoculated in 10 mL peptone water and incubated for 48-72 h at 28±2 ºC. 0.5 mL of Nessler's reagent (Sigma-Aldrich® - HgI4K2 0.09m/L) was added after incubations and the color change to brown yellow color which was a positive result (Cappuccino and Sherman 1992).

Phosphate solubilization. The isolates' ability to solubilize tri-calcium phosphate was assayed using methods of Wahyudi et al. (2011). The isolates was inoculated to Pikovskaya's Agar (Compositons per litre glucose 10g, Ca3 (PO4)2 5g, (NH4)2SO4 0.5g, KCl 0.2g,

MgSO4.7H2O 0.1g, MnSO4.H2O 0.01g, Yeast extract 0.5g, FeCl3.6H2O 0.01 g, Agar 15g, pH 7) separately and was incubated at room temperature for five days with formation of halo indicating positive results.

Protease activity. Protease activity was assayed on Luria Bertani Broth medium (compositions per litre contains Casein enzymic hydrolysate 10g, Yeast extract 5g, NaCl 10g, pH 7.5(HiMedia®)), and was amended with 2% of skim milk powder (Nestle) and 15g of agar based on methods of Bakker and Schippers (1987). Clearing zones appear around isolates expressed activity of protease.

Root colonization of rifampicin-resistant (Rif) mutants of selected *Bacillus*

Root colonization ability of *Bacillus* strains was assayed with firstly mutated strains accompanied with Rifampicin which was gradually streaked on TSA (Himedia®) (compositions Tryptone 15 g/L, soya peptone 5g/L, NaCl 5 g/L and agar 15g/L) with concentration of 0, 10, 20, 50 and 100 ppm rifampicin each for 24-36 h. Culture streaked on the last concentration (100ppm) was used for assaying Chili seedlings and then was dipped into mutated *Bacillus* spp. (10^6 CFU/mL) for 5 min and was planted in sterilized soil. Roots of chili then were harvested after 9 days. *Bacillus* spp. then was re-isolated from external root (rhizospheric) with dipping methods to 10 mL of sterilized aquadest and vortexed for 2 minutes, and from internal root (endophytic), first, the surface was sterilized sequentially for 1 minute with aquadest, for 1 minutes with NaOCl 1%, and for 3 times 1 minutes with aquadest, then was macerated and both (external and internal suspension) was diluted to 10^{-4}. 0.1 mL of each was homogenized with TSA (when medium was still in liquid state before pouring, and temperature was 45-50^{o}C) containing 100 ppm rifampicin and was plated to petri dishes for 48 h. Colonies growth with the same morphologies then counted.

RESULTS AND DISCUSSION

Biocontrol mechanism

In vitro production of HCN by the six-selected *Bacillus* spp. was carried out using the picric acid assay. None of these isolates produced HCN (Table 1). Siderophores provide a competitive advantage to organism producer over plant pathogens for the absorption of available iron. Only *B. subtilis* CIFT MFB 4158A produced siderophore on CAS agar medium. while the others were negative. The hemolytic activity showed by lipopeptides can be used for selecting lipopeptide-producing microorganisms. Therefore, hemolytic activity tests were performed to investigate the possible roleof these compounds in the antimicrobial activity of the *Bacillus* isolates. None of these isolates showed hemolytic activity. None of these isolates produced biosurfactant. In agar well diffusion assays for antibacterial activity, two *Bacillus* strains inhibited the growth of *R. solanacearum,* namely *B. cereus* C38/15 and *B. subtilis* CIFT-MFB-4158A.

Biofertilizer

Production of IAA by all the selected *Bacillus* isolates was detected by the production of pink color by all of them. All the *Bacillus* isolates produced indole acetic acid when grown in media containing tryptophan and it is obvious by the production of pink color by all isolates (Table 2). Using spectrophotometer (Thermo Spectronic, Merck, SA), absorbance at 535 nm revealed that *Bacillus subtilis* BSn5 had the highest IAA production (29.25 ppm) while *Bacillus subtilis* CIFT-MFB-4158A had the lowest IAA production (8.3 ppm). Bacterial isolates were tested for the production of ammonia in peptone water. A total five selected *Bacillus* isolates produced ammonia and only *Bacillus* sp. Y81-1 did not produce ammonia. Qualitatively phosphate solubilization was detected on Pikovskaya agar plate, indicated by halo around the inoculated spot. Only *Bacillus subtilis* CIFT-MFB-4158A showed positive results. All the others were negative without any clear zones. In the study of protease detection, it was found that four-selected *Bacillus* spp. secreted protease.

Root colonization

All selected *Bacillus* spp. have colonized the chili's rhizosphere until 21 days after introduction or after planting, but their population densities have fluctuated from 1.1×10^4 to 8.48×10^5 cfu g^{-1} (Figure 1). All *Bacillus* spp. had ability to colonize and have a good persistence in both rhizospheres and endophytic root.

Colonization of external and internal roots of chili by *Bacillus* spp. shown various amounts (Figure 1 and 2). All strains showed its ability to colonize both on external and internal (endophyte) of chili seedlings roots. All strains shown decreasing trends based on days after introduction. Although, some strains still showed an increase of populations from time to time, which shown its ability to maintain its populations, others showed inversely.

Discussion

Our previous study has demonstrated that six-selected *Bacillus* spp. controlled 100 % of *R. solanacearum* on chili with no Wilt symptom and improved the growth of *R. solanacearum*-inoculated chili plants (Yanti et al. 2017). These may have been happened by direct antagonism of pathogens, production of antibiotic, or competition with pathogens for nutrients (Gamliel and Katan 1993). The present study demonstrates that biocontrol characters of the selected *Bacillus* spp. were presented in Table 1. As shown in Table 1, only *B. subtilis* CIFT-MFB-4158A produced siderophore and antibiotic for growth inhibition of *R. solanacearum*. The other *Bacillus* spp. which also produced antibiotic was *Bacillus cereus* C38/15. All selected *Bacillus* spp. showed negative for the other characters, such as: HCN, siderophore, hemolytic activity and biosurfactant. This study showed that direct mechanism's characters of *Bacillus* spp. have not played an important role to control *R. solanacearum* on chili.

Table 1. Characters of selected *Bacillus* spp. as a biocontrol agent.

Bacterial strains	*Bacillus* species	Characteristics as biocontrol agent				
		HCN production	Siderophore production	Hemolytic activity	Biosurfactant production	Antibiotic production
RZ2.1.AP3	*Bacillus subtilis* BSn5	-	-	-	-	-
RZ2.1.AP1	*Bacillus* sp. RD-AZPVI-03	-	-	-	-	-
RZ2.2.AG2	*Bacillus* sp. Y81-1	-	-	-	-	-
RZ2.1.AG1	*Bacillus cereus* C38/15	-	-	-	-	+
RZ1.2.AP1	*Bacillus cereus* APSB-03	-	-	-	-	-
RZ1.1.AP1	*Bacillus subtilis* CIFT MFB 4158A	-	+	-	-	+

Table 2. Characters of selected *Bacillus* spp. as biofertilizer

Bacterial strains	*Bacillus* species	Characteristics as biofertilizer			
		IAA Production (ppm)	Ammonia production	Phosphate solubilization	Protease activity
RZ2.1.AP3	*Bacillus subtilis* BSn5	29.25	+	-	-
RZ2.1.AP1	*Bacillus* sp. RD-AZPVI-03	24.05	+	-	+
RZ2.2.AG2	*Bacillus* sp. Y81-1	23.05	-	-	+
RZ2.1.AG1	*Bacillus cereus* C38/15	15.75	+	-	+
RZ1.2.AP1	*Bacillus cereus* APSB-03	22.05	+	-	-
RZ1.1.AP1	*Bacillus subtilis* CIFT-MFB-4158A	8.30	+	+	+

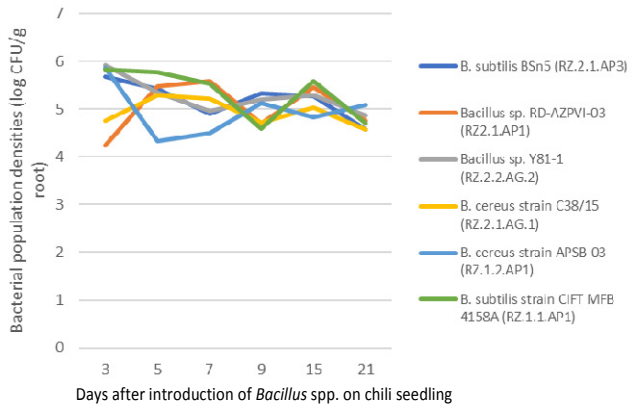


Figure 1. Population densities of selected *Bacillus* spp. in the rhizospheres of chili at 3 until 21 days after planting (DAP)

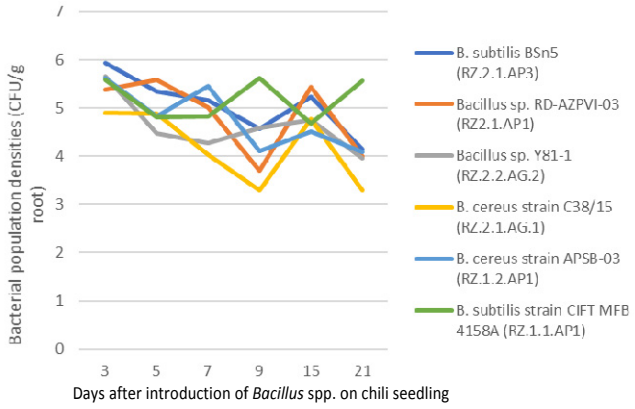


Figure 2. Population densities of *Bacillus* spp. as an endophyte in the chili root tissue from 3 until 21 days after planting (DAP)

The biofertilizer characters of the selected *Bacillus* spp. were as shown in Table 2. All selected *Bacillus* spp., could induced IAA productions. The IAA productions of selected *Bacillus* spp. were varied from 8.30 to 29.25 ppm. *Bacillus subtilis* BSn5 produced higher IAA than the other *Bacillus* spp.. IAA production by PGPR had been reported to be varied among different species and was influenced by culture condition, growth stage and substrate ability (Mirza et al. 2001; Mishra et al. 2010).

The IAA produced by *Bacillus* played a major role in roots elongation and could directly promote roots growth by cells elongation and cells division stimulation or could indirectly influence ACC-deaminase activity in rhizobacteria (Rathaur et al. 2012). Patten and Glick (2002) also reported that various IAA concentrations could induce different stimulations to plants. Low levels of IAA could stimulate roots elongation, while high levels of IAA would stimulate the lateral and adventitious roots formation. IAA was part of many secondary metabolites produced by PGPR in abundance during the stationary phase (Wahyudi, et al. 2011). This explained that the ability of *B. subtilis* CIFT MFB 4158A with low level of IAA production and its combination with phosphate solvent could also increase growth rate. *B. amyloliquefaciens* strain FZB42 was also found producing IAA and stimulating the growth of plant (Idris et al. 2007).

Bacillus subtilis strain produced a variety of powerful antibiotic metabolites (Peyopux et al. 1999) and

lipopeptides belonging to surfactin, iturin and fengycin families (Rahman et al. 2002). Hemolysis assay is the sole method widely used to screen biosurfactant production (Yonebayashi et al. 2000). In this study, all strains did not show any ability to produce biosurfactants. However, all strains showed a good ability to colonize roots, and it is showed that there is any other ability that helps the strains to colonize rhizosphere and endophyte of roots. Bacterial endophytes are well known for its beneficial effects for host plants (Puente et al. 2009). The ecological niches that are similar with phytopathogens, makes endophytic bacteria more suitable as biocontrol agents (Berg et al. 2005) and more stable to control pathogens. The growth reductions of *R. solanacearum* by *Bacillus* spp. and its in vitro inhibition zones are probably due to its ability to produce lytic enzymes like protease and a synthesis of antibiotics, which are most common character associated in PGPR (Mazurier et al. 2009).

Based on the properties of strains, only little direct activities showed relation to biocontrol activity, whereas from previous research (Yanti et al. 2017), all isolates have shown best biocontrol activity with no *R. solanacearum* symptoms appears. Based on these results, it can be concluded that most of all strains' ability in controlling *R. solanacearum* are not via direct mechanisms. According to Kloepper et al. (1999), ISR could be one of the most important mechanisms against systemic pathogens such as *R. solanacearum*. Other studies had reported that ISR can be triggered by inoculation of bacteria (Van Peer et al. 1990; Benhamou et al. 1996; Wilhelm et al. 1998). A further research is needed to be done to characterize all the bacterium activity in inducing systemic resistance of chili plants.

Our research concluded that these *Bacillus* strains had good traits characters to both promote plant growth and control pathogens. All strains also had ability to colonize internal (endophyte) and rhizosphere of chili roots. These *Bacillus* strains have potential to be used as biofertilizer and biocontrol agent of bacterial wilt of chili and further research for the commercial production is needed.

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Species richness, conservation status, and potential uses of plants in Segara Anakan Area of Sempu Island, East Java, Indonesia

SITI NURFADILAH♥, LIA HAPSARI, ILHAM KURNIA ABYWIJAYA
Purwodadi Botanic Garden, Indonesian Institute of Sciences. Jl. Surabaya-Malang Km. 65 Purwodadi, Pasuruan 67163, East Java, Indonesia.
Tel.: +62-343-615033; Fax: +62-341-426046, ♥email: siti.nurfadilah@lipi.go.id; fadilahzr@gmail.com

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Abstract. *Nurfadilah S, Hapsari L, Abywijaya IK. 2017. Species richness, conservation status, and potential uses of plants in Segara Anakan Area of Sempu Island, East Java, Indonesia. Biodiversitas 18: 1568-1588.* Sempu Island is a small Island in Indonesia that has an important functional role as a nature reserve to protect unique landscapes and high plant species richness. Within the Island, one of the most frequently visited areas, Segara Anakan, needs careful attention in order to conserve its plant diversity. The objectives of the present study were to investigate the species richness, conservation status, and potential uses of plants in Segara Anakan, Sempu Island. The aim was to support management of the area and to reveal plants that have potential to be developed for useful products. A total of 77 plant species belonging to 43 families were recorded within the study area. These consisted of trees (55.84 %), shrubs (33.77%), herbs (9.09%) and epiphytes (1.3%). Two species were listed in the IUCN Red List as endangered (*Cycas circinalis* L. and *Myristica teijsmannii* Miq.). Plant species in Segara Anakan have many potential uses; namely for staple food, fruits, vegetables, flavouring, beverages, herbal medicines, timbers, ornamental plants, natural dyes, and other uses. The study has implications for biodiversity conservation and management of Sempu Island. Plant species on the IUCN Red List should receive priority in conservation efforts. Data concerning the potential uses of plants can serve as a basis for biodiversity conservation on Sempu Island.

Keywords: Biodiversity, conservation status, species richness, potential uses of plants, Sempu Island

INTRODUCTION

Understanding diversity, conservation status, and potential uses of plants is important for conservation planning (Kreft et al. 2004; Pearce and Moran 1994; IUCN 2013). Compilation of an inventory of plant diversity can be used to assess the richness of flora and the rarity of species within an area. It is considered as a basis for prioritisation in the conservation of biodiversity in an area, focussing efforts on the most species-rich ecosystems and on species threatened by environmental pressures (Gordon and Newton 2006; Torres-Miranda et al. 2011). Recording the conservation status of plants based on assessment by the IUCN, leads to identification in the IUCN Red List of those species that requre high priority in conservation efforts.

Plants play important roles fulfilling human needs, including staple foods, medicines, sources of timber, fruits, beverages, and so on. Many plant species have been recorded and documented to have several uses based on the empirical experience and local knowledge of indigenous people (Trimanto et al. 2016, Frei et al. 1998). The ethnobotany and local knowedge of indigenous peoples has been widely studied across the world in an endeavour to identify the value and uses of plants in context. Such ethnobotanical studies have been conducted in places like Brazil (de Oliveira et al. 2007; Peroni et al. 2008), Central America (Audet et al. 2013), South Africa (Dahlberg and Trygger 2009), West Africa (Etongo et al. 2016), Albanian Alps (Pieroni, 2008), Nepal (Uprety et al. 2011), South Korea (Ong et al. 2016), Indian Himalayas and the Slovenian Alps (Kala and Ratajc 2012), Solomon Islands (Furusawa et al. 2014), Patagonia, South America (Ladio and Lozada 2004), Australia (Edwards and Heinrich 2006), Cyprus (Ciftcioglu 2015), Bolivian Amazon (Thomas 2012), the Pacific Islands (da Silva et al. 2004), and in Italy (Motti et al. 2009).

Information on plant utilization derived from local knowledge and ethnobotanical data provides a basis to support biochemical, pharmacological, and pharmaceutical research for the development of plant products with prospective use in food, medicine, and in the industrial sector. For example, the industrial development of certain medicines has quite often been based on the knowledge and empirical experience of local and indigenous peoples that has stimulated laboratory identification of biologically active compounds suitable for formulation as new drugs (Jaric et al. 2014; Razak et al. 2014; Dahlberg and Trygger 2009; Balick and O' Brien 2004; Sabandar et al. 2016; Frei et al. 1998).

Indonesia is one of the world's biodiversity hotspots, comprising a wide diversity of plant life with multiple potential uses (Myers et al. 2000; Heyne 1987). Many ethnic groups in Indonesia, including the Javanese, Sundanese, Madurese, Dayakese and others, have various traditional uses for plants growing in their regions and have their own local names for each particular plant (Abdillah et al. 2014, Setyowati et al. 2005, Trimanto et al. 2016, Hapsari et al. 2017). Ethnobotanical research has been carried out to increase our understanding of the plant

species in terms of their uses and interaction with human life, and for further development of natural products. Botanical research on plant biodiversity needs to be coupled with research in the human sciences, in order to learn the traditional cultural knowledge of uses, conservation and preservation of plant life accomplished over countless generations by indigenous peoples in Indonesia.

One of many important conservation areas in Indonesia is Sempu Island Nature Reserve. It possesses a high richness and diversity of plants, with many potential uses that warrant exploration for bioprospecting purposes. Despite the fact that this very small island ecosystem is still relatively protected and is in quite good condition (Suhardjono 2012; Risna 2009), the study area is currently facing various ecological threats from uncontrolled tourism (Purnomo 2013) and invasion by alien species (Abywijaya et al. 2014). The aims of the present study were to investigate the species richness, conservation status, and potential uses of plants in Sempu Island, to support biodiversity conservation management, and to bioprospect for plant resources that can be developed into potential new products.

MATERIALS AND METHODS

Study area

Sempu Island is a very small island off the south coast of East Java province (Indonesia) with a total area of 877 ha. It is administratively located in Malang Regency, East Java. The coastline is mainly composed of limestone cliffs in the southern part of East Java. The present study focussed on the Segara Anakan area within Sempu Island (Figure 1). Its ecosystem is lowland tropical forest.

The topography of Sempu Island is mainly undulating, with rocky hills. The altitude is between 0 and 102 m asl. Based on Ministry of Forestry Regulation No. 837/Kpts/II/1980, the slope of the flat area in the island ranges between 0 and 8%, while the slopes of the hilly area can reach > 45%.

Wet season in Sempu Island occurs from October to April with an annual precipitation of 2,132 mm with 90.3 rainy days. The dry season occurs between July and September. The temperature in the island ranges from 22°C to 30 °C and relative humidity from 53% to 92% (between 08.00 am and 14.00 pm). The soil pH ranges from 6.0 to 7.0 (Risna 2009).

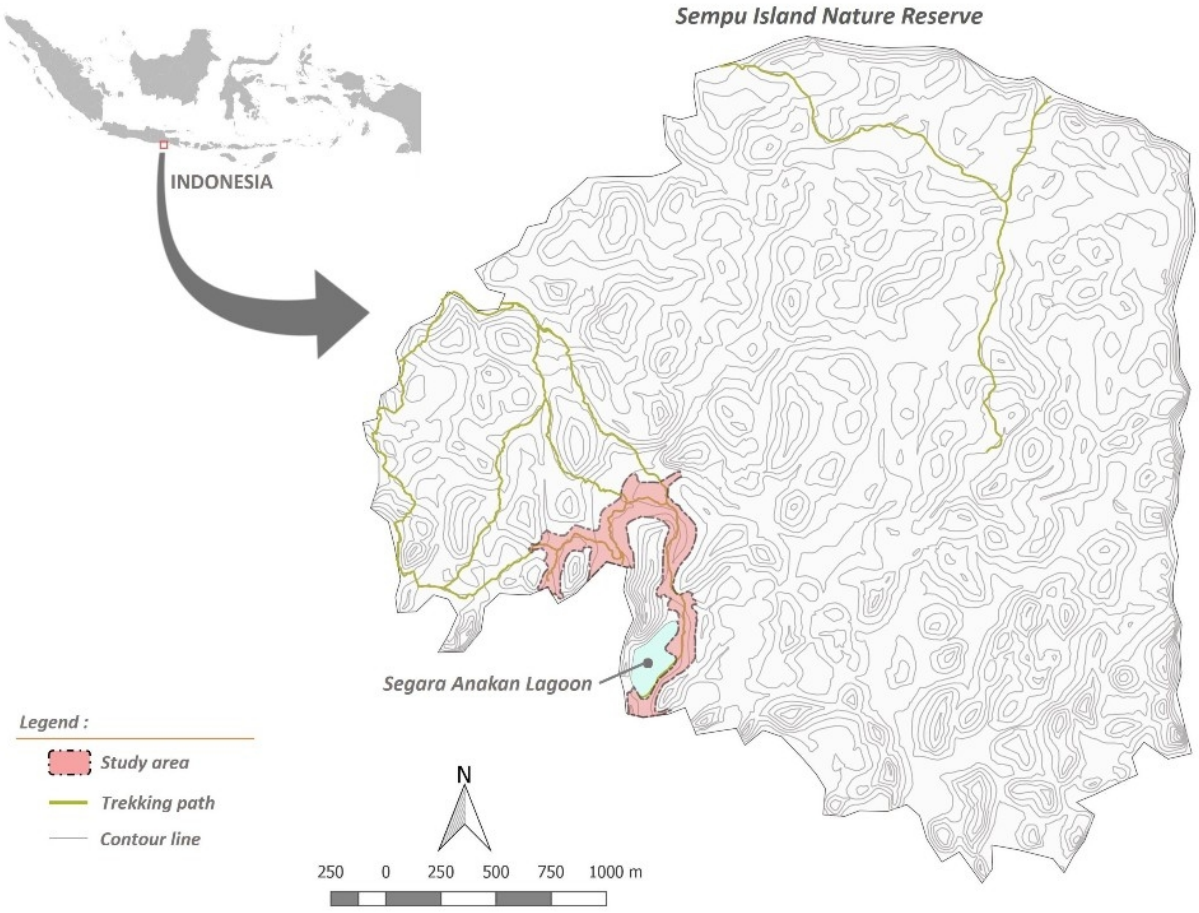


Figure 1. Study area in Segara Anakan area within Sempu Island, East Java, Indonesia

Procedures

To investigate the plant species richnesss in the Segara Anakan area, we explored along tracks. Within each 100 m of the exploration tracks we observed, identified and documented each plant in the area. The conservation status of the plants was evaluated based on the IUCN Red List (http://www.iucnredlist.org/). Potential uses and vernacular names for the plant species were verified by local informants and through scientific literature (Jaric et al. 2014).

Data analysis

The relative abundance (percentage) of trees, shrubs, herbs, and epiphytes on Sempu Island was calculated using a MS Excel spreadsheet.

RESULTS AND DISCUSSION

Plant species richness

There were 77 plant species recorded in the present study belonging to 68 genera and 43 plant families (Table 1). The plant classification system used in this study was that of the Angiosperm Phylogeny Group (Stevens 2001). The four plant families in Segara Anakan Area with the highest number of species (4-5) were Anacardiaceae, Euphorbiaceae, Leguminosae, and Rutaceae.

The 77 plant recorded species comprised trees, shrubs, herbs, and epiphytes. The highest percentage was trees (55.84%), followed by shrubs (33.77%), and herbs (9.09%), while the lowest percentage was epiphytes (1.3%) (Fig.2).

Conservation status of plants on Sempu Island

Of all 77 plant species recorded in Segara Anakan area, Sempu Island, two species (i.e. *Cycas circinalis* and *Myristica teijsmannii*) were listed on the IUCN Red List as endangered plant species (Table 2).

Potential uses of plants

Each plant species has several potential uses such as for staple food, fruits, vegetables, timber, and for other uses. The species with these potential uses are categorized and described below:

Staple food

There is one species that has potential use as a staple food *i.e. Cycas circinalis* (Table 3). The trunk and seeds of *Cycas circinalis* can be prepared as a food source. The inner part of the trunk (pith) of *C. circinalis* contains starchy substances that can be processed into a kind of sago. Various food and culinary items can be made from this sago flour. The seeds of *C. circinalis* can also be processed into flour. The seeds need to be leached by boiling in water more than three times to remove the poisonous substances contained in the seeds. After leaching, the seeds are dried and pounded, and then processed into flour. The flour is a source for making a variety of foods, such as noodles (Saneesh 2009). The potential uses of *C. circinalis* is important to support food security and food diversification. Research is required into suitable technology for processing *C. circinalis* into various culinary items. The uses of *C. circinalis* need to take into account conservation status, beacuse it is listed as an endangered species on the IUCN Red List. Species recovery is required for *C. circinalis* before the development of its potential uses.

Fruits

There are 12 plant species that have potential to be used for fruits, either fresh or processed (as jam, jelly, candy, juices, pickles, chutneys, and dried slices) (Table 4). *Antidesma bunius, Antidesma montanum*, *Ardisia crispa, Hernandia nymphaeifolia, Mangifera indica, Phaleria capitata, Sandoricum koetjape, Streblus asper, Syzygium polyanthum, Vitex glabrata,* and *Spondias pinnata* can be eaten as fresh fruits. Furthermore, some of these fruits can be processed into jams (such as *A. bunius, S. koetjape, S. pinnata*), jelly (*A. bunius, S. koetjape*), chutneys (*M. indica* and *S. pinnata*), candy (*S. koetjape*), pickles (*Fagraea ceilanica, M. indica, S. pinnata*), and dried slices (*M. indica*).

These edible fruits have high potential to be developed as popular local fruits. Research on the development and domestication of these edible fruit plants is required. This will promote the conservation and cultivation of of local fruits to counter competition from imported fruits from other countries. We have a lot of plant resources that can be used to meet the requirement for fruit in Indonesia. We have to encourage the development and popularisation of local fruits. The conservation of plants species with potential for such local fruit production is an important priority in Indonesia.

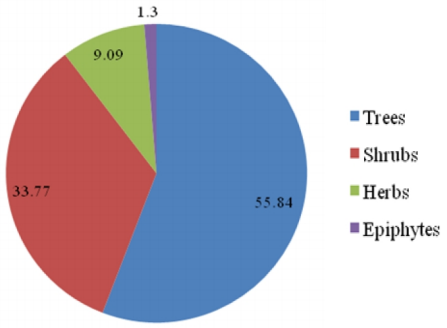


Figure 2. Relative abundance (percentage) of tree, shrub, herb, and epiphyte species in Segara Anakan area, Sempu Island, East Java, Indonesia

Table 2. Conservation status of plants in Segara Anakan, Sempu Island (IUCN Red List)

Species	Family	Habit	Conservation status
Cycas circinalis L.	Cycadaceae	Tree	Endangered
Myristica teijsmannii Miq.	Myristicaceae	Tree	Endangered

Table 1. Plant species recorded in Segara Anakan area, Sempu Island, East Java, Indonesia

Family	Number of species	Plant species
Anacardiaceae	5	*Buchanania arborescens* (Blume) Blume; *Dracontomelon dao* (Blanco) Merr. &Rolfe; *Mangifera indica* L.; *Semecarpus heterophylla* Blume**; *Spondias pinnata* (L. f.) Kurz
Annonnaceae	2	*Mitrephora polypyrena* (Bl.) Miq.*; *Polyalthia lateriflora* (Blume) Kurz
Apocynaceae	1	*Rauvolfia sumatrana* Jack
Araliaceae	1	*Schefflera elliptica* (Blume) Harms
Aspleniaceae	1	*Asplenium nidus* L.
Burseraceae	2	*Canarium hirsutum* Willd.; *Canarium indicum* L.
Calophyllaceae	1	*Mammea odorata* Kosterm.
Celastraceae	2	*Euonymus indicus* B.Heyne ex Wall.; *Lophopetalum javanicum* (Zoll.) Turcz.**
Clusiaceae	1	*Garcinia celebica* L.
Combretaceae	2	*Terminalia bellirica* (Gaertn.) Roxb.; *Terminalia catappa* L.
Compositae	3	*Ageratina riparia* (Regel) R.M.King & H.Rob.; *Cyanthillium cinereum* (L.) H.Rob.; *Synedrella nodiflora* (L.) Gaertn.
Convolvulaceae	1	*Ipomoea pes-caprae* (L.) R. Br.
Cycadaceae	1	*Cycas circinalis* L.
Cyperaceae	1	*Cyperus elatus* L.
Dioscoreaceae	1	*Dioscorea hispida* Dennst.
Ebenaceae	1	*Diospyros maritima* Blume
Euphorbiaceae	4	*Baccaurea dulcis* (Jack) Müll.Arg.; *Baccaurea javanica* (Blume) Müll.Arg.; *Croton tiglium* L.; *Macaranga tanarius* (L.) Müll.Arg.
Gentianaceae	1	*Fagraea ceilanica* Thunb.
Goodeniaceae	1	*Scaevola taccada* (Gaertn.) Roxb.
Hernandiaceae	1	*Hernandia nymphaeifolia* (J.Presl) Kubitzki
Lamiaceae	1	*Anisomeles indica* (L.) Kuntze
Lamiaceae	2	*Premna serratifolia* L.; *Vitex glabrata* R. Br.
Lauraceae	2	*Cinnamomum iners* Reinw. ex Blume; *Dehaasia caesia* Blume
Lecythidaceae	2	*Barringtonia racemosa* (L.) Spreng.; *Chydenanthus excelsus* (Blume) Miers
Leguminosae	4	*Cynometra ramiflora* L.; *Dendrolobium umbellatum* (L.) Benth.; *Derris elliptica* (Wall.) Benth.; *Pongamia pinnata* (L.) Pierre.
Magnoliaceae	1	*Magnolia liliifera* (L.) Baill
Malvaceae	2	*Hibiscus tilliaceus* L.; *Pterospermum diversifolium* Blume*
Meliaceae	2	*Aglaia lawii* (Wight) C.J.Saldanha; *Sandoricum koetjape* (Burm.f.) Merr
Moraceae	3	*Artocarpus elasticus* Reinw. ex Blume; *Ficus microcarpa* L.f; *Streblus asper* Lour.
Myristicaceae	1	*Myristica teijsmannii* Miq.****
Myrtaceae	3	*Syzygium polyanthum* (Wight) Walp.; *Syzygium racemosum* (Blume) DC.; *Syzygium syzygoides* (Miq.) Merr. & L.M.Perry
Pandanaceae	2	*Pandanus furcatus* Roxb.; *Pandanus tectorius* Parkinson ex Du Roi
Phyllanthaceae	3	*Antidesma bunius* (L.) Spreng.; *Antidesma montanum* Blume; *Glochidion obscurum* (Roxb. ex Willd.) Blume
Primulaceae	3	*Ardisia crispa* (Thunb.) A.DC .; *Ardisia fuliginosa* Blume; *Ardisia humilis* Vahl
Putranjivaceae	1	*Drypetes longifolia* (Blume) Pax & K.Hoffm
Rhizophoraceae	1	*Carallia brachiata* (Lour.) Merr.
Rubiaceae	2	*Guettarda speciosa* L.; *Ixora nigricans* R.Br. ex Wight & Arn.
Rutaceae	4	*Acronychia pedunculata* (L.) Miq.; *Glycosmis pentaphylla* (Retz.) DC.; *Harrisonia perforata* (Blanco) Merr.; *Zanthoxylum rhetsa* (Roxb.) DC.** and ***
Smilacaceae	1	*Smilax zeylanica* L.
Stemonaceae	1	*Stemona tuberosa* Lour.
Sterculiaceae	1	*Sterculia cordata* Blume*
Thymelaceae	1	*Phaleria capitata* Jack
Vitaceae	1	*Cissus javana* DC.

Note: * Backer CA and van den Brink jr RCB (1963); ** Backer CA and van den Brink jr RCB (1965); *** Backer CA and van den Brink jr RCB (1968); **** de Wilde (2000)

Vegetables

There are 12 plant species that can be used for vegetables (especially the young leaves of some plants). The leaves can be eaten as fresh vegetables or cooked vegetables. Other parts of the plants can in some cases be used for vegetables such as the young shoots of *Cyanthillium cinereum*, flowers of *Dracontomelon dao*, and the fruit of *Spondias pinnata* (Table 5).

These plant species from the present study have potential to be developed and popularised as alternative vegetables and to support diversification of vegetables sources. These would make an addition to common

vegetables daily consumed, such as spinach, water spinach (kangkung), long beans, green beans, cabbage, carrot, and lettuce.

Flavouring

There are five plant species that have potential use for flavouring foods (fish or meat stew) and confectionary (Table 6). The leaves of *A. pedunculata, D. dao, S. pinnata,* and *S. polyanthum* can be used as spices for flavouring various type of food. The leaves of *Antidesma bunius* can be used to flavour fish or meat stew; both immature fruits and young leaves can substitute for vinegar, and the fruits in sour fish sauce. In addition to flavouring foods, there is one species that also has been recorded as providing a flavouring in sweets, candies and confectionary, i.e. the oil from *Cinnamomum iners.* Cinnamon can be added as a flavouring in the making of some cakes and fudges.

Beverages

There are two plant species that have potential use in beverages *i.e. Antidesma bunius* and *Mangifera indica* (Table 7). A refreshing drink can be made from fully ripened fruits of *A. bunius.* Juices and syrup can be made from fruits of *M. indica.*

Herbal medicines

A total of 49 plant species were recorded that have potential uses for herbal medicines to treat a wide range of ailments and diseases; such as malaria, headache, stomach ache, dysentery, chickenpox, fever, and itch. (Table 8). *Fagraea ceilanica* (roots, bark, twigs, leaves, and flowers), *Rauvolvia sumatrana* (bark), *Scaevola taccada* (leaves), and *Antidesma montanum* (roots) have potential uses for treating malaria. Liver complaints can be cured using *Glycosmis pentaphylla* and *Ficus microcarpa*.

Plants that have potential use in treating stomach ache are *Canarium hirsutum* (roots), *Cyanthilium cinereum* (roots), *Cissus javana* (leaves), *Glochidion obscurum* (roots), *Glycosmis pentaphylla* (leaves), *Ipomoea pes-caprae* (seeds), *Premna serratifolia* (leaves and roots), and *Sandoricum koetjape* (roots). Some plants can also be used to treat diarrhoea, such as *Barringtonia racemosa* (peeled seeds), *Cyanthilium cinereum* (root decoction), *Glycosmis pentaphylla, Harrisonia perforata* (young shoots, root bark, dried roots, and stems), *Hibiscus tiliaceus* (leaves), *Pandanus furcatus* (leaf sap), *Sandoricum koetjape* (roots), *Streblus asper* (stem bark), *Synedrella nodiflora* (roots and leaves), *Syzygium polyanthum* (bark) and *Terminalia bellirica* (fruits).

To treat coughs, some plants can be used such as *Barringtonia racemosa* (fruits and scraped nuts), *Glycosmis pentaphylla*, *Hibiscus tiliaceus* (leaves), *Pandanus furcatus* (young top leaves), *Premna serratifolia* (leaves), *Scaevola taccada* (young leaves), *Stemona tuberosa* (tuberous roots), and *Synedrella nodiflora* (roots). Some plants can also be used for treating wounds, such as *Cyanthilium cinereum* (ground or poultice of the leaves), *Ficus microcarpa* (roots, bark, and leaf latex), *Guettarda speciosa* (bark), *Harrisonia perforata* (dried roots), *Ipomoea pes-caprae* (crushed leaves), *Pterospermum diversifolium* (leaves and bark), *Scaevola taccada* (stem sap), and *Streblus asper* (bark).

Itch can be treated by plants such as *Acronichya pedunculata* (bark extract), *Barringtonia racemosa*, *Carallia brachiata* (bark), *Derris elliptica*, *Harrisonia perforata* (crushed leaves), *Pterospermum diversifolium* (leaves and bark) and *Syzygium polyanthum* (bark, roots, and leaves). Plant species that can be used to treat rheumatism include *Acronichya pedunculata* (root extracts), *Dioscorea hispida*, *Glycosmis pentaphylla*, *Ipomoea pes-caprae*, *Pongamia pinnata* (extracts of the leaves, bark, and seeds), *Premna serratifolia*, *Smilax zeylanica* (roots), *Synedrella nodiflora* (leaves), and *Terminalia catappa* (leaves).

Tuberculosis can be treated by *Hibiscus tiliaceus* (leaves) and *Scaevola taccada* (young leaves; chewed or extracts from the leaves). Asthma can be treated by *Barringtonia racemosa* (fruits), *Cyanthilium cinereum* (leaves) and *Scaevola taccada* (leaves extracts). Some species can also be used to treat fever, such as *Asplenium nidus* (decoction or infusion), *Ficus microcarpa* (young leaves), *Glycosmis pentaphylla* (juice of the leaves), *Premna serratifolia* (extracts of leaves), and *Sandoricum koetjape* (fresh leaves).

Timber

There are 42 plant species that have potential use for timber as important sources of material for housings, buildings, furniture, cooking utensils, canoes, and handicrafts (Table 9). Some plant species that have potential use for housing/buildings are *Acronichya pedunculata, Baccaurea dulcis, B. javanica, Cinnamomum iners, Cynometra ramiflora, Dehaasia caesia, Drypetes longifolia, Euonymus indicus, Fagraea ceilanica, Glochidion obscurum, Guettarda speciosa, Macaranga tanarius, Mammea odorata, Polyalthia lateriflora, Terminalia bellirica, T. catappa,* and *Vitex glabrata.*

Some species have potential use for furniture, such as *Acronichya pedunculata, Baccaurea dulcis, B. javanica, Buchanania arborescens, Drypetes longifolia, Euonymus indicus, Fagraea ceilanica, Glochidion obscurum, Guettarda speciosa, Hernandia nymphaeifolia, Magnolia liliifera, Polyalthia lateriflora, Pterospermum diversifolium, Terminalia bellirica, T. catappa*, and *Vitex glabrata.* Timbers of some other plant species also have potential uses for making canoes and boats, such as timbers of *Baccaurea dulcis, B. javanica, Dehaasia caesia, Fagraea ceilanica, Hernandia nymphaeifolia, Macaranga tanarius, Magnolia liliifera, Mammea odorata, Spondias pinnata,* and *Terminalia catappa.*

Ornamental plants

Fifteen species can be used as ornamental plants because of their beautiful flowers, leaves, and/or plant shapes (Table 10). Some of the plants have attractive flowers e.g. *Fagraea ceilanica* and *Pongamia pinnata*. *Cissus javana* has beautiful leaves. Some of the plants have unique plant shapes of ornamental value e.g. *Cycas circinalis* and *Pandanus tectorius*.

Table 3. Plant species with potential use of plants from Segara Anakan area, Sempu Island, East Java, Indonesia

Latin name	Vernacular names (Indonesian) *	Family	Uses	References
Staple food				
Cycas circinalis L.	Pakis Haji, Pakis Raja[1]	Cycadaceae	A kind of sago from the trunk and flour from the seeds	Anon. (1992)
Edible fruit				
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	Fresh fruits, or made into jam or jelly	Lemmens (2003)
Antidesma montanum Blume	Ande-ande[1], Kenyan Pasir, Wunen[3],	Phyllanthaceae	Fresh fruits	Lemmens (2003)
Ardisia crispa (Thunb.) A.DC.	Mata Ayam[4]	Myrsinaceae	Fresh fruits	Heyne (1987)
Fagraea ceilanica Thunb.	Tamosu[8], Kolaki, Nosu[10]	Gentianaceae	Fruits are sometimes pickled and eaten	Hildebrand et al. (1995)
Hernandia nymphaeifolia (J.Presl) Kubitzki	Kampak, Kampis[2].	Hernandiaceae	Fresh fruits	Irwanto (1998)
Mangifera indica L.	Mangga, Mempelam[1]	Anacardiaceae	Fresh fruits when ripe, or processsed fruits (in pickles, chutneys, dried slices) when unripe	Boer et al. (1995)
Phaleria capitata Jack	Kakapasan[2], Lawe[3]	Thymelaceae	Fresh fruits (sweet fruit), the seed cotyledon contains a biting poison	Anon. (2003)
Sandoricum koetjape (Burm.f.) Merr	Kecapi, Kentuat, Sentul[1]	Meliaceae	Fresh fruits, or processed into candy, chutney, jam, jelly, marmalade or preserve, or used in flavouring native dishes	Sotto (1992)
Streblus asper Lour.	Serut[2], Peleh[12]	Moraceae	Fresh fruits	Kalima (2002); Ba (1998)
Syzygium polyanthum (Wight) Walp.	Salam, Manting[3], Ubar Serai[8]	Myrtaceae	Fresh fruits	Haron et al. (1995)
Vitex glabrata R. Br.	Ketileng, Bigbul, Gentileng[3]	Lamiaceae	Fresh fruits	Sunarno et al. (1995)
Spondias pinnata (L. f.) Kurz	Kedongdong[1], Kedongdong Leuweung[2], Kacemcem[9]	Anacardiaceae	Fresh fruit when ripe, or processed in chutneys, stews, pickles, and jams	Anon. (1999)
Vegetables				
Acronychia pedunculata (L.) Miq.	Jejerukan[2], Kayu Semidra, Sarirah[3],	Rutaceae	Young vegetables as condiments	Tue and Sosef (1998)
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	Young leaves for salads and cooked with rice	Lemmens (2003)
Asplenium nidus L.	Paku Sarang Burung[1]	Aspleniaceae	Young curled leaves as a vegetable	Rusea (2003)
Barringtonia racemosa (L.) Spreng.	Penggung[2, 3], Putat Sungai[4], Butun Darat[5].	Lecythidaceae	The fresh or cooked leaves as a vegetable	Yaplito (2002)
Cyanthillium cinereum (L.)	Sasawi Langit[2], Maryuna[3]	Compositae	The young shoots as a cooked vegetable	Utomo and van Valkenburg (1999)
Cycas circinalis L.	Pakis Haji, Pakis Raja[1]	Cycadaceae	The youngest leaves as a vegetable	Anon. (1992)

Cissus javana	Beubeureumen[2], Mirah[3], Rabet Dara[12]	Vitaceae	The sour leaves and young shoots mixed with other vegetables.	Anon. (1994)
Dracontomelon dao (Blanco) Merr. & Rolfe	Dahu[1], Sengkuang[6], Basuong[13]	Anacardiaceae	Cooked flowers and leaves as a vegetable	Louman et al. (1995)
Mangifera indica L.	Mangga, Mempelam[1]	Anacardiaceae	Young leaves as a vegetable	Boer et al. (1995)
Premna serratifolia L	Ki Pahan[2], Singkil Alas[3],	Lamiaceae	Cooked leaves are as a vegetable	Cardenas (1999)
Synedrella nodiflora (L.) Gaertn.	Jotang Kuda[2], Bruwan[3], Gofu Makeang[20]	Compositae	Tender leaves in salads	Hidayat (2002)
Spondias pinnata (L. f.) Kurz	Kedongdong[1], Kedongdong Leuweung[2], Kacemcem[9]	Anacardiaceae	Unripe fruit as a vegetable	Anon. (1999)
Flavouring				
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	Fruit in sour fish sauce Young leaves to flavour fish or meat stew, and both immature fruit and young leaves serve as substitutes for vinegar	Lemmens (2003)
Cinnamomum iners Reinw. ex Blume	Ki Kacengal[12], Medang Kalong[21]	Lauracaeae	The oil from the leaves for flavouring sweets and confectionary	Wiselius et al. (1995)
Dracontomelon dao (Blanco) Merr. & Rolfe	Dahu[1], Sengkuang[6]	Anacardiaceae	Flowers and leaves for food flavouring	Louman et al. (1995)
Spondias pinnata (L. f.) Kurz	Kedongdong[1], Kedongdong Leuweung[2], Kacemcem[9]	Anacardiaceae	The leaves for flavouring	Anon. (1999)
Syzygium polyanthum (Wight) Walp.	Salam, Manting[3], Ubar Serai[8]	Myrtaceae	The aromatic leaves for spice in food	Haron et al. (1995)
Beverages				
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	Juice of fully ripened fruit as a refreshing drink	Lemmens (2003)
Mangifera indica L.	Mangga, Mempelam[1]	Anacardiaceae	Fruits are processed into syrup, juice	Boer et al. (1995)
Herbal medicines				
Acronychia pedunculata (L.) Miq.	Jejerukan[2], Kayu Semidra, Sarirah[3],	Rutaceae	Root extract applied to the skin for rheumatism Bark extract for itch. The leaves for aroma therapy	Tue and Sosef (1998)
Aglaia lawii (Wight) C.J. Saldanha	Langsat Lutung[3], Lasih[8], Kayu Jangan[10], Aisnepapir[13, 16]	Meliaceae	The leaves for headache	Ba et al. (1995)
Anisomeles indica (L.) Kuntze	Ki Hileud, Patuk Bangkong[2], Celangking[3]	Lamiaceae	The whole plants, especially leaves and roots are used as a powerful astringent, carminative (to reduce flatulence), febrifuge (to reduce fever), and tonic (to promote general health)	Ng and Ling (2002)
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	The leaves as a stimulant and to treat syphilis	Lemmens (2003)
Antidesma montanum Blume	Ande-ande[1], Kenyan Pasir, Wunen[3]	Phyllanthaceae	Roots for measles, chickenpox, and malaria The leaves externally apllied for headache and thrush in children Roots and stems as a diuretic (to promote diuresis)	Lemmens (2003)

Ardisia fuliginosa Blume	Getah Adjag[1]	Myrsinaceae	Dried sap from the stem for scabies	Lemmens (2002 a) Quattrocchi (2012)
Ardisia humilis Vahl	Lampeni[2], Lempeni[3], Jambulan Pante[4]	Myrsinaceae	The leaves for scabies The fruits as anthelmintic (to expel parasitic worms) and cardiotonic (having a tonic effect on the heart)	Lemmens (2002 b)
Asplenium nidus L.	Paku Sarang Burung[1]	Aspleniaceae	Decoction or infusion of the plant to ease labour pain and against fever	Rusea (2003)
Barringtonia racemosa (L.) Spreng.	Penggung[2, 3], Putat Sungai[4], Butun Darat[5]	Lecythidaceae	Leaves, roots or bark are externally applied to chickenpox A poultice for itch The roots as a febrifuge; an infusion for measles A decoction of the scraped nut for cough, sinusitis, or bronchitis The fruit for coughs and asthma and the peeled seed mixed with flour and oil for diarrhoea The seed is internally applied for colic and externally for ophthalmia The fruit for poulticing sore throat and skin eruptions The leaves for high blood pressure and as purgative (to stimulate evacuation of the bowels)	Yaplito (2002)
Buchanania arborescens (Blume) Blume	Getasan[3], Rawa-rawa Pipit[6]	Anacardiaceae	Pounded leaves as a poultice for headache	Sulistiarini (1998)
Canarium hirsutum Willd.	Ki Bonteng[2], Mede-mede[5], Kanari Jaki[10],	Burseraceae	A decoction of the roots for stomach ache	Kochummen et al. (1995)
Carallia brachiata (Lour.) Merr.	Kitamiyang[2], Sepat[3], Ringgit Dareh[8]	Rhizophoraceae	The bark for itch	Hou (1998 b) Wardani (2003)
Cyanthillium cinereum (L.)	Sasawi Langit[2], Maryuna[3]	Compositae	Ground or poultice of leaves for headache and wounds The ground or decoction of leaves for skin diseases Root decoction for diarrhoea and stomach ache An infusion of the plant for coughs The leaves for asthma and bronchitis	Utomo and van Valkenburg (1999)
Cycas circinalis L.	Pakis Haji, Pakis Raja[1]	Cycadaceae	A poultice of the seeds and bark for sores and skin complaints	Anon. (1992)
Cissus javana	Beubeureumen[2], Mirah[3], Rabet Dara[12]	Vitaceae	The leaves for stomach ache	Anon. (1994)
Dendrolobium umbellatum (L.) Benth	kanyere laut[2] ,blanakan[3], gowou[20].	Leguminosae	A decoction of the leaves as a general tonic. The young leaves are an ingredient of medicine for women after child delivery	Aguilar (2002)
Derris elliptica (Wall.) Benth.	Tuwa Leteng[2], Tuba, Oyod Tungkul[3],		For antisepsis and applied to abscesses and against leprosy and itch. The roots as an emmenagogue (to increase menstrual flow) and the stems as a blood tonic	Hamid (1999)
Dioscorea hispida Dennst.	Gadung[1], Sikapa[9,10], Ondo[11]	Dioscoreaceae	The pounded tubers, used externally as an antiseptic, and a decoction for chronic rheumatism	Onwueme (1996)
Dracontomelon dao (Blanco) Merr. & Rolfe	Dahu[1], Sengkuang[6], Basuong[13]	Anacardiaceae	The bark for dysentery	Louman et al. (1995)
Fagraea ceilanica Thunb.	Tamosu[8], Kolaki, Nosu[10]	Gentianaceae	Decoctions of roots, bark, twigs, leaves, and flower for malaria and dysentery	Hildebrand et al. (1995)
Ficus microcarpa L. F	Preh[1]	Moraceae	The root, bark, and leaf latex for wounds, headache and toothache The bark and leaf latex for colic and liver trouble Perspire in the steam of boiling young leaves for fever and headache	Rojo et al. (1999)

Glochidion obscurum (Roxb. ex Willd.) Bl.	Ki Pare Lalaki[2], Uris-urisan[3]	Phyllanthaceae	The roots for stomach ache	Irwanto (1998)
Glycosmis pentaphylla (Retz.) DC.	Gongseng[2], Jerukan, Totoan[3]	Rutaceae	A decoction of the roots for bilious attacks A decoction of roots and leaves for intestinal trouble The leaves to stimulate appetite, and for stomach ache. An infusion of roasted leaves for women after delivery as an appetite stimulant. To treat diarrhoea, coughs, rheumatism, anaemia, and jaundice. Juice of the leaves for fever, liver complaints and as a vermifuge (anthelminthic) A paste of the leaves mixed with ginger is applied for eczema and skin affections	Chua and van Valkenburg (2002)
Guettarda speciosa L.	Jati Pasir, Titi Laut[11], Kenjang-kenjang[12]	Rubiaceae	The bark for chronic dysentery, wounds and abscesses	Ong (1998)
Harrisonia perforata (Blanco) Merr.	Garut[2], Ri Kengkeng[3], Sesepang[14]	Rutaceae	Young shoots for diarrhoea A decoction of the root bark for diarrhoea, dysentery, cholera Ashes of the roasted leaves mixed with oil or crushed leaves are applied to relieve itch The dried root as antipyretic and anti-inflammatory, for wound and diarrhoea The stems for diarrhoea	Kiew (2002)
Hernandia nymphaeifolia (J.Presl) Kubitzki	Kampak, Kampis[2].	Hernandiaceae	The blackish corewood at the base of the trunk for haemorrhaging The leaves and the fruit are purgatives An extract of the leaves for painless depilation.	Irwanto (1998)
Hibiscus tilliaceus L.	Waru, Waru Laut, Baru[1]	Malvaceae	The fresh macerated bark makes water mucilaginous, and for dysentery The leaves as a laxative, and the pulverized roots as a vomitive A decoction of the leaves is emetic (to cause vomiting) A decoction of the leaves for sore throat, pneumonia, coughs, tuberculosis and diarrhoea The leaves and roots are crushed in water and drunk to ease labour pain	Dasuki (2003)
Ipomoea pes-caprae (L.) R. Br.	Daun Katang[5], Katang-katang[9], Batata Pantai[15]	Convolvulaceae	The plant as astringent, tonic, alterative (to restore normal health), diuretic, and laxative, useful in skin infections. A decoction of the root as emollient and diminishes the irritation caused by bladder infections A paste of the leaves for ulcers and boils The seed is chewed and swallowed as a remedy for cramp and stomach ache A decoction is anodynal (to soothe pain) in rheumatism Leaf sap for jellyfish stings The seeds for stomach ache The crushed leaves for boils, ulcers, piles, haemorrhoids, swellings and wounds The leaves as astringent, alterative, tonic, and diuretic, and as an external application for rheumatism, dropsy, and colic The seeds for stomach ache and cramp The juice from the stem for sting of jellyfish and toadfish	Dibiyantoro and Schmelzer (2002)

Ixora nigricans R.Br. ex Wight & Arn.	Soka[1]	Rubiaceae	The leaves as anti-dysenteric	Ysrael and Van Valkenbonurg (1999)
Mangifera indica L.	Mangga, Mempelam[1]	Anacardiaceae	Bark, kernels, and flowers as astringents Various parts of the tree have antibiotic properties	Boer et al. (1995)
Pandanus furcatus Roxb.	Harashas[2], Panan Kowang[3],	Pandanaceae	The young top leaves as an antidote, the leaf sap for diarrhoea and dysentery	Brink and Jansen (2003)
Pandanus tectorius Parkinson ex Du Roi	Pandan Pudak[1]	Pandanaceae	The bark is scraped into a wild ginger leaf, water is added and the solution to sedate mental patients	Brink and Jansen (2003)
Phaleria capitata Jack	Kakapasan[2], Lawe[3],	Thymelaceae	The seeds for scurf in children	Anon. (2003)
Pongamia pinnata (L.) Pierre	Ki Pahang Laut[2], Bangkong[3], Kranji[12] .	Leguminosae	Extracts from the leaves, bark, and seeds as antiseptic against skin diseases and rheumatism	van Valkenburg (2002)
Premna serratifolia L.	Ki Pahan[2], Singkil Alas[3],	Lamiaceae	The leaves and roots as a diuretic, stomachic and febrifuge The leaves as a galactogogue (to stimulate lactation), and for rheumatic arthritis, colic, and flatulence A decoction of roots and leaves as a febrifuge Extracts of the leaves for coughs, headache, and fever A tea from the boiled bark for neuralgia	Cardenas (1999)
Pterospermum diversifolium Blume	Bayur[1], Wadang[3].	Malvaceae	Leaves and bark as a poultice for itch and wounds, and internally for dysentery	Boer and Lemmens (1998)
Rauvolfia sumatrana Jack	Lame Lalaki[2], Tampa Badak[8], Polay Lakek[12],	Apocynaceae	The bark for dysentery. The dried bark as an anti-malarial	Ly and Mai (1999)
Sandoricum koetjape (Burm.f.) Merr	Kecapi, Kentuat, Sentul[1]	Meliaceae	The fresh leaf: when applied to the skin is sudorific (causing sweat): and in decoction, for fever The powdered bark: effective treatment for ringworm The roots: anti-diarrhetic, anti-spasmodic, carminative, stomachic, and as a general tonic after childbirth	Sotto (1992)
Scaevola taccada (Gaertn.) Roxb.	Babakoan[2], Dudulan[3], Subong-subong[17].	Goodeniaceae	Diluted sap from the leaves or sap from the ripe berries to clear opacity of the eye and in the treatment of eye infections The bitter leaves for indigestion, and as a poultice for headache The root as an antidote after eating poisonous fish or crab The roots in decoction for beri-beri and in certain syphilitic affections, also in dysentery Roots and leaves for skin affections. The young leaves for coughs, colds, tuberculosis, and malaria. The sap directly applied to sores. An extract from the leaves as a form of long-term contraception by women and for earache, asthma, and tuberculosis The juice squeezed from young stems and ripe fruits applied directly to bites and stings The leaves for skin ailments. Swellings, elephantiasis, scrotal swellings, oedema, chill, and indigestion; the roots for cancer therapy; the bark for abscesses, menstrual complaints and bone fractures; the stem for abdominal complaints Stem sap to cleanse a sore eye and on wounds, and when hot on topical ulcers	Wardini (2002)

Schefflera elliptica (Blume) Harms	Tangana[3], Panakomo [18], Putiana Ma Gitipi[19]	Araliaceae	The bark as a bechic (cough-supressant), the resin as a vulnerary (to heal wounds) A decoction of the leaves is an effective antiscorbustic (to prevent scurvy) and for aromatic baths The wood chewed to relieve toothache The roots mixed with rice are eaten to cure dropsy	Tap and Sosef (1999)
Smilax zeylanica L.	Kayu Cina Utan, Saihe Maruani, Asaihe Tuni[5]	Smilacaceae	The roots against rheumatism, urinary complaints and venereal diseases	Teo (1999)
Spondias pinnata (L. f.) Kurz	Kedongdong[1], Kedongdong Leuweung[2], Kacemcem[9]	Anacardiaceae	The fruit as an astringent, antiscorbustic (to prevent scurvy), and against bilious dyspepsia The juice is applied against earache	Anon. (1999)
Stemona tuberosa Lour.	Kanyalut[5], Ngabalo[19], Isoratu[20]	Stemonaceae	The tuberous roots as insecticides and therapeutical agents (especially for coughs). The ground fresh tuberous roots to repel lice from body and clothing The tuberous roots to treat scabies and kill head lice The tuberous roots used internally as an antitussive (cough-supressant) and anthelmintic, and externally as an insecticide.	Thin (2002)
Streblus asper Lour.	Serut[2], Peleh[12]	Moraceae	The leaves with a little salt are given after childbirth as depurative (detoxifier); in a mixture with other plants as a galactagogue (to stimulate lactation) The bark as a disinfectant for wounds, and as a depurative (detoxifier) in certain skin diseases A decoction of stem bark is anti-diarrhoeal, anti-dysenteric, and anti-pyretic (to reduce fever) The seed as tonic and carminative, as well as appetizer An infusion of the leaves as a purgative The smoke from the wood for nose polyps The root bark powdered or in decoction for toothache and periodontitis (gum disease) A decoction of the branchlets to relieve a swollen abdomen, and a decoction of the roots to treat diphteria	Kalima (2002) Ba (1998)
Synedrella nodiflora (L.) Gaertn.	Jotang Kuda[2], Bruwan[3], Gofu Makeang[20]	Compositae	The leaves as a poultice for sore legs and rheumatism, as an embrocation (liniment) for diffferent oedemas. An infusion of young leaves as a laxative The juice of the leaves to treat earache, mouth affections such as infected gums The root is chewed against diarrhoea, together with some other herbs Dislocated bones are massaged daily with sap from the squeezed leaves A decoction of the leaves for haemorrhoids and diarrhea A decoction of the pounded and cooked roots as a cough mixture The entire plant as an emmenagogue (to increase menstrual flow)	Hidayat (2002)
Sterculia cordata Blume	Hantap Heulang[2], Kayu Binong[3], Gelumpang Padang[4]	Sterculiaceae	The seeds are used in traditional medicine	Lemmens et al. (1995)

Syzygium polyanthum (Wight) Walp.	Salam, Manting[3], Ubar Serai[8]	Myrtaceae	An extract of the bark for diarrhea Bark, roots, and leaves for poulticing against itch	Haron et al. (1995)
Terminalia bellirica (Gaertner) Roxb	Jaha Kebo, Jaha Sapi[3], Ulu Belu[8].	Combretaceae	The unripe fruit is purgative and the ripe fruit astringent The fruit for dropsy, haemorrhoids and diarrhea	Sosef et al. (1995)
Terminalia catappa L.	Ketapang[1]	Combretaceae	The leaves cause sudorific (sweating) action and are applied to rheumatic joints The tannin from leaves and bark is used as an astringent in dysentery and thrush, also as a diuretic and cardiotonic agent, and applied externally on skin eruptions A decoction of the leaves as a vermifuge (anthelmintic) Crushed flowers to induce sterility	Sosef et al. (1995)

Timber

Acronychia pedunculata (L.) Miq.	Jejerukan[2], Kayu Semidra, Sarirah[3]	Rutaceae	The wood for house building, utility furniture, flooring, lining, panelling, mouldings, turnery, carving, and tool handles	Tue and Sosef (1998)
Aglaia lawii (Wight) C.J. Saldanha	Langsat Kayu Jangan[10], Aisnepapir[16, 13]	Meliaceae	The wood an important source of timber	Ba et al. (1995)
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	The wood for timber	Lemmens (2003)
Baccaurea dulcis (Jack) Müll.Arg.	Kepundung, Menteng, Tampui[1]	Euphorbiaceae	The wood for poles, construction of houses, boats, and furniture	Uji (1992)
Baccaurea javanica (Blume) Müll.Arg.	Heucip[2], Jirek Emprit, Kapundung Lanang[3],	Euphorbiaceae	The wood for poles, construction of houses, boats, and furniture	Idris (1998)
Buchanania arborescens (Blume) Blume	Getasan[3], Rawa-rawa Pipit[6].	Anacardiaceae	For light construction, canoes, furniture, drawers, mouldings, light framing, interior finishes, household implements, cigar boxes, turnery articles, veneer and blockboard, and also for pulp and as firewood	Sulistiarini (1998)
Canarium indicum L.	Kenari Ambon[2], Kanari Bagea[5], Kanari Ternate[10],	Burseraceae	For light construction, mouldings, interior finishes, and as firewood	Kochummen et al. (1995)
Carallia brachiata (Lour.) Merr.	Kitamiyang[2], Sepat[3], Ringgit Dareh[8]	Rhizophoraceae	Timber and fuelwood	Hou (1998 b); Wardani (2003)
Cinnamomum iners Reinw. ex Blume	Ki Teja [2, 3], Kacengal[12], Medang Kalong[21],	Lauraceae	For house building, and cabinet work	Wiselius et al. (1995)
Cynometra ramiflora L.	Kateng, Kepel, Wunut[3]	Leguminosae	The wood for house building, tool handles, woodcraft and ornamental purposes	Soerianagara et al. (1994)
Artocarpus elasticus Reinw. ex Blume	Teureup [2], Benda[3], Mengko[8]	Moraceae	The wood for light construction	Djarwaningsih et al. (1995)
Dehaasia caesia Blume	Huru Kacang[2], Madang Intalo[6], Medang Batu[8].	Lauraceae	For light construction under cover, house posts, house piling, interior finishes, panelling, partitioning and ceiling, picture framing, oars, boat building Musical instruments (pianos), tools, and knife sheaths	Wiselius (1998 a)
Dracontomelon dao (Blanco) Merr. & Rolfe	Dahu[1], Sengkuang[6], Basuong[13]	Anacardiaceae	For timber	Louman et al. (1995)

Drypetes longifolia (Blume) Pax & K.Hoffm	Buniyaga[2], Batung[3], Kikir Daun Besar[6]	Putranjivaceae	The exceptionally tough wood is used for general construction under cover (beams, joists, rafters, flooring), temporary construction, bridge, and wharf superstructure, industrial flooring, furniture and cabinet making, rice mortars, tool handles, posts, piles, poles and railway sleepers	Boer and Sosef (1998a)
Euonymus indicus B.Heyne ex Wall.	Ki Keuyeup[2], Jerukan[3], Awa Kudang-kudang[8]	Celastraceae	The wood for house building, temporary constructions, bridges, small furniture, carving and turnery and as a substitute for boxwood (*Buxus* spp) for making small articles such as spindles, pegs, spoons, and toothpicks	Kochummen (1998)
Fagraea ceilanica Thunb.	Tamosu[8], Kolaki, Nosu[10]	Gentianaceae	The strength and durability wood for heavy constructions, in exposed conditions and in contact with the ground. For house posts, bridges, boats, railway sleepers, piles,poles, fence posts, door and window sills, heavy-duty flooring, barrels, chopping blocks and coffins, and considered a first-class turnery timber for furniture cabinet making and carving, high-quality firewood and charcoal	Hildebrand et al. (1995)
Garcinia celebica	Manggu leuweung[2], Baros[3], Kirasa[10]	Clusiaceae	The wood to build houses	Noor (1998); Heyne (1987)
Glochidion obscurum (Roxb. ex Willd.) Blume	Ki Pare Lalaki[2], Uris-urisan[3],	Phyllanthaceae	The wood to build native and temporary houses, mainly as poles and rafters, and for tool handles for light framing, flooring, moulding, interior trim, and utility furniture The trees yield a good hot fuelwood and the boles used in mushroom cultivation	Irwanto (1998)
Glycosmis pentaphylla (Retz.) DC.	Gongseng[2], Jerukan, Totoan[3],	Rutaceae	The pale wood for tool handles.	Chua and van Valkenburg (2002)
Guettarda speciosa L.	Jati Pasir, Titi Laut[11], Kenjang-kenjang[12]	Rubiaceae	For house-blocks, very durable,and for heavy furniture	Ong (1998)
Hernandia nymphaeifolia (J.Presl) Kubitzki	Kampak, Kampis[2]	Hernandiaceae	The wood for furniture and cabinet work, light interior construction, mouldings, canoe building, fishing net floats, wooden sandals, drawing boards, carving, model making, chopsticks, musical instruments and for core veneer	Irwanto (1998)
Lophopetalum javanicum *(Zdl.) Turcz.	Mandalaksa[3], Madang-gambici [8, 23], Tatokwa[13].	Celastraceae	The wood for timber	Kartasubrata et al. (1994)
Macaranga tanarius (L.) Müll.Arg.	Mara[2], Tutup Ancur[3], Hanuwa[5]	Euphorbiaceae	Peeled poles for temporary construction and especially for parts of native houses not in contact with the ground The wood for light framing, interior trim, moulding, shingles, packing cases, outriggers for canoes and, especially, match splints A high quality pulp and produces high quality particle board, cement-bonded board, and wood-wool board, and for the production of plywood. It provides good fuelwood, wooden shoes	Lim (1998)
Magnolia liliifera (L.) Baill	Kembang Tunjung[2], Cempaka Gonda[3]	Magnoliaceae	The wood for general construction under cover, bridge building, interior finishes, panelling, partitioning, flooring, door and window frames, furniture, mouldings, sporting goods, musical instruments, handicrafts, canoe building, and pencil slats	Sambas and Sosef (1998)

Mammea odorata Kosterm.	Kembang Satu[1, 11], Kapurancak[3], Lolang Waran[11],	Calophyllaceae	The wood for boat building (planks and pins), house posts, flooring and cabinet work	Wiselius (1998 b)
Mangifera indica L.	Mangga, Mempelam[1]	Anacardiaceae	The wood as machang, e.g. in door construction, it makes excellent charcoal, also used to culture mushrooms	Boer et al. (1995)
Mitrephora polypyrena (Bl.) Miq.	Janglot, Kalak Sapi[3], Tapai Bunga[8]	Annonaceae	The wood for interior joinery, poles, tool handles, matchboxes, splints, packing cases, and firewood	Boer and Sosef (1998b)
Polyalthia lateriflora (Blume) Kurz	Kalak [2], Kalak Ucet[3], Janglot[12]	Annonaceae	The generally tough and flexible wood for temporary construction, house building, furniture, interior trim, mouldings, light framing, decorative wall panelling, masts, oars, wooden shoes, tool handles, sporting goods, novelties, toys, match splints, packing cases and boxes	Ban (1998)
Pongamia pinnata (L.) Pierre	Ki Pahang Laut[2], Bangkong[3], Kranji[12]	Leguminosae	The wood provides timber for cabinet work and chartwheels and paper pulp	van Valkenburg (2002)
Pterospermum diversifolium Blume	Bayur[1], Wadang[3].	Malvaceae	The wood for joinery, flooring furniture, cladding, striking tool handles, implements and plywood also used in ship and bridge building suitable for construction under cover; e.g. for boards, beams, joists and rafters	Boer and Lemmens (1998)
Rauvolfia sumatrana Jack	Lame Lalaki[2], Tampa Badak[8], Polay Lakek[12],	Apocynaceae	The lightweight wood is sometimes used for planks and small objects such as knife handles	Ly and Mai (1999)
Sandoricum koetjape (Burm.f.) Merr	Kecapi, Kentuat, Sentul[1]	Meliaceae	The wood for construction, carpentry, household utensils and implements	Sotto (1992)
Semecarpus heterophylla Blume	Rengas Gunung[2] , Ingas[3], Lungas Delok[8]	Anacardiaceae	The wood for temporary construction, mouldings, interior finishes, boxes and crates	Hou (1998 a)
Spondias pinnata (L. f.) Kurz	Kedongdong[1], Kedongdong Leuweung[2], Kacemcem[9]	Anacardiaceae	Timber for packing cases, floats, canoes, matches, and non-ornamental plywood	Anon. (1999)
Sterculia cordata Blume	Hantap Heulang[2], Kayu Binong[3], Gelumpang Padang[4]	Sterculiaceae	The wood for timber	Lemmens et al. (1995)
Syzygium polyanthum (Wight) Walp.	Salam, Manting[3], Ubar Serai[8]	Myrtaceae	The timber for house building	Haron et al. (1995)
Syzygium racemosum (Blume) DC	Kopo Mangud, Resep[3], Klampok Bato[12]	Myrtaceae	The timber for house building	Haron et al. (1995)
Syzygium syzygoides (Miq.) Merr. & L.M.Perry	Ki Sereum[2], Manting[3], Mengkelingan[21]	Myrtaceae	The timber for house building	Haron et al. (1995)
Terminalia bellirica (Gaertn.) Roxb.	Jaha Kebo, Jaha Sapi[3], Ulu Belu[8]	Combretaceae	The wood for boxes, furniture, and house construction after being steeped in water to make it more durable The tree also yields a good-quality firewood and charcoal	Sosef et al. (1995)
Terminalia catappa L.	Ketapang [1]	Combretaceae	The wood for house and boat construction, furniture, and cabinet-making	Sosef et al. (1995)
Vitex glabrata R. Br.	Ketileng, Bigbul, Gentileng[3]	Lamiaceae	The timber for house construction and furniture	Sunarno et al. (1995)
Zanthoxylum rhetsa (Roxb.) DC	ki tanah[2], kayu lemah[3], kaju tana[12]	Rutaceae	The wood to build small houses, tools, and knife sheaths	Boer et al. (1998); Heyne (1987)

Ornamental plants

Ageratina riparia (Regel) R.M.King & H.Rob.	Teklan[3]	Compositae	As an ornamental plant but in some areas has become a troublesome weed	Anon. (1997)
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	Ornamental tree with changing colour of fruits	Lemmens (2003)
Asplenium nidus L.	Paku Sarang Burung[1]	Aspleniaceae	Ornamental plants	Rusea (2003)
Barringtonia racemosa (L.) Spreng.	Penggung[2, 3], Putat Sungai[4], Butun Darat[5].	Lecythidaceae	Planted as a roadside tree	Yaplito (2002)
Cycas circinalis L.	Pakis Haji, Pakis Raja[1]	Cycadaceae	Ornamental plant	Anon. (1992)
Cissus javana DC.	Beubeureumen[2], Mirah[3], Rabet Dara[12]	Vitaceae	Ornamental plant	Anon. (1994)
Dracontomelon dao (Blanco) Merr. & Rolfe	Dahu[1], Sengkuang[6], Basuong[13]	Anacardiaceae	The tree is planted as an ornamental in roadside plantings	Louman et al. (1995)
Fagraea ceilanica Thunb.	Tamosu[8], Kolaki, Nosu[10]	Gentianaceae	Planted as ornamental trees along roads and avenues, because of their handsome, slender appearance and their fragrant or large flowers.	Hildebrand et al. (1995)
Guettarda speciosa L.	Jati Pasir, Titi Laut[11], Kenjang-kenjang[12]	Rubiaceae	The fragrant flowers are used as decoration (garlands)	Ong (1998)
Mitrephora polypyrena (Bl.) Miq.	Janglot, Kalak Sapi[3],Tapai Bunga[8]	Annonaceae	Cultivated as an ornamental and has been used as cover crop in forest plantations	Boer and Sosef (1998 b)
Pandanus tectorius Parkinson ex Du Roi	Pandan Pudak[1]	Pandanaceae	Grown worldwide as ornamentals for their attractive yellow and green striped leaves, indoors in temperate regions and outdoors in the tropics	Brink and Jansen (2003)
Pongamia pinnata (L.) Pierre	Ki Pahang Laut[2], Bangkong[3], Kranji[12]	Leguminosae	Ornamental plants because of its attractive flowers	van Valkenburg (2002)
Sandoricum koetjape (Burm.f.) Merr	Kecapi, Kentuat, Sentul[1]	Meliaceae	Excellent shade trees with ornamental values	Sotto (1992)
Scaevola taccada (Gaertn.) Roxb.	Babakoan[2], Dudulan[3], Subong-subong[17]	Goodeniaceae	Ornamental plant	Wardini (2002)
Terminalia catappa L.	Ketapang [1]	Combretaceae	The trees as shade trees in gardens and along roads.	Sosef et al. (1995)

Natural dyes

Mangifera indica L.	Mangga, Mempelam[1]	Anacardiaceae	The bark yields a yellowish-brown dye for silk	Boer et al. (1995)
Mammea odorata Kosterm.	Kembang Satu[1, 11], Kapurancak[3], Lolang Waran[11]	Calophyllaceae	The heartwood yields a red dye	Wiselius (1998 b)
Myristica teijsmannii Miq.	Kosar, Sibungan[2], Durenan[3]	Myristicaeae	The bark yields a red dye to colour paper	Heyne (1987) Risna (2009)
Pterospermum diversifolium Blume	Bayur[1], Wadang[3].	Malvaceae	The bark yields a yellow dye	Boer and Lemmens (1998)
Syzygium polyanthum (Wight) Walp.	Salam, Manting[3], Ubar Serai[8]	Myrtaceae	The bark for tanning fishing-nets and for dyeing matting	Haron et al. (1995)
Syzygium racemosum (Blume) DC.	Kopo Mangud, Resep[3], Klampok Bato[12]	Myrtaceae	The bark yields a black dye	Haron et al. (1995)
Syzygium syzygoides (Miq.) Merr.& Perry	Ki Sereum[2], Manting[3],	Myrtaceae	The bark yields a brown or black dye and for tanning fishing-nets	Haron et al. (1995)

Terminalia bellirica Gaertner (Roxb)	Jaha Kebo, Jaha Sapi[3], Ulu Belu[8]	Combretaceae	Fruits containing tannin and a dye are used to tan leather and dye cloth and matting, and to prepare ink	Sosef et al. (1995)
Terminalia catappa L.	Ketapang[1]	Combretaceae	The bark and the leaves are used for tanning leather and for dyeing cloth, and making ink, Green fruits are used for the same purpose	Sosef et al. (1995)
Other purposes				
Acronychia pedunculata (L.) Miq.	Jejerukan[2], Kayu Semidra, Sarirah[3],	Rutaceae	The bark for caulking boats and toughening fishing nets The wood for firewood	Tue and Sosef (1998)
Ageratina riparia (Regel) R.M.King & H.Rob.	Teklan[3]	Compositae	As green material for composting, and as a cover crop to check erosion on slopes and terrace walls and the spread of *Imperata cylindrica* L. Raeuschel	Anon. (1997)
Antidesma bunius (L.) Spreng.	Buni[1], Huni[2], Wuni[3]	Phyllanthaceae	Applied in reforestation projects, it has high adaptability to dry environment	Lemmens (2003) Rindyastuti and Hapsari (2017)
Asplenium nidus L.	Paku Sarang Burung[1]	Aspleniaceae	Young *A. nidus* leaves as a contraceptive To improve hair growth by frequent washing of the hair with a decoction of a mixture of ground leaves and coconut milk	Rusea (2003)
Buchanania arborescens (Blume) Blume	Getasan[3], Rawa-rawa Pipit[6].	Anacardiaceae	Tannin from the bark for toughening fishing nets As a shade tree for dry, sunny sites and poor soils	Sulistiarini (1998)
Canarium hirsutum Willd.	Ki Bonteng[2], Mede-mede[5], Kanari Jaki[10]	Burseraceae	The resin for torches and as a glue	Kochummen et al. (1995)
Canarium indicum L	Kenari Ambon[2], Kanari Bagea[5], Kanari Ternate[10],	Burseraceae	An oil extracted from the seeds serves as a substitute for coconut oil	Kochummen et al. (1995)
Chydenanthus excelsus (Blume) Miers	Besole, Blundeng, Brosol, Emprak, Leprak[3].	Lecythidaceae	Fuelwood	Heyne (1987)
Croton tiglium L.	Kemalakian[2], Ceraken[3], Simalakian[7]	Euphorbiaceae	For production of soap	van Welzen and Esser (2002)
Cinnamomum iners Reinw. ex Blume	Ki Teja [2, 3], Kacengal[12], Medang Kalong[21]	Lauraceae	The mucilage has found technical applications; e.g in the manufacture of mosquito coils, fragrant joss-sticks, plastic products, formica, glue, inner layering of tyres, coating of high quality paper products, joints, and fibre glass The tree as a shade tree	Wiselius et al. (1995)
Cyperus elatus L.	Lilisungan[2], Wlingi[3], Tintilohuangga[10]	Cyperaceae	Weaving hats and mats Forage for cattle	Dasuki (2003)
Artocarpus elasticus Reinw. ex Blume	Teureup [2], Benda[3], Mengko[8]	Moraceae	The latex for birdlime and the bark is prepared for cloth and rope by aboriginal tribes.	Djarwaningsih et al. (1995)
Derris elliptica (Wall.) Benth.	Tuwa Leteng[2], Tuba, Oyod Tungkul[3]	Leguminosae	The powdered root as an insecticide	Hamid (1999)
Diospyros maritima Blume	Kunyit[3], Kayu Itam Lewo[10] , Belu Itam Perempuan[22]	Ebenaceae	The pale wood: used locally; e.g. for musical instruments, furniture, inlaying and novelties good quality charcoal	Soerianagara et al. (1995)

Drypetes longifolia (Blume) Pax & K.Hoffm	Buniyaga[2], Batung[3], Kikir Daun Besar[6]	Putranjivaceae	For manufacture of paper	Boer and Sosef (1998a)
Fagraea ceilanica Thunb.	Tamosu[8], Kolaki, Nosu[10]	Gentianaceae	The latex under the epidermis of the fruits as a glue used as a fly trap The leaves for feed stock	Hildebrand et al. (1995)
Ficus microcarpa L. f	Preh[3]	Moraceae	Shade trees	Rojo et al. (1999)
Pandanus furcatus Roxb.	Harashas[2], Panan Kowang[3]	Pandanaceae	The wood is used to make floats for fishing nets, covers for quivers For matting, woven, untrimmed leaves Pandanus leaves are usually reduced to strips for weaving a range of articles, from small hand bags and containers to large floor mats or light interior wall panels. Leaves potential sources of weaving material, and serve for thatching The leaves, stems or prop roots may be retted to obtain fibres which are used for twine and ropes	Brink and Jansen (2003)
Pandanus tectorius Parkinson ex Du Roi	Pandan Pudak[1]	Pandanaceae	The leaves are made into hats, mats, sacks, cordage, baskets, umbrellas, and other articles	Brink and Jansen (2003)
Phaleria capitata Jack	Kakapasan[2], Lawe[3]	Thymelaceae	The tough fibre from the inner bark is used as tying material and for making cordage	Anon. (2003)
Pongamia pinnata (L.) Pierre	Ki Pahang Laut[2], Bangkong[3], Kranji[12] .	Leguminosae	Dried leaves are stored with grain to repel insects The bark can be made into rope The oil is applied as a lubricant, as a leather dressing in the traditional Indian tanning industry, and in manufacturing soap, varnish, and paint For reforestation of marginal land, its extensive root system making it valuable for checking erosion	van Valkenburg (2002)
Premna serratifolia L	Ki Pahan[2], Singkil Alas[3]	Lamiaceae	The wood is used for implements and paddles, the bark as binding material. The plant is usually used as hedges.	Cardenas (1999)
Pterospermum diversifolium Blume	Bayur[1], Wadang[3]	Malvaceae	The wood is suitable for matches and for the production of wood-wool boards The pulp is suitable for making paper The bark is locally used for toughening fishing nets	Boer and Lemmens (1998)
Scaevola taccada (Gaertn.) Roxb.	Babakoan[2], Dudulan[3], Subong-subong[17]	Goodeniaceae	The wood from the base of the mature stems is used as nails in traditional boat building. The plant is used for soil stabilization and wind and salt spray protection in coastal zones	Wardini (2002)
Spondias pinnata (L. f.) Kurz	Kedongdong[1] Leuweung[2], Kacemcem[9]	Anacardiaceae	For unbleached wood pulp	Anon. (1999)

*) Note: 1. Indonesia 2. Sunda 3. Java 4. Bangka 5. Moluccas 6. Kalimantan 7. Minangkabau 8. Sumatra 9. Bali 10. Sulawesi 11. Ambon, 12. Madura 13. Irian Jaya 14. Lampung 15. Manado 16. Biak 17. Bengkulu 18. Timor 19. Halmahera 20.Ternate 21. Belitung 22. Seram 23. Batak

Natural dyes

Nine plant species have been recorded to be used for natural dyes (Table 11): the bark of *Mangifera indica* provides a yellowish-brown dye, the heartwood of *Mammea odorata* provides a red dye, the bark of *Pterospermum diversifolium* provides a yellow dye, the bark of *Syzygium racemosum* provides a black dye, and *Syzygium syzygoides* provides a brown or black dye. These natural dyes have potential to be developed as natural colouring for fabrics such as batik, for food and for beverages. Further research is required into the efficacy and safety of their use in colouring food, beverages, fabrics, and for other purposes. Natural dyes are in high demand as environmental friendly products, as a result of concern over the adverse impacts of synthetic dyes on the environment and on health.

Other uses

There were 25 plant species that have other uses; such as for shampoo and hair improvement, shade trees, woven hats and mats, reforestation, wood pulp, forages, and other uses (Table 12). A decoction of a mixture of ground leaves of *Asplenium nidus* and coconut milk can be used for shampoo and hair growth improvement. Some plant species can also be used for shade trees, such as *Buchanania arborescens, Cinnamomum iners, Ficus microcarpa, Sandoricum koetjape,* and *Terminalia catappa*. Woven hats and mats can be made from leaves of *Cyperus elatus* and *Pandanus tectorius*. Some other plant species are useful for reforestation, such as *Pongamia pinnata*. The leaves of *Fagraea ceilanica* can be used as forage to feed livestock. Wood of *Spondias pinnata* is a good source for unbleached pulp.

Implications for conservation

Overall, the present study, based on the knowledge of local informants and on a review of ethnobotanical literature, provides empirical data on the plant species of Segara Anakan area, Sempu Island, East Java; on their conservation status; and on potential uses of the plants. Further research is required on the biochemical, pharmaceutical, and pharmacological value of the plants as well as on the possible development from them of prospective products for the industrial sector. Developing useful products from the local plant life has the potential to raise the socio-economic level of communities near the island.

The rich diversity of plant life in the Segara Anakan area needs to be conserved, with priority to be given to plant species on the IUCN Red List. Developing new potential uses of the plants for the local communities near Sempu Island can provide a stong incentive for conservation of the plant life. However, development of the potential uses of the plant species requires pro-active consideration of their conservation in order to avoid the kind of over-exploitation that would result in decline in biodiversity, or even threaten the survival of the plant species (Lopez-Pujol et al. 2006).

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The diversity of locally utilized plants and local botanical knowledge in Central Bengkulu District, Bengkulu Province, Indonesia

WIRYONO♥, JAPRIYANTO, ERNIWATI
Department of Forestry, Faculty of Agriculture, University of Bengkulu. Jl. WR Supratman, Bengkulu, Indonesia. Tel./Fax. +62-736-21170, ♥email: wiryonogood@unib.ac.id, wiryonogood@yahoo.com

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Abstract. *Wiryono, Japriyanto, Erniwati. 2017. The diversity of locally utilized plants and local botanical knowledge in Central Bengkulu District, Bengkulu Province, Indonesia. Biodiversitas 18: 1589-1595.* For thousands of years rural communities have derived from their surrounding plants many products to meet their daily life. Maintaining botanical knowledge is, therefore, essential for the sustainability of rural communities. The objectives of this study were to know the diversity of plants utilized by villagers in Tanjung Terdana Village, Central Bengkulu District, Indonesia, and to describe the botanical knowledge of the villagers. Data were collected through interview with two key informants and 64 respondents. The results showed that 75 of plant species were used by villagers for ten purposes, namely medicine (53 species), firewood (41), food (32), construction (23), ornament (8), handycraft (6), hedge (6), custom (6) forage (3), and food coloring (1). On average, respondents could identify 70% of the 50 plants shown to them, and almost all respondents (98%) could identify plants for food. Older respondents spent more time in garden and knew more plant names and uses than the younger ones. Gender or time spent in electronic entertainment did not affect the botanical knowledge. Respondents unfinishing elementary school knew more plants than those finishing schools, may be because they were older. This study implied that activities in the garden can maintain the local botanical knowledge.

Keywords: Biodiversity, ethnobotany, traditional ecological knowledge

INTRODUCTION

In rural areas, plants provide daily needs as well marketable products that may generate income for communities. For example, villagers in Batu Ampar Village of South Bengkulu District used 83 plant species for eight purposes (Wiryono and Lipranto 2013), in Harapan Makmur Village of Central Bengkulu District, 79 species for ten purposes (Wiryono et al. 2016), and in Banten 163 species for nine purposes (Wardah 2003).

The use of plants for food may be the most important one for human survival, followed by the use for medicine. Searching for food and other basic necessities in the environment for thousands of years, human has accumulated knowledge of local plants, animals and their physical environment and this knowledge is passed from generation to generation (Pilgrim et al. 2008). This knowledge is called traditional ecological knowledge or local ecological knowledge (hereafter referred to as TEK or LEK).

Essential for the survival of human, TEK is currently under threat of modernization. Studies show that older generation fails to pass TEK to the younger generation in many parts of the world (Quinlan and Quinlan 2007; Sousa et al. 2012; Saynez-Vaquest et al. 2016). With modernization young people may be allienated from their environment and consequently lose their knowledge of nature. In the US, the visit to nature parks decreased drastically due to the emergence of electronic entertainment, such as video games and the internet (Pergams and Zaradic 2006). In Mexico, the children of Zapotecs who attended school had little TEK because they did not have enough time to accompany their parents collecting traditional plants and the language used in school was different from that used for TEK (Saynez-Vaquez et al. 2016), while in Doñana, Spain, farmers lost their TEK due to market integration and mechanization and intensification of agriculture, so that the farmers abandoned their traditional farming (Gómez-Baggethun and Reyes-Garcia 2013).

There have been many concerns for the preservation of TEK and integrate it in the management and conservation of natural resources (Drew and Henne 2006; Charnley et al 2007). It is encouraging that some studies showed that some communities managed to preserve their TEK despite cultural changes they experienced. In New York, migrants from the Dominican Republic maintained their knowledge of their traditional food medicines because they still consumed them (Vandebroek and Balick 2012). In Bolivia, the Tsimane' managed to maintain their TEK because it was still the basis of their livelyhood and they still practiced farming and sold products in traditional way (Gómez-Baggethun and Reyes-Garcia 2013).

In Sumatera, economic development has caused the conversion of natural forest into single-species plantation, mainly oil palm (Saxon and Roquemore 2011). The disappearing of tropical forest has caused the loss of biodiversity and botanical knowledge among indigenous people, so it is important to document this knowledge in order to slow or reverse the loss of it (Ramirez 2007). The

objectives of this study were to know the diversity of plants utilized by villagers in Central Bengkulu District, Bengkulu Province, Indonesia, and to know the ability of villagers to identify plants and their uses.

MATERIALS AND METHODS

Location and time of study

The study was conducted in Tanjung Terdana Village, Pondok Kubang Sub-district, Central Bengkulu District, Bengkulu Province, Sumatra Island, Indonesia (Figure 1). Data collection was done in February-March, 2012. The study site is a lowland area with altitude of 20-30 m above sea level. The climate is wet tropical, with average annual rainfall of 2500-4000 mm and temperature of 25-27^{o}C. The total residents of the villagers was 606, mostly native to the area, belonging to Lembak tribe who worked as farmers. The settlement is adjacent to state forest area, but there has been land tenurial conflict in the forest area, and most of the forest area has been cleared and cultivated with agricultural crops by villagers.

Data collection

Step 1. Selecting key informants and gathering data of utilized plants

To know the plants commonly used by the villagers, two key informants were choosen to be interviewed. They were traditional medicine men in the village who had good knowledge of local plants and their uses. Using key informants for gathering data is commonly done in ethnobotany study (Tongco 2007). They were taken to the field to show the plants and their uses. Each plant was photographed and the local name and uses were recorded in tally sheets. Identification of plants was done using photographs as well as plant specimens. A total of 75 plant species were identified as useful for villagers.

Step 2. Selecting plants to be shown to respondents

Of the 75 species used by villagers, a total of 50 species commonly found in the field were selected and their images were printed. Each plant was represented by two or more pictures showing the whole plant and a close-up showing the characteristics of the plant.

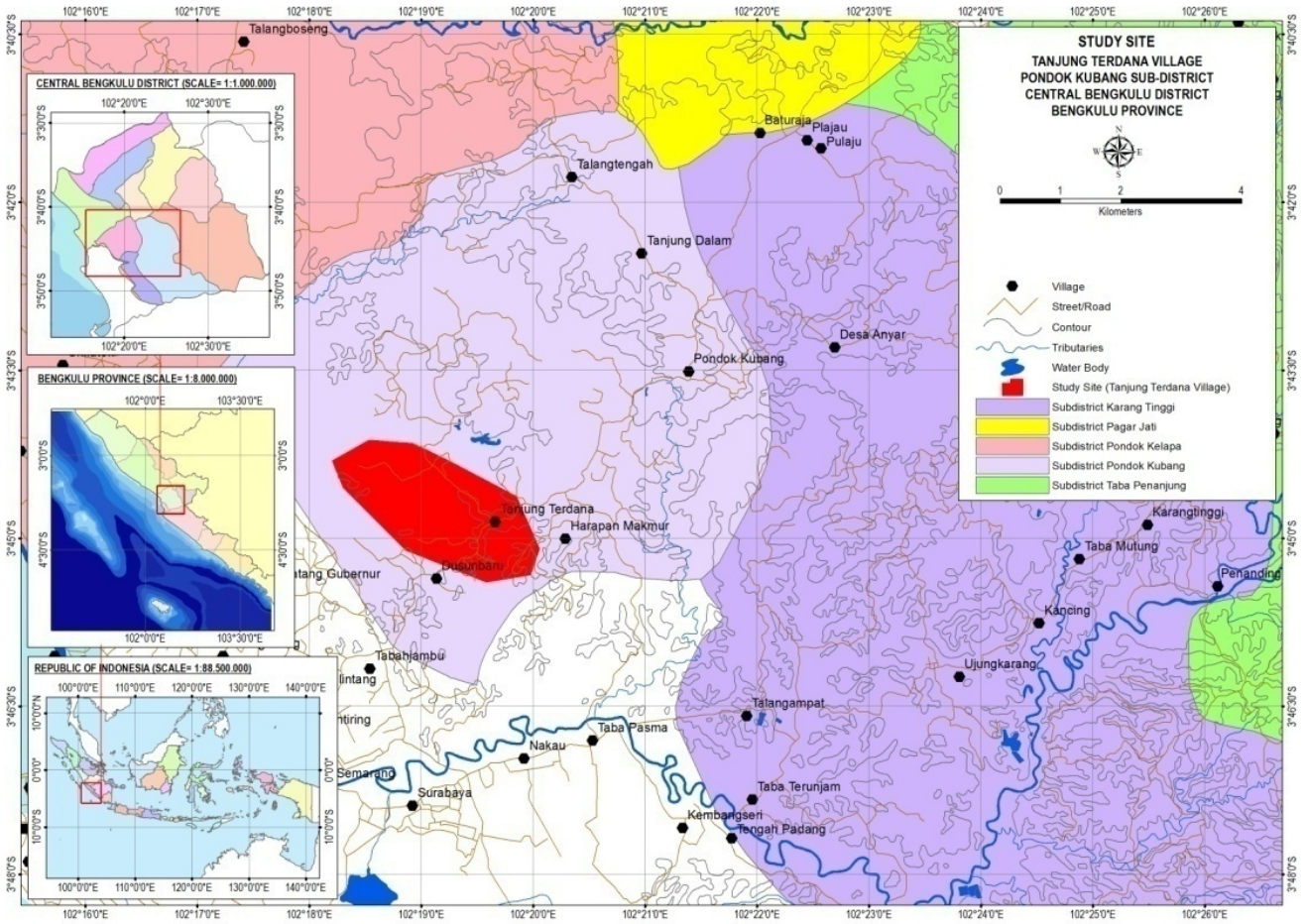


Figure 1. Location of study, Tanjung Terdana Village, colored red on the map, Pondok Kubang Sub-district, Central Bengkulu District, Bengkulu Province, Indonesia

Step 3. Selecting respondents

A total of 64 respondents, consisting of 32 males and 32 females, were selected from the total of 606 villagers, 15-64 years old. From each age class, with 5-year interval, approximately 10% was selected as respondents, except for the elderly whose population was very small; they were represented by 20% samples.

Step 4. Interviewing respondents

Each respondent was interviewed to gather their personal data, namely age, gender, family profession, education, hobby, time spent in garden (field), and time spent enjoying electronic entertaiment (watching TV, video, playing video games). Then, each photograph of the selected plants was shown to them to identify its name and uses. Photographs have been used successfully by Setalaphruk and Price (2007), Pilgrim et al. (2008) and Wiryono and Nurliana (2011) to test the ability of respondents to recognize plants.

Data analysis

All data were tabulated and analyzed descriptively. To determine whether gender affected the knowledge of plant names and uses, t-tests were conducted. Stepwise multiple regressions were done to know the correlation between knowledge of plant names and uses (dependent variables) and three other variables, namely age of respondents, the length of time they spent for electronic entertainment, and the length of time they spent in garden (independent variables). Simple regressions were done to know the correlation between age and time spent in garden, between use values and the identifiability of plants, and between knowledge of plants and knowledge of plants uses. The statistical analyses were done using statistical software SPSS, except for t-tests which were done using Excel. Family profession was not tested because 91% of respondents were farmers. Education data did not fit for a statistical test, so they were tabulated and averaged.

The usefulness (use value) of each species according to respondents was calculated using this formula (Albuquerque et al. 2006).

$$UV = (\Sigma Ui)/n$$

Whereby, Ui is the number of uses of a given species mentioned by each respondent and n is the total number of respondents.

RESULTS AND DISCUSSION

Diversity of locally used plants

A total of 75 plant species from 39 families were recognized by key informants as having been used by villagers for ten purposes (Figure 2 and Table 1). Most of the plants were trees (48 species), followed by shrub (14), herb (5), palm (4), climber and grass (2 species each). Medicinal use had the highest number of plant species (53), followed by firewood (41), food (32), construction (23), and ornament (8). Handycraft, hedge and custom uses had the same number of species, which was 6, while 3 species could be used for forage and one species for food coloring.

The number of plants used by villagers in this study was fewer than that used by villagers in Batu Ampar, South Bengkulu District, which was 83 species (Wiryono and Lipranto 2013), in Harapan Makmur Village, Central Bengkulu District, 79 species (Wiryono et al. 2016), and in Banten, 163 species (Wardah 2003). However, it was higher than 41 species used by villagers in Keramat Mulya, Soreang District of West Java (Okubo et al. 2010) and 65 species in Kabena Island, Southeast Celebes Province (Rahayu and Rugayah 2010). The high diversity of utilized species shown by the key informants reflected the high diversity of plants growing in the village and the wide knowledge of the key informants of the plants.

It is not surprising that the highest number of plant use was for medicines because the key informants in this study were medicine men. The number of plants for medicine in this study was higher than 47 species used in Rajegwesi Village, Banyuwangi District, East Java (Pamungkas et al. 2013), 33 species in Mamuju, in West Sulawesi (Syamsiah et al. 2016) and 45 species in Seram Island, Molluccas (Susiarti 2015), but lower than 127 species by Serampas tribe, Jambi Province (Hariyadi and Ticktin 2012). Serampas tribe is indigenous people living in the border of Kerinci Seblat National Park who still practices traditional life. In many parts of the world, despite the advance of biomedicine, traditional medicine is still used (Rathore et al. 2015; Zank and Hanazaki 2017).

Among the 75 species used by villagers, key informants mentioned coconut as having the highest uses (6), followed by bamboo (5). Correspondingly, the determination of use value from the respondents'answer showed that these two species had the highest use values, but bamboo being the number one (2.7) and coconut second (2.5). Similarly, these two species were also the most important useful species, in Batu Ampar village, Bengkulu Province (Wiryono and Lipranto 2013). In Rajegwesi Village, Banyuwangi, East Java Province (Pamungkas et al. 2013) coconut also had the highest cultural important value, but bamboo didn't.

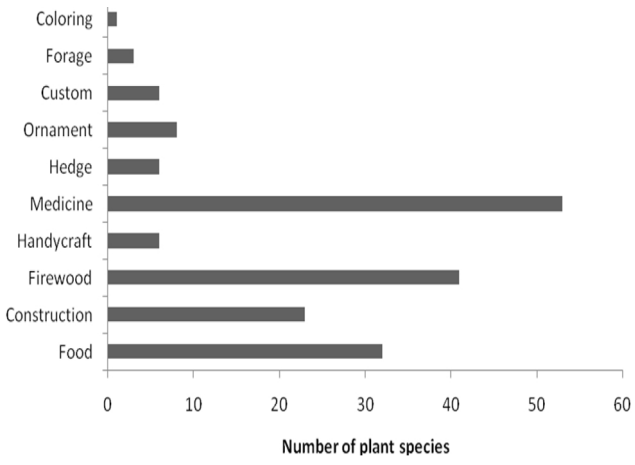


Figure 2. The number of plant species for each type of use

Table 1. The diversity of locally utilized plants and local botanical knowledge in Central Bengkulu District, Bengkulu Province, Indonesia

Local names	Scientific names	Family	Growth form	Types of use	Total uses	Use value	% identification
Bambu	*Bambusa* sp.	Poaceae	Grass	Construction, custom, firewood, food, handycraft, hedge	5	2.7	100
Kelapa hijau	*Cocos nucifera* Var. Viridis.	Arecaceae	Palm	Construction, custom, firewood, food, handycraft, medicine	6	2.5	100
Durian	*Durio zibetinus* L.	Malvaceae	Tree	Construction, firewood, food	3	2.3	97
Mangga	*Mangifera indica* L.	Anacardiaceae	Tree	Construction, firewood, food, forage	4	2.2	100
Jering/Jengkol	*Archidendron pauciflorum* (Benth) I.C. Nielsen	Fabaceae	Tree	Construction, firewood, food, medicine	4	2.0	95
Sirsak	*Annona muricata* L.	Annonaceae	Tree	Firewood, food, medicine	3	1.8	100
Manggis	*Garcinia mangostana* L.	Clusiaceae	Tree	Firewood, food medicine	3	1.7	92
Bangka/Pinang	*Areca catechu* L.	Arecaceae	Palm	Custom, food, hedge, medicine	4	1.5	98
Kapuk	*Ceiba pentandra* L. Gaertn.	Bombacaceae	Tree	Construction, firewood, hedge, medicine	4	1.5	77
Belimbing wuluh/Besi	*Averrhoa bilimbi* L.	Oxalidaceae	Tree	Food, medicine	2	1.4	97
Jambu biji	*Psidium guajava* L.	Myrtaceae	Tree	Food, medicine	2	1.4	97
Kayu gadis	*Cinnamomum parthenoxylon* (Jack) Meisn.	Lauraceae	Tree	Construction, firewood, medicine	3	1.3	81
Tebu Hitam	*Saccharum officinarum* L.	Poaceae	Grass	Food, medicine	2	1.3	100
Terap/Lantung	*Artocarpus elasticus* Reinwe. Ex. Blume	Moraceae	Tree	Construction, firewood, hedge	3	1.3	70
Langsat	*Lansium domesticum*.	Meliaceae	Tree	Firewood, food	2	1.2	86
Nangka	*Artocarpus heterophyllus* Lam.	Moraceae	Tree	Construction, firewood, food, forage	4	1.2	100
Keduruk/Sendud uk	*Melastoma malabahtricum* L.	Melastomaceae	Shrub	Firewood, food, medicine	3	1.1	91
Sungkai	*Peronema canescens* Jack.	Verbenaceae	Tree	Construction, firewood, medicine	3	1.1	84
Bungo rayo	*Hibiscus rosa-sinensis* L.	Malvaceae	Tree	Hedge, medicine, ornament	3	1.1	87
Jeruk purut	*Citrus hystrix* D.C.	Rutaceae	Tree	Food, medicine	2	1.1	98
Jeruk nipis	*Citrus aurantifolia* (Christm.) Swingle.	Rutaceae	Tree	Food, medicine	2	1.0	95
Lengkuas	*Alpinia galanga* (L.) Wild.	Zingiberaceae	Herb	Food, medicine	2	1.0	91
Jarak pagar	*Jatropha curcas* L.	Euphorbiaceae	Shrub	Hedge, medicine	3	1.0	97
Sedingin	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	Shrub	Medicine, ornament	2	1.0	92
Tegang/Rimbang	*Solanum torvum* Sw.	Solanaceae	Shrub	Food, medicine,	2	1.0	97
Pandan Wangi	*Pandanus amaryllifolius* Roxb.	Pandanaceae	Shrub	Coloring, custom, ornament	3	0.9	92
Rumput Malesia	*Chromolaena odorata* (L.) R.M.King &.H.Rob	Asteraceae	Shrub	Medicine	1	0.9	94
Gelinggang	*Senna alata* (L.) Roxb.	Fabaceae	Shrub	Medicine	1	0.9	89
Pulai/Plawi	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	Tree	Construction, firewood, medicine	4	0.9	81
Nilau	*Commersonia bartramia* (L.) Merr.	Malvaceae	Tree	Construction, firewood, hedge	3	0.8	45
Rambutan	*Nephelium lappaceum* L.	Sapindaceae	Tree	Construction, firewood, food, medicine	4	0.8	67
Kayu Kapung	*Oroxylum indicum* (L.) Benth. ex Kurz	Bignoniaceae	Tree	Firewood, medicine	2	0.7	70
Merampuyan	*Rhodamnia cinerea* Jack.	Myrtaceae	Tree	Construction, firewood, medicine	3	0.7	55
Capo/Sembung	*Blumea balsamifera* (L). DC.	Asteraceae	Tree	Medicine	1	0.7	67
Pelangas	*Aporosa dioica* (Roxb.) Müll.Arg.	Phyllanthaceae	Tree	Construction, firewood	2	0.7	45
Tukup bumi	*Elephantopus scaber* L.	Asteraceae	Herb	Medicine	1	0.7	67
Simpur	*Dillenia excelsa* (Jack.) Martelli ex Gilg	Dilleniaceae	Tree	Firewood, medicine	2	0.6	53
Kenidai	*Bridelia monoica* (Lour.) Merr	Phyllanthaceae	Tree	Firewood, medicine	2	0.5	30
Salung	*Psychotria viridiflora* Reinw ex Blume	Rubiaceae	Shrub	Firewood, medicine	2	0.5	52
Inai kayu	*Lawsonia inermis* L.	Lythraceae	Shrub	Custom, medicine, ornament	3	0.5	41
Balik angin	*Mallotus paniculatus*. (Lam.) Müll.Arg.	Euphorbiaceae	Tree	Construction, firewood	2	0.5	28
Cemetir/Tembelekan	*Lantana camara* L.	Verbenaceae	Tree	Medicine	1	0.4	36
Leban	*Vitex pinnata* L.	Lamiaceae	Tree	Construction, firewood	2	0.4	25
Johar	*Senna siamea* (Lam.) H.S. Irwin + Barneby	Fabaceae	Tree	Construction, firewood	2	0.4	36
Daun sesiap	*Ficus septica* Burm.f	Moraceae	Tree	Medicine	1	0.4	33
Kagas/Gharu	*Aquilaria malaccensis* Lamk.	Thymelaeaceae	Tree	Construction, firewood	2	0.3	28
Kayu Angit	*Clausena excavata* Burm. F.	Rutaceae	Tree	Firewood, medicine	2	0.3	30
Gelam	*Syzygium* sp.	Myrtaceae	Tree	Construction, firewood	2	0.2	14
Kemutun	*Cratoxylon formosum* Benth & Hook.f. ex Dyer.	Hypericaceae	Tree	Construction, firewood, medicine	3	0.2	12

Semanik	*Ixonanthes icosandra* Jack.	Ixonanthaceae	Tree	Firewood, medicine	2	0.1	12
Sirih	*Piper betle* L.	Piperaceae	Climber	Coloring, food, medicine, ornament	4	NA	NA
Alpukat/jambu mentega	*Persea americana* Mill.	Lauraceae	Tree	Firewood, food, medicine	3	NA	NA
Jambu air	*Syzygium aqueum* (Burm.f.) Alston.	Myrtaceae	Tree	Firewood, food, medicine	3	NA	NA
Jarak abang	*Jatropha gossypifolia* L.	Euphorbiaceae	Shrub	Hedge, medicine, ornament	3	NA	NA
Mbacang	*Mangifera foetida* Lour.	Anacardiaceae	Tree	Construction, firewood, food	3	NA	NA
Pisang	*Musa* sp.	Musaceae	Shrub	Custom, food, medicine	3	NA	NA
Seris	*Muntingia calabura* L.	Muntingiaceae	Tree	Firewood, food, medicine	3	NA	NA
Daup putih	*Bauhinia acuminata* L.	Fabaceae	Tree	Medicine, ornament	2	NA	NA
Kandis	*Garcinia dioica* Blume	Clusiaceae	Tree	Firewood, food	2	NA	NA
Kayu Bawang	*Dysoxylum mollisimum* Blume	Meliaceae	Tree	Construction, firewood	2	NA	NA
Kayu Belalang	*Dysoxylum arborescens* (Blume) Miq.	Meliaceae	Tree	Firewood, medicine	2	NA	NA
Kayu tulang	*Euphorbia tirucalli* L.	Euphorbiaceae	Shrub	Medicine, ornament	2	NA	NA
Mengkudu	*Morinda citrifolia* L.	Rubiaceae	Tree	Food, medicine	2	NA	NA
Pegagan/kaki kudo	*Centella asiatica* (L.) Urban	Apiaceae	Herb	Food, medicine	2	NA	NA
Rotan getah	*Doemonorops angustifolia* (Griff) Mart.	Arecaceae	Palm	Food, handycraft	2	NA	NA
Rotan kesur	*Calamus ornatus* Blume	Arecaceae	Palm	Food, handycraft	2	NA	NA
Tupak	*Baccaurea brevipes* Hook.F	Phyllanthaceae	Tree	Firewood, food	2	NA	NA
Ubi hitam	*Dioscorea esculenta* (Lour) Burkill	Dioscoreaceae	Climber	Food, medicine	2	NA	NA
Belai banglai	*Zingiber purpureum* Roscoe.	Zingeberaceae	Herb	Medicine	1	NA	NA
Karet/para	*Hevea brasiliensis* Müll.Agr.	Euphorbiaceae	Tree	Firewood	1	NA	NA
Kayu Gambo	Unidentified		Tree	Firewood	1	NA	NA
Nuing	*Ficus hispida* L.f.	Moraceae	Tree	Medicine	1	NA	NA
Pepulut	*Urena lobata* L.	Malvaceae	Shrub	Medicine	1	NA	NA
Rumput angit/ bandotan	*Ageratum conyzoides* L.	Asteraceae	Herb	Medicine	1	NA	NA
Rumput babi	*Cyanthillium cinereum* (L.) H. Rob.	Asteraceae	Shrub	Medicine	1	NA	NA

Note: NA: not available (not shown to respondents)

Table 2. The ability of respondents to identify plant names.

Number of respondents	Ability to identify plants (%)
6	≥ 90
14	80-89
16	70-79
17	60-69
4	51-59
6	41-49
0	31-39
1	28
Average ability to identify plants = 70.5%	

Table 3. The percentage of respondents knowing the types of plant uses

Types of uses	% of respondents knew
Food	98
Medicine	77
Construction	57
Handycraft	42
Firewood	37
Hedge	13

The knowledge of plant names and uses

No respondent could identify all the 50 plant species in the photographs, but six of them could identify ≥ 90% of the plants. On average, they could recognize 70% of the plants and only one respondent had little knowledge of plant names, which was 28% (Table 2). Six species, namely, coconut, bamboo, jackfruit, manggo, sugar cane, and pandanus palm, were successfully identified by all respondents, fourten other species by more than 90% respondents and only 15 species were recognized by less than 40% respondents (Table 1). The identifiability of a plant had possitive correlation with its use value, and the correlation was strong (Table 4).

The knowledge of plant names among villagers in this study was higher than that among students of forestry in Bengkulu city, just 10 km from the study site, who could identify less than 60% of tree names in their campus (Wiryono and Nurliana 2011). It was likely that villagers in this study had more intensive interaction with the plants than the university students. Local knowledge of plants in rural area is the results of long and intimate interaction between people and their surrounding flora in search of food, medicine and other daily needs for their survival and this knowledge is passed from generation to generation (Pilgrim et al. 2008).

Almost all respondents (98%) recognized the dietary use of edible plants, and majority respondents recognized the medicinal use of medicine plants (77%) and constructional use of plants (57%) (Table 3). Food and medicine are the most important need for human survival, so it is logical that most respondents knew the dietary and medicinal uses of plants.

In this study, key informants who were medicine men recognized more plants for medicine than for food, while respondents recognized more dietary use than medicinal use of plants. In an interesting study, Vandebroek and Balick (2012) found that young people of Dominican Republic who have migrated to New York City, far from their home country, maintained their knowledge of plants used for food and medicine simultaneously.

Factors affecting botanical knowledge

Gender

The knowledge of plant names was positively correlated with the knowledge of plant uses and the correlation was strong, meaning that respondents knowing more about the plant names also knew more about the plant uses (Table 4). The knowledge of plant names and uses was not affected by gender. On average, male respondents correctly identified 70% of the plants and females 71%, and there was no difference on t-test. The average number of plant uses mentioned by males was 49 and by females 52, but this was not significant either.

Previous study (Wiryono and Nurliana 2011) also showed that gender did not affect knowledge of plants among forestry students in Bengkulu. Likewise, in Parnaíba Delta Environmental Protection Area, Brazil, Sousa et al. (2012) did not find any effect of gender on the knowledge of plant diversity.

Age and time spent in garden

The regression analyses showed that knowledge of plant names as well as plant uses was highly significantly correlated positively with ages, meaning that older people had more knowledge than the younger one, although the correlation was not strong (Table 4). The results of this study confirmed other studies that younger generation had less TEK than the older ones (Quinlan and Quinlan 2007; Sousa et al. 2012; Saynez-Vaquest et al. 2016). The loss of TEK among the youth is often attributed to cultural changes due to economic development (Pilgrim et al. 2008), modernization and globalization (Brosi et al. 2007; Ramirez et al. 2007; Turner and Turner 2008). With modernization younger people may be allienated from their environment and consequently lose their knowledge of nature. Pergams and Zaradic (2006) and Pergams and Zaradic (2008) had shown that the visit to the US parks had decreased drastically due to the emergence of electronic entertainment. The farmers of Doñana, Spain, lost their TEK because the TEK system had been replaced by modern knowledge and the farming system had been affected much by mechanization and market economy (Gómez-Baggethun and Reyes-Garcia 2013)

In our study, the time spent for electronic entertainment was not correlated with knowledge of plant names and uses, but the time spent in garden was significantly correlated positively with the knowledge of plants and the time spent in the garden was also correlated positively with ages (Table 4). So, in our study, older people visited garden more often than the younger generation. Similarly, Kai et al. (2014) also found that in Mengsong Township, Xishuangbanna, China, the elders visited forest more often than the youth, and so older people were better able to name birds at the species level.

We can assume that it is the decline in interaction with nature that causes the TEK loss and not the modernization or economic development itself. Furusawa (2009) studying in Solomon Islands concluded that modernization was not directly related to TEK loss. Gómez-Baggethun and Reyes-Garcia (2013) from their study in Bolivia and Spain also concluded that response to globalization depends on the TEK system held by the small societies. They found that the Tsimane' in Bolivia managed to maintain their TEK because it was still the basis of their livelihood and they still practiced farming and sold products in a traditional way. In another study, Vandebroek and Balick (2012) found that young people from the Dominican Republic, who had moved to New York City maintained the knowledge of food medicines because they continued consuming the food.

Education

Table 5 implied that level of education had no effect on the knowldege of plants. The higher knowledge of respodents unfinishing school than the others was likely due to their ages, because they were old people, 49-63 years old. However, other studies in Caribbean (Quinlan and Quinlan 2007) and in Mexico (Saynez-Vaquez et al. 2016) found that knowledge of plants was negatively correlated with formal education level. Saynez-Vaquez et al. (2016) explained that Zapotecs children in Mexico who attended school did not have much time to accompany and help their parents collecting plants and they lost their skill in their mother tongue, the language of their TEK, because the schools used different language.

Table 3. Results of regression analyses showing correlation among variables

Variables correlated	Sig.	R
Independent variables (X1, X2): age of respondents, time spent in garden Dependent variable (Y1): knowledge of plant names	0.000	0.511
X1, X2: Age of respondents, time spent in garden Y2: Knowledge of plant uses	0.000	0.640
X3: Time spent for electronic entertainment Y1: Knowledge of plant names	0.264	0.144
X3: Time spent for electronic entertainment Y2: Knowledge of plant uses	0.371	0.116
X4: Use value of plants Y3: The identifiablity of plant	0.000	0.722
X: Age of respondents Y: Time spent in garden	0.053	0.24

Note R: correlation coefficient

Table 5. The level education and the knowledge of plant names and uses.

Education level	% of plant names correctly identified	The number of plant uses known
Not finishing Elementaray school	41	66
Elementary School	34	50
Junior High school	35	47
Junior High School	34	49
College	34	47

Note: those unfinishing elementary school were 49-63 years old

In conclusion, the villagers used 75 plant species for ten categories, namely medicine, firewood, food, construction, ornament, handycraft, hedge, custom, forage, and food coloring. On average, respondents could identify 70% of plants shown to them. The local botanical knowledge was positively correlated with age and time spent in the garden, and time spent in the garden was positively correlated with age. The decrease of botanical knowledge among the youth was presumably due to the less time they interacted with plants in the garden than the older people.

To get a better result of the study, we should have investigated how villagers acquired botanical knowledge, so we would have known for sure, what caused the decline of that knowledge among the youth. Based on this information, we could plan learning activities involving the local people, young and old, collecting botanical data from their environment. Engagement in this project may increase ecological awareness and therefore may help preserve the LEK among the youth.

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Short communication: Seagrass diversity and carbon sequestration: Case study on Pari Island, Jakarta Bay, Indonesia

TEGUH HUSODO[1,2,3], SAHBUDDIN DG. PALABBI[2], OEKAN S. ABDOELLAH[2,3], MOHAMAD NURZAMAN[1], NURULLIA FITRIANI[1], RUHYAT PARTASASMITA[1,♥]

1Biology Department, Math and Science Faculty, Universitas Padjadjaran. Jl. Raya Jatinangor KM 21, Sumedang 45363, West java, Indonesia. Tel./fax.: +62-22-7796412. ♥email: ruhyat.partasasmita@unpad.ac.id

[2] Programe of Environmental Science, School of Graduates, Universitas Padjadjaran. Jl. Sekeloa, Coblong, Bandung 40134, West Java, Indonesia.

[3]Institute of Ecology, Directorate of Reseach, Community Services and Innovation, Universitas Padjadjaran. Jl. Sekeloa, Coblong, Bandung 40134, West Java, Indonesia

Manuscript received: 5 July 2016. Revision accepted: 10 October 2017.

Abstract. *Husodo T, Palabbi SDG, Abdoellah OS, Nurzaman M, Fitriani N, Partasasmita P. 2017. Seagrass diversity and carbon sequestration: Case study on Pari Island, Jakarta Bay, Indonesia. Biodiversitas 18: 1596-1601.* Pari Island is located in Kepulauan Seribu chain. As a tourist destination, the seagrass ecosystem at the island is constantly under pressure from time to time. An effort to prevent widespread damage is to designate parts of the coastline as a limited access research centre. Efforts to study seagrass ecosystem on Pari Island are ongoing, including studies on seagrass' ability to sequestrate carbon. This quantitative study was conducted in December 2014. Surveys are done on the abundance and density of seagrass in 122 plots (area = 50 × 50 cm^2) which spread in 6 research stations. Biomass measurements were performed by extractive method on 4 types of seagrass (*Cymodocea serrulata*, *Halophila ovalis*, *Halodule uninervis* and *Syringodium isoetifolium*). The assessment of seagrass capability in storing carbon has been done by measuring the primary productivity of above ground and below ground, while the amount of carbon released is measured from littered leaves and the leaves eaten by herbivores.The findings show that there are 7 of 12 species of seagrass identified in Indonesia, i.e. *Cymodoceae rotundata, Cymodoceae serrulata, Thalassia hemprichii, Enhalus acoroides, Syringodium isoetifolium, Halodule uninervis* and *Halophila ovalis*. The dominant species is *Thalassia hemprichii* (density 200±153, 261±107 and 117±52 stands/m^2) and *Halodule uninervis* (82±59 stands/m^2).Seagrass' carbon sequestration capacity in the waters surrounding Pari Island, Jakarta Bay, is approximately 0.006-1.048 gC/m^2/day whereas carbon reserves are between 0.24-346.10 gC/m^2.

Keywords: Carbon sequestration, carbon reserve seagrass ecosystem, Jakarta Bay, Pari Island

INTRODUCTION

The seagrass ecosystem is a coastal ecosystem like coral reefs and mangrove. The ecosystem is facing sustained damages in terms of quality and coverage. The Indonesian seagrass ecosystem covers some 30,000km^2, although the area is believed to have been reduced by 30-40%. The Pari Island's seagrass ecosystem in DKI Jakarta has diminished by 25% between 1999 and 2004. A number of factors, such as reclamation and physical developments along coastlines, pollution, and blast and cyanide fishing, are the cause of degradation and shrinking of the ecosystem. In the case of Pari Island, Kiswara (1999) found that seagrass damage is caused by water whirlpool due to the fishing boats' propellers and tourism activities.

Quality of the ecosystem can be assessed, among others, by the species abundance and species distribution that expressed interaction of abiotic (chemical and physical) and biotic components (Pearson and Rosenberg 1978). Similarly in the distribution and abundance of seagrass species, it is influenced by salinity, temperature, tidal and muddy substrate or muddy sand (Hemminga and Duarte 2000). The seagrass ecosystem has many functions, such as a habitat of the coastal aquatic organisms, and a regulator of pollutants and carbon (Liberatus 2016). Fourqurean et al. (2012) explain that seagrass could store up to 83,000 metric tons of carbon per km^2 or can save twice as much as forest. It is estimated that the seagrass ecosystem is able to sequestrate 10% of the total carbon received by the oceans each year.

The study of potential seagrass as carbon storage and carbon sequestration have been carried out at various coastal waters locations on Indonesia, such as on the coastal waters of Pari Island at Jakarta Bay by Kiswara (2010) from the Oceanography Research Center (The Indonesian Institute of Sciences) especially for species of *Enhalus acoroides*, *Cymodocea rotundata* and *Thalassia hemprichii*. Rahmawati and Kiswara (2012) have continued *Enhalus acoroides* research in sequestrating, releasing and storing carbon. Study at the community level has been done by Rahmawati (2011). A similar study has also been conducted in Barrang Lompo Island Makassar (Supriya 2014) and Banten bay Waters (Rustam 2016), but both did not measure the ability of carbon storage which was assessed by the difference between the amount of carbon absorbed and the amount of carbon released.

Based on the findings in Kiswara (2010) and Rahmawati (2011) researches, the research on carbon

storage capability in seagrass ecosystems on the coastal waters of Pari Island might be used as a reference for similar studies in other coastal waters in Indonesia. Referring to the research that has been conducted in Pari Island, this study answers the following questions : a) what are the species, abundance, and density of seagrass ecosystem at Pari Island; b) how much the carbon sequestration capacity ($gC/m^2/day$) of the 4 selected seagrass species (*Cymodocea serrulata*, *Halophila ovalis*, *Halodule uninervis* and *Syringodium isoetifolium*) in the waters surrounding Pari Island, Jakarta Bay; and, c) how much carbon is stored (gC/m^2) in those 4 selected seagrass species in the waters surrounding Pari Island, Jakarta Bay.

MATERIALS AND METHOD

Study area

This study was conducted in December 2014 in seagrass communities in waters surrounding Pari Island, Jakarta Bay. Surveys are done on the abundance and density of seagrass in 122 plots (size = 50×50 cm^2) spreading across 6 research stations**.**

Procedure

Quantitative method is used in this study, and data collection is done by survey using 122 square plots technique (50 x 50 cm) along imaginary transects. Transects for data collection (composition, abundance and density of seagrasses) were placed on 6 stations, namely, North Station I (20 plots); North Station II (14 plots), West Station (10 plots), South Station I (21 plots), South Station II (27 plots), and South Station III (30 plots). Carbon sequestration capacity was measured from four species of seagrass (*Cymodocea serrulata*, *Halophila ovalis*, *Halodule uninervis* and *Syringodium isoetifolium*) which were found only at the South Station. Parameters taken at each station were the percentage of seagrass covering using visual estimation method based on the SeagrassWatch Standard Guidance for Seagrass Covering (McKenzie et al. 2003) (Figure 1).

Data analysis

Species identification was done by following method of Husni (1999). Seagrass abundance and density were calculated using the Seagrass Watch Standard (McKenzie *et* al. 2003, English et al 1997). The carbon storage and carbon sequestration measurements were conducted using the approach of density, biomass and carbon content (% C) relationship in different organs of seagrass plants by Rahmawati and Kiswara's (2012) method and its conversion into organic carbon used the Walkley and Black's methods (Schumaker 2002).

RESULTS AND DISCUSSION

Seagrass structure

In general, the seagrass community on Pari Island was composed by two or more species, except in the western coast (West Station/A) where only one species was found. Of the seven species recorded in this study, *Thalassia hemprichii* (Sum Dominance Ratio/SDR = 25.5%) and *Enhalus acoroides* (SDR = 58.4%) were the most dominant. Seagrass species was distributed across the sampling plots (total = 122) at six research stations. *Enhalus acoroides* was found in every research station and *Thalassia hemprichii* was found in four research stations. On the other hand, other seagrass species (*Cymodocea rotundata*, *Cymodocea serrulata*, *Halodule uninervis*, *Halophila ovalis*, and *Syringodium isoetifolium*) were only found in a few plots at the southern coast of Pari Island (South Station/B) (Figure 1).



Figure 1. Location map in the coastal waters of Pari Island, Jakarta Bay, Indonesia. A. West Station, C-D. South Station, and E-F. North Station. The black dotted line is a square plot transects to show the plot location for density and biomass sampling. The sign (☼) indicates the location of leaf growth measurement and seagrass litter traps for calculation of carbon sequestration capability

The seagrass covering in plot area referred to Rahmawati (2011), the average of seagrass covering on Southern Beach and Western Coast was 22.86% and 23.17%. For the seagrass covering at Northern Coast with the range from 21.50% to 30.50% referred to Kiswara's method (2010). From the result from these four locations, it can be concluded that the highest seagrass density recorded at South Station I with 261±107 stands/m^2 was *Thalassia hemprichii*, while the lowest density of seagrass recorded at South Station II with 22±3 stands/m^2 was *Enhalus acoroides* (Figure 2). The same picture showed that the seagrass species at each study site had different density values, for example *Enhalus acoroides* at North Station II and West Station ranging from 67±28 stands/m^2 and 62±28 stands/m^2. This value was lower than the average density in some other smaller seagrass species found in the southern part of Pari Island, such as *Cymodocea serrulata* (84±34 stands/m^2), *Halodule uninervis* (82±59 stands/m^2), *Syringodium isoetifolium* (118±71 stands/m^2) and *Halophila ovalis* (67±12 stands/m^2) at South Station III. The data indicated that seagrass density per unit area was highly dependent on species covering it. Nienhuis et al. (1989) noted that seagrasses with large morphological features, such as *Enhalus acoroides*, tended to have lower coverage density compared to those with smaller morphological features such as *Halodule uninervis*.

Seagrass biomass

Seagrass biomass is closely related with the size of seagrass' morphological features. Seagrass with large morphological features, such as *Enhalus acoroides,* has higher biomass values compared to those with smaller morphological features, such as *Halophila ovalis*. Biomass of the seagrass are shown in Table 1. The highest seagrass biomass average was found in North Station II (772.65±461.85 gBK/m^2) and West Station (536.46±275.68 gBK/m^2), largely populated by *Enhalus acoroides*. The lowest seagrass biomass average was found in South Station III (1.05±0.42 gBK/m^2), largely populated by *Halophila ovalis.* The findings in the western coast were consistent with Rahmawati (2011) that the dominant *Enhalus acoroides* population resulted in larger biomass values than that in the mixed population at southern coast.

Biomass value is closely linked with extracted parts of seagrass to calculate its overall biomass. Seagrass rhizome was part of the seagrass with the highest biomass value (361.11±196.53 gBK/m2 for *Enhalus acoroides*) and the lowest was 0.14±0.04 gBK/m2. Similarly, for *Halophila ovalis,* the highest was 0.45±0.28 gBK/m2 and the lowest was 0.32±0.16 gBK/m2 compared with biomass of its shoot (lamina and bract) of the same species (265.97±167.01 gBK/m2 and 36.11±27 gBK/m2) for *Enhalus acoroides*, while for *Halophila ovalis* the highest was 0.5±0.44 gBK/m2 and the lowest was 0.33±0.13 gBK/m2. Similar findings were found in Kiswara's research (2010) that the average biomass of seagrass rhizome is higher than other parts of the plant. Nienhuis et al. (1989) reported that the biomass of seagrass lower parts (roots and rhizomes) are six to ten times greater than the upper parts (bract and lamina).

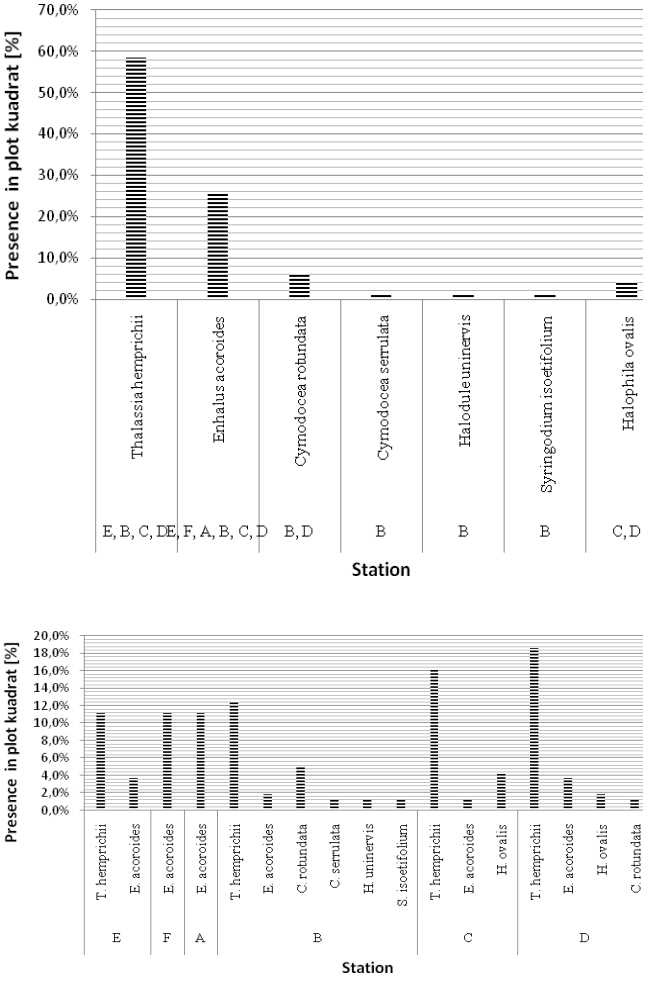


Figure 1. Abundance (in %) of seagrass species at any location / station in the coastal waters of Pari Island. West Station (A); South Station (C, D) and North Station (E, F)

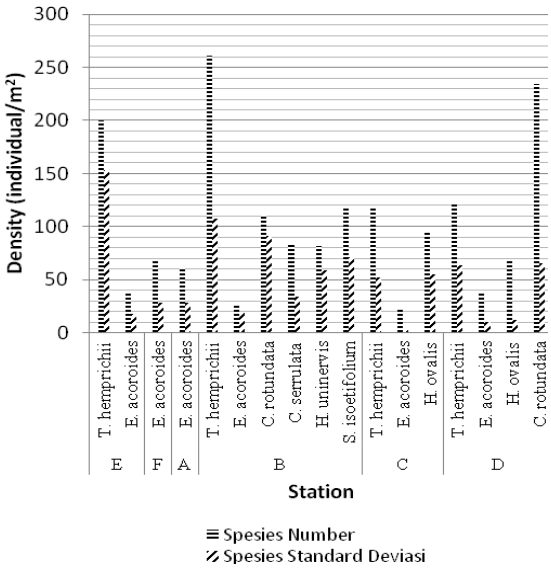


Figure 2. Density (stand/m^2) at each location/station on Pari Island coastal waters. West Station (A); South Station (C, D) and North Station (E, F)

Seagrass carbon sequestration capacity

Carbon sequestrated via photosynthesis process is stored and distributed into the seagrass compartments, among others, in its biomass both above and below the substrate. Since carbon stored in biomass, particularly in below the substrate part of plants, it will be kept for long periods of time. This made the long-term role of seagrass in carbon sequestration is important (Kiswara and Ulumuddin 2009). Based on carbon storage capacity calculations, measured by the amount of carbon captured (from primary productivity above and below ground) and released (from leaf litter and consumption by herbivores), seagrass in coastal waters surrounding Pari Island is able to store from 0.007 to 1.825 gC/m^2/day while releasing only 0.001 to 0.777 gC/m^2/day (Table 2). The overall capacity of the seven species of seagrass is between 0.006 and 1.048 gC/m^2/day.

Table 1. Species composition and seagrass Bbomass research area Pari Island in Jakarta Bay, Indonesia

Station	Species	Number of plot	Par.	Seagrass biomassa (gBK/m^2)				Total of biomassa (gBK/m^2)
				Leaf strand	Leaf base	Stem	Root	
North 1	*T. hemprichii*	18	X	44.33	56.99	76.85	51.80	229.96
(N = 20)	*(n=18)*		Sd	44.09	49.33	76.22	45.70	215.34
	E. acoroides	6	X	77.40	33.18	189.99	61.04	361.61
	(n=6)		Sd	60.65	17.11	151.95	37.61	267.32
North 2	*E. acoroides*	14	X	265.97	71.47	361.11	74.09	772.65
(N = 14)	*(n=14)*		Sd	167.01	53.69	196.53	44.61	461.85
West	*E. acoroides*	10	X	195.39	50.99	233.02	57.06	536.46
(N = 10)	*(n=10)*		Sd	96.48	23.97	118.77	36.47	275.68
South 1	*T. hemprichii*	20	X	33.35	45.38	65.68	21.95	166.37
(N = 22)	*(n=20)*		Sd	30.98	37.51	57.68	18.50	144.68
	E. acoroides	3	X	68.04	24.74	85.73	2.96	181.47
	(n=3)		Sd	61.99	21.87	78.11	3.18	165.14
	C. rotundata	8	X	3.97	7.89	9.74	3.09	24.68
	(n=8)		Sd	3.51	7.37	8.16	2.95	21.99
	C. serrulata	2	X	4.01	2.53	14.34	2.40	23.28
	(n=2)		Sd	1.86	1.31	7.66	1.22	12.05
	H. uninervis	2	X	5.13	2.20	6.78	0.46	14.57
	(n=2)		Sd	3.97	1.77	5.35	0.22	11.31
	S. isoetifolium	2	X	1.29	0.93	2.11	2.55	6.88
	(n=2)		Sd	0.91	0.52	1.24	1.65	4.32
South 2	*T. hemprichii*	26	X	11.45	14.04	31.73	15.68	72.89
(N = 27)	*(n=26)*		Sd	10.79	11.59	26.83	13.19	62.40
	E. acoroides	2	X	36.11	13.93	97.81	24.81	172.66
	(n=2)		Sd	27.00	1.02	74.53	31.47	134.02
	H. ovalis	7	X	0.50	0.38	0.45	0.38	1.72
	(n=7)		Sd	0.44	0.31	0.28	0.33	1.37
South 3	*T. hemprichii*	30	X	17.28	22.59	28.02	12.92	80.81
(N = 30)	*(n=30)*		Sd	16.98	18.98	23.51	8.90	68.37
	E. acoroides	6	X	83.44	30.45	113.78	72.69	300.37
	(n=6)		Sd	31.86	22.00	45.06	49.63	148.54
	H. ovalis	3	X	0.33	0.14	0.32	0.25	1.05
	(n=3)		Sd	0.13	0.04	0.16	0.09	0.42
	C. *rotundata*	2	X	9.90	19.71	14.12	6.00	49.73
	(n=2)		Sd	0.64	1.42	10.66	1.94	14.67

Table 2. Seagrass carbon storage (gC/m^2/day) in Pari Island, Jakarta Bay, Indonesia

Species	Productivity *above ground*	Productivity *below ground*	Absorption	Herbivore consumption	Litter	Discharge	Storage
	(gC/m^2/hr)	(gC/m^2/hr)	(gC/m^2/hr)	(gC/m^2/hr)	(gC/m^2/hr)	(gC/m^2/hr)	(gC/m^2/hr)
1	2	3	4	5	6	7	8
			(= 2 + 3)			(= 5 + 6)	(= 4 - 7)
E. acoroides	1.368	0.457	1.825	0.339	0.438	0.777	1.048
T. hemprichii	1.235	0.192	1.427	0.265	0.490	0.756	0.671
C. rotundata	0.313	0.076	0.388	0.072	0.123	0.195	0.193
C. serrulata	0.029	0.065	0.094	0.018	0.033	0.050	0.044
H. uninervis	0.014	0.035	0.049	0.009	0.012	0.021	0.028
S. isoetifolium	0.027	0.028	0.055	0.010	0.004	0.014	0.040
H. ovalis	0.002	0.005	0.007	0.001	0.000	0.001	0.006

Discussion

Seve species out of 12 species of seagrass species found in Indonesian waters (Syukur 2015) were recorded on the coastal waters of Pari Island and they were commonly found in the Kepulauan Seribu Marine National Park (BTNLKpS 2014). While, compared with the number of species found in the seagrass ecosystems in some other coastal areas, such as the coast of Tanjung Luar Lombok Timur (9 species recorded, Syukur et al. 2011); Tanjung Lesung Teluk Miskam Banten, (7 species recorded, Rustam et nal. 2013) and Kuta Bali beach (8 species recorded, Graha 2015), the diversity of seagrass species on coastal waters of Pari Island is categorized to be generally existed in other coastal waters of Indonesia. From the dominant species found in the coastal waters seagrass ecosystem of Pulau Pari and compared with the previously mentioned ecosystem, it can be seen that the diversity and dominant of seagrass species are not similar. It is strongly influenced by various environmental factors in the ecosystem, such as the type of substrate, sunlight intensity, water depth and water current velocity. As well as seagrass species found in other coastal ecosystems in Indonesia, on Pari Island the *Enhalus*, *Thalassia*, *Syringodium* and *Halophila* are easily found in the upper sublitoral and intertidal (Bengen 2004) with less than 5-10 m water depth.

Based on data of covering level on each plot, *T. hemprichii* (699 individuals/m^2) was estimated to have the highest density and followed by *C. rotundata* (344 individuals/m^2), the lowest density was *C. serrulata* and *H. uninervis* (80 individuals/m^2). Different from the seagrass ecosystems on coastal water of Sanur Bali, where *H. uninervis* and *S. isoetifolium* were the species with the highest covering (2189 individuals/m^2 and 1660 individuals/m^2) and the lowest was *C. serrulata* (35 individuals/m^2). Density conditions of each seagrass ecosystem would provide carbon storage data at each site, where the type and density of each species were important. Liberatus (2016) and Björk et al. (2008) explained that not only the species and its covering but also the storage of organic carbon was affected by the productivity of seagrass and its carbon sequestration capability. Nevertheless, Graha (2015) showed that seagrass ecosystem stored carbon more in the root and rhizome than on the substrate itself.

The seven species of seagrass with their sequestration capability showed that total seagrass carbon storage on Pari Island was influenced by the species with the largest carbon storage capability (*E. acroides* and *T. hemprichii*). This was because *E. acroides* had a high biomass because of its large morphological size, whereas *T. hemprichii* had a high biomass value because it was distributed widespread on the area of Pari Island. These results were similar to Graha (2015) about the species *E. acroides* and Liberatus (2016), Björk et al. (2008) about the productivity of each seagrass species (Table 2).

The carbon sequestration capability of seagrass on Pari Island contributed to the importance of seaweed as a world carbon storage (blue carbon) especially for the tropical area that contributed between 0.23-346.10 gC/m^2 of carbon sequestration capabilities of the world's seagrass ecosystem (1.684-110.819 gC/m^2). This showed that seagrass in the tropical area are good climate change mitigation agents (Liberatus 2016).

Seven seagrass species were found on the seagrass ecosystem in the coastal waters of Pari Island with *T. hemprichii* as a most dominant species. Carbon storage capacity ranged from 0.006-1.048 gC/m^2/day, depended on species and species abundance (density). The ability to store carbon on the coastal water of Pulau Pari, Jakarta Bay ranged between 0.23-346.10 gC/m^2 and was dominantly stored in the root, rhizomes and dead organs of seagrass lies in its substrate.

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Collaborative planning for development of the Pelawan Biodiversity Park in Bangka, Indonesia

DIAN AKBARINI[1,2], JOHAN ISKANDAR[1,3,4], RUHYAT PARTASASMITA[3,4,♥]

[1]Postgraduate of Environmental Science (PSMIL & DIL), Universitas Padjadjaran. Jatinangor, Sumedang 45363, West Java, Indonesia
[2]Board of Development Planning and Regional Develoment Research of the Central Bangka District. Jl. Titian Puspa 2, Koba, Central Bangka District, Bangka-Belitung Archipelago Province, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia. Tel.: +62-22-77912. ♥email: ruhyat.partasasmita@unpad.ac.id, rp2010rikkyo@gmail.com
[4]Institute of Ecology (PPSDAL), Universitas Padjadjaran. Jatinangor, Sumedang 45363, West Java, Indonesia

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Abstract. *Akbarini D, Iskandar J, Partasasmita R. 2017. Collaborative planning for development of the Pelawan Biodiversity Park in Bangka, Indonesia. Biodiversitas 18: 1602-1610.* Pelawan Biodiversity Park is located in the village of Namang, Sub-district of Namang, District of Central Bangka. The Pelawan Biodiversity Park with a size of approximately 47 hectares is intended as a conservation area in Bangka to protect various distinctive plants and animals, especially the plant species *Tristaniopsis merguensis* (Griff.) Peter G.Wilson & J.T.Waterh. A preliminary review of Pelawan Biodiversity Park management planning was undertaken between January and March 2017. The purpose of this preliminary review was to obtain information for planning and managing the Pelawan Biodiversity Park which has an important function for conservation, ecotourism and research in the Central Bangka District. The method used for the study was qualitative with field data collection; namely observation, intensive interviews with informants and analysis of documentation. The results of the study show that the management planning for Pelawan Biodiversity Park has not been implemented collaboratively with stakeholders. Furthermore, the potential for biodiversity in the Palawan Biodiversity Parks has not been properly analyzed and used to develop strategies for conversation, ecotourism and research. The proposed solution to this deficiency in the planning of the Pelawan Biodiversity Park is to invite relevent stakeholders comprising village government, community leaders, universities, researchers, non-governmental organisations and private organizations to actively collaborate in the process.

Keywords: Biodiversity park, collaborative planning, conservation *in-situ*, ecotourism*, Tristaniopsis merguensis*

INTRODUCTION

Indonesia is a tropical archipelago situated between the continents of Asia and Australia, and also located between two oceans, the Pacific and Indian Ocean. As a result, Indonesia has a high diversity of flora and fauna, as well as a highly diverse human culture (Iskandar 2016). Based on recent data from the Indonesian Institute of Sciences (LIPI), flora diversity in Indonesia recorded 1,500 species of algae 1,500 species of algae; 80,000 species of organisms with spores (i.e. Cryptograms) for example fungi; 595 species of moss and 2,197 species of ferns; and 30,000-40,000 species of seed plants (15.5 % of the total number of the world's flora). Meanwhile, in terms of fauna, it is documented that Indonesia has 8,157 vertebrate species (mammals, birds, herpetofauna, and fish) and 1,900 species of butterflies (10% of the world's species) (Widjaja et al. 2014).

Biodiversity has an important role in human life, either directly or indirectly (Koziell 2001, Iskandar 2015). Biodiversity has direct functions for human society in terms of providing subsistence and trade-able goods for village people. Indirect functions of biodiversity consist of ecosystem services such as the production of oxygen and the fixing of carbon by vegetation, and the maintenance of gene pools for constituent organisms. There are currently unused functions of biodiversity such as genetic resources that can allow for development of future options in agriculture. Biodiversity also provides for various other purposes such as the development of ecotourism.

To conserve various species of flora and fauna, the Indonesian government has developed both *ex-situ* species conservation and in-situ species conservation strategies. Facilities for ex-situ species conservation consist of botanical gardens, zoological gardens, safari parks, and gene banks. Facilities for *in-situ* species conservation comprise the nature reserves, wildlife reserves, national parks, national recreational parks, hunting parks, and protection forest areas (Supriana and Sukandar 1996; Wiratno et al. 2004). In addition, with the purpose of conserving various species and their genetic resources in some places of Indonesia, the Government of the Republic of Indonesia through the Minister of Environment issued the Regulation of the Minister of Environment of the Republic of Indonesia Number 03 Year 2012 on development of Biodiversity Parks (*Taman Kehati*) (Anonymous 2012). A Biodiversity Park may be defined as a reserve area of local biological natural resources outside official forest areas, which have *in-situ* and/or *ex-situ* conservation functions, especially for pollination of plants and/or seed dispersal. The Biodiversity Parks achieve this

by maintaining the structure and composition of vegetation that can support resident populations of pollinating animals and seed dispersers.

Currently, there are 72 Biodiversity Parks (*Taman Kehati*) in 22 Provinces of Indonesia. One of the Biodiversity Parks is the Pelawan Biodiversity Park located in Namang Village, Namang Sub-district, Central Bangka District of Bangka Belitung Archipelago Province. The Pelawan Biodiversity Park has many functions. One of them is to conserve the special tree of Pelawan/Pelawan Merah (*Tristaniopsis merguensis* (Griff.) Peter G.Wilson & J.T.Waterh) This species has been recognized as one of the key species for biodiversity sustainability in Central Bangka district, since the Pelawan Tree can guarantee the growth of the fungus Heimioporus sp. and as a feed tree by Apis dorsata (honeybee) because the nectar of the tree is used as the main feed (Akbarini 2016).

The development of Pelawan Biodiversity Park was initiated by the Regional Government of Central Bangka District in 2013. Pelawan was chosen as the location for the Biodiversity Park because this area has various plant species that are beneficial for the livelihood of the community, especially the *Pelawan Merah* Tree (*Tristaniopsis merguensis*). This plant has an important role as host of the fungus (*Heimiporus* sp), and its nectar is food for *Apis dorsata* bees that are known to produce 'bitter honey'. This honey is a popular product of the Bangka Belitung Archipelago Province. Moreover, the bees are important pollinators of various local plant species in the region.

Since Pelawan was first proposed as the location for a Biodiversity Park, so an area was proposed by the Namang Village Government as a protected area in Namang Village. In addition, it was officially established through the Decree of the Central Bangka Regent No.188.45/586/DPK/2009 on the Determination of the *Kalung* Area of Namang Village, Namang Sub-district, Central Bangka District as a Protected Area.

Although biodiversity is known to play an important role for ecological, socioeconomic and cultural functions for human well-being, loss of various species of flora and fauna have occurred both within conservation and non-conservation areas in Indonesia. Indeed, in some instances, conservation areas have not properly guaranteed protection of biodiversity.

Despite the fact that biodiversity conservation has direct socio-cultural consequences for the lives of local people, its role in the management of flora and fauna, including whole ecosystems has often been neglected, even ignored (Cunningham 2001; Newing et al. 2011). As a result, in some cases, local people have not obtained appropriate benefits from such conservation programs (Iskandar et al. 2016; Partasasmita et al. 2016).

This paper reports recent preliminary research to elucidate a model for collaborative planning in the development of the Pelawan Biodiversity Park of Bangka. This model will be improved upon and completed by further, more intensive study in the near future. Aspects discussed in this paper focus particularly on a general view of the Pelawan Biodiversity Park, on history of the area, on its current biodiversity, and on planning for management of the Palawan Biodiversity Park.

MATERIALS AND METHODS

Study area

The study was conducted at Pelawan Biodiversity Park (*Taman Kehati Pelawan*) situated in Kalung Area, Namang Village, Namang Sub-district, Central Bangka District of Bangka Belitung Archipelago Province, Indonesia, located $2^0$19'18" South latitude and $106^0$09'51" East Longitude (Figure 1). Pelawan Biodiversity Park (*Taman Kehati Pelawan*) with a total area of 47 hectares was built by the Government of Central Bangka District. The Pelawan Biodiversity Park is a forest area that lies in Namang Village. The location is about 40 km from the central district. Namang village is an area of 37.76 km^2 (18.47% of Namang District). Geographically, the location of Namang District is considered as interior, not close to the coast. Physically, Namang area is located in the lowlands at an altitude of between 0 and 10 meters above sea level. Topographically, it is considered flat. Namang has an average rainfall of about 1,200 mm per year (Statistics Bureau of Bangka Tengah 2016).

Procedure

The study method used was qualitative (Newing et al. 2011; Creswell 2014). Data collection included field observations, in-depth interviews with several informants, and an analysis of relevent documents. The field observations were undertaken to determine the general conditions of the forest area of Pelawan Biodiversity Park, Intensive interviews were conducted with competent informants. The informants were purposively chosen, namely key representative staffs of the Environment Agency of the Central Bangka District (*Bappelitbangda*), the Forestry Service of the Provincial Government (*Dinas Kehutanan Provinsi*), the Namang Sub-district (*Kecamatan*), the Namang Village, the managers of the Pelawan Biodiversity Park management unit, and several local people.

Data analysis

Data resulting from the documents, field observations and deep-interviews were analyzed by cross-checking, summarizing, synthesizing, and building up a narrative account based on descriptive and evaluative analysis (cf. Newing et al. 2011).

RESULTS AND DISCUSSION

A model of Pelawan Biodiversity Park management was constructed based on the principles of Community-Based Resource Management (MacKinnon 1981; Richardson and Pugh III 1981; RCCO-IPB 1998). Steps in such a procedure include obtaining a general understanding of the Pelawan Biodiversity Park system; problem definition; system conceptualization; model formulation; simulation of the model; policy analysis, and policy

implementation. In this paper, however, only the preliminary steps were carried out: in particular, the development of an understanding of the system, of the Park's potential biodiversity, and of the various obstacles and constraints; followed by elucidation of a planning model for the Pelawan Biodiversity Park.

System of the Pelawan Biodiversity Park

The Pelawan Biodiversity Park ecosystem is composed of several sub-ecosystems: namely flora and fauna (the forest), visitors, local people, managers of the biodiversity park, and government (Figure 2).

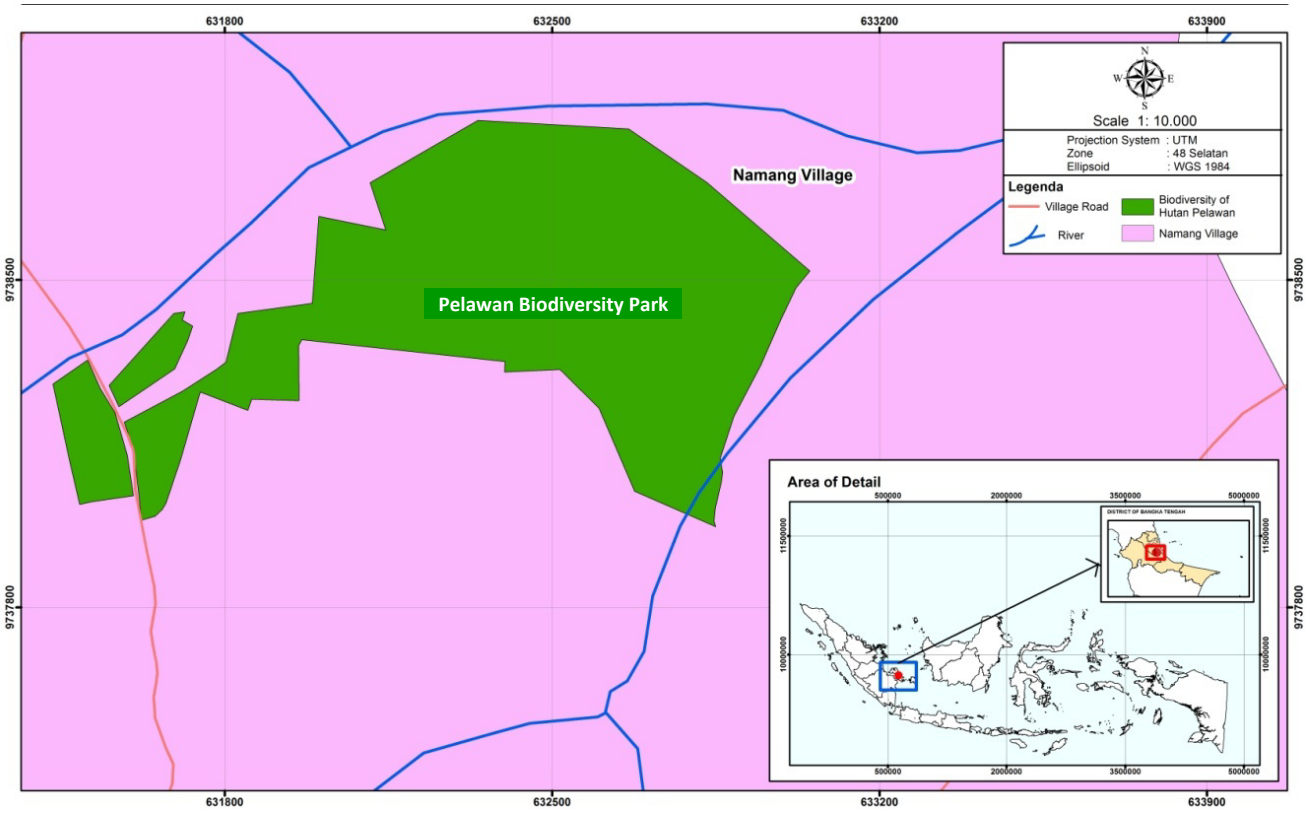


Figure 1. Research location at Pelawan Biodiversity Park in the Kalung Area, Namang Village, Namang Sub-district, Central Bangka District, Bangka Belitung Archipelago Province, Indonesia

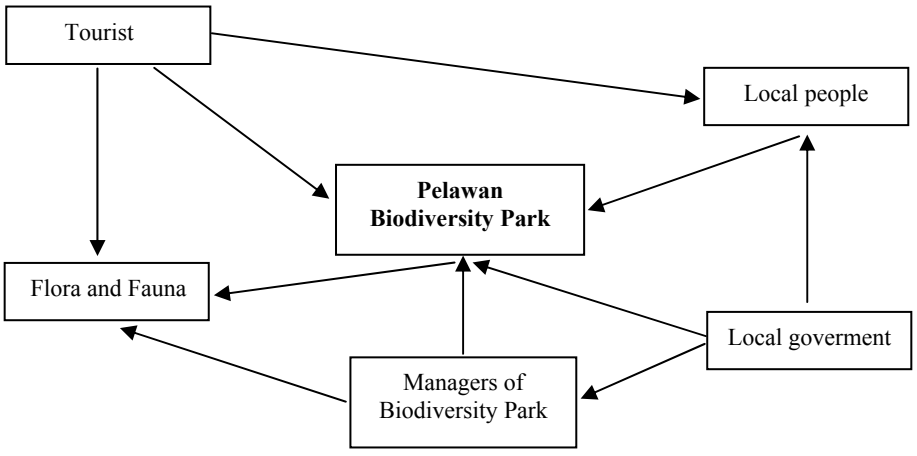


Figure 2. System of the Palawan Biodiversity Park, Central Bangka District, Bangka Belitung Archipelago Province, Indonesia consists of the flora and fauna, local government, local people, and managers

The components that make up the ecosystem of Biodiversity park are interconnected with each other. Therefore, changes in one component may cause changes in other components. The management objectives of Pelawan Biodiversity Park are primarily to protect the flora and fauna, as well as the area, so that the park can be useful in providing ecological services as well as socio-economic and cultural benefits to society. The ecological benefits include conservation of the living species and their genetic diversity; the preservation of the integrity of an oxygen-producing, carbon-fixing forest ecosystem; protection of the soil from erosion; regulation of the hydrological system; and preservation of wildlife habitats. At the same time, socio-economic and cultural benefits to society include among others the strengthening of regional science, education of the community in the values of eco-conservation; and promotion of different kinds of forest products, such as mushrooms and medicinal herbs.

Various kinds of potential disturbances must be taken into account in developing a management model for the Pelawan Biodiversity Park, including the impacts of activities by local people or by visitors.

The history of Pelawan Biodiversity Park

Information gained from intensive interviews with Namang village elders indicates that in the era before Indonesian independence the Kalung area was recognized as a primary forest area. However, over time, with increasing populations in the post-independence era, the Kalung area began to change; forest was cleared and used as places to plant upland rice fields or swidden rice (*hume* or *ladang*) as well as gardens. The activity of swidden rice farming (*hume*) was practiced until about the early 1990s. After that, the Kalung area was considered to be village forest consisting only of secondary forest and shrub land (*blukar*) along with residual planted crops such as rubber and fruit-bearing trees. Moreover, the area became a place for people to obtain various incidental subsistence needs, such as stakes to support pepper plants (*junjung* in the language of Bangka), traditional medicinal plants, timber for building materials, honey from bees, mushrooms and ornamental plants.

After declaration of Decree of the Minister of Trade and Industry no. 146 of 1999, issued in 2001, tin is no longer considered a strategic commodity, so its mining and trade should not be overseen by the central government (Nordholt and Klinken 2007). Therefore, in the area around the Kalung area, which is an informal tin mining site by the people of Namang Village, became more developed in 2004. This area is located not so far from Kalung and is called by local people as the Aik Layang area. In that year, also, many village households used wood from the forest for building materials, either to supply their own needs or else to sell to earn cash income.

Due to the large number of people involved in mining tin at that time, local people, including the village head, became worried that the Kalung area would be expanded to serve the interests of both miners and private companies. Therefore, in 2002 the head of the village decided that the Kalung area had to be preserved. His concern was also shared by the Village Consultative Board (BPD). At that time, the forest area of Kalung was also being encroached upon by illegal cutting. So, in 2008, because of the environmental destruction caused both by tin mining and forest encroachment, the villagers of Namang Village through their village head proposed that the Namang forest be declared a protected area. Agreement for this proposal resulted in the formal setup of a Protected Area in *Kalung Area* under the Namang Village Rule No. 3 Year 2008.

At the same time, on the basis of the Decision of the District Government of Bangka Tengah Number 188.45/586/DPK/2009 on the Determination of *Kalung Area* Namang Village, the area in Namang was declared to be a Protected Area. This determination was in line not only with the dynamics of regional development, but also took into account the potential gain from managing this protected area of *Kalung* to be utilized more optimally for the the welfare of the community. It envisaged that the Kalung forest area would function not only as a site for environmental conservation, but also as an opportunity for the development of ecotourism and regional research particularly for the development of the area's pelawan tree population (*Tristaniopsis merguensis*). As a result, the Government of Central Bangka District determined that this area become a Biodiversity Park (*Taman Kehati*) by the Decree of District of Central Bangka Number 188.45/403/KLH/2013 on the Determination of Kalung Area as the Pelawan Biodiversity Park (Decree Bangka Tengah 2013).

The decision of the Central Bangka Government to nominate the Kalung Protection Area as a Biodiversity Park (*Taman Kehati*) was also supported by the Government of the Republic of Indonesia, through the Minister of Environment, via its Regulation of the Minister of Environment No. 3 of 2012 on Biodiversity Parks (Anonymous 2012), in accordance with the implementation of Law Number 32 Year 2009 on Protection and Environmental Management (Anonymous 2009). It stated that in order to implement development of natural resource reserves, central Government, provincial governments, district/municipal governments or individuals can build Biodiversity Parks outside of formal forest areas, thus allowing for determination of the change in status of the *Kalung Area* from Protected Area to Biodiversity Park (*Taman Kehati*), by implementation of Regulation of Environment Minister No. 3 Year 2012. At the time of the development of the Biodiversity Park in Namang Village, there were no private initiatives in the area, such as by the Aqua Danone Group (Gunawan and Sugiarti 2015; Gunawan et al. 2016); nor initiatives from universities such as Semarang State University (Priyono et al. 2015).

The potential of flora and fauna diversity in Pelawan Biodiversity Park

The area of Pelawan Biodiversity Park has a high diversity of flora and fauna, with the potential to attract the interest of visitors and to stimulate the development of ecotourism in the area (Wistaria et al. 2016). In terms of

the flora, for example, it has been recorded that the precinct contains at least 93 species in the seedling stage, 69 species at the stake stage, 29 species at the pole stage, and 9 species in the tree stage. In addition, this area is known to have a rich fauna. Based on the primary study of the area, there has been recorded 6 species of amphibians, 8 species of reptiles, 13 species of mammals, 33 species of birds and 6 types of insects (Wistaria et al. 2016). As examples of this biodiversity, the birds commonly found in the Pelawan Biodiversity Park are listed in Table 1, while a listing of the common flora in the Park is presented in Table 2.

Among the fauna, there is a species of tarsier that is distinctive of the local fauna in Bangka Belitung Archipelago Province; namely *Cephalopachus bancanus* (local Bangka name: *Mentilin*; Indonesian name: *Krabu Ingkat*). Based on the conservation status listings of IUCN, this is considered to be a vulnerable species.

In addition, a wide variety of bird species have been recorded in the Palawan Biodiversity Park (Table 1). Some of the birds are known as protected species based on National Indonesian Law and international assessment by the IUCN. For example, *Setornis criniger* is considered a vulnerable species on the IUCN Red List. Because this species is restricted to low-lying forests in regions where this habitat type is being cleared and degraded at a catastrophic rate, rapid and continuing population decline is suspected (Birdlife International 2017 b). Other bird species, including *Rollulus rouloul, Lophura ignita, Phaenicophaeus sumatranus* and *Otus rufescens*, are considered as Near Threatened on the IUCN Red List. In addition, though the Oriental Honey-buzzard, *Pernis ptilorhyncus,* is documented as of least concern based on the Birdlife International (2017 a) it is classified as a protected animal by the Indonesian Government, based on the law no 5 of 1999 on protection of Biodiversity and Ecosystems, and Government Regulation no 7/1999 on fauna preservation (Anonymous 1999).

Among the unique and distinctive flora in the area,is the Pelawan tree *Tristaniopsis merguensis*. The presence of the Palawan forest in the Kalung area was considered a basis for the establishment of the Biodiversity Park (*Taman Kehati*) in the Province of Bangka. *Tristaniopsis merguensis* is a recognised habitat for the growth of mushrooms around its roots. The *Pelawan* mushrooms include a *Heimiporus* sp. (a member of the *Boletaceae* family of mushrooms), popularly named by local people as *kulat/jamur pelawan*. These mushrooms grow in symbiosis to form an ectomycorrhizal association with Pelawan trees. The Pelawan mushrooms are recognized as a food source of omega-6 and omega-9 fatty acids. The Pelawan mushrooms contain seven essential amino acids namely valine, methionine, threonine, isoleucine, phenylalanine and lysine. The Pelawan mushrooms are also a source of natural antioxidants with the ability to capture free radicals. The antioxidant components present in the fungus are phenolic components (4.77mg GAE/gbb), β-carotene (15.37 μg/gb) and lycopene (6.34 μg/gb) (Rich 2011).

Table 1. Biodiversity of birds in Pelawan Biodiversity Park, Central Bangka District, Bangka Belitung Archipelago Province, Indonesia

Family	Species	English name	Indonesian name
Accipitridae	*Pernis ptilorhynchus* (Temminck, 1821)	Oriental Honey-buzzard	*Sikep Madu Asia*
Ardeidae	*Gorsachius melanolophus* (Raffles, 1822)	Malayan Night-heron	*Kowak Melayu*
Ardeidae	*Ixobrychus eurhythmus* (Swinhoe ,1873)	Schrenk's Bittern	*Bambangan Coklat*
Caprimulgidae	*Caprimulgus affinis* (Horsfield, 1821)	Savannah Nightjar	*Cabak Kota*
Caprimulgidae	*Lyncornis temminckii* (Gould, 1838)	Malaysian Nightjar	*Taktaran Melayu*
Columbidae	*Chalcophaps indica* (Linnaeus, 1758)	Emerald Dove	*Delimukan Zamrud*
Colimbidae	*Geopelia striata* (Linnaeus, 1766)	Zebra Dove	*Perkutut Jawa*
Columbidae	*Treron vernans* (Linnaeus, 1771)	Pink-necked Green Pigeon	*Punai Gading*
Columbidae	*Spilopelia chinensis* (Scopoli, 1768)	Spotted Dove	*Tekukur biasa*
Cuculidae	*Cacomantis merulinus* (Scopoli, 1786)	Plaintive Cuckoo	*Wiwik Kelabu*
Cuculidae	*Cacomantis sepulclaris* (Müller S., 1843)	Rusty-breasted Cuckoo	*Wiwik Uncuing*
Cuculidae	*Cacomantis sonneratii* (Latham, 1790)	Banded Bay Cuckoo	*Wiwik Lurik*
Cuculidae	*Centropus bengalensis* (Gmelin, 1788)	Lesser Coucal	*Bubut Alang-alang*
Cuculidae	*Chrysococcyx minutillus* (Gould, 1859)	Little Bronze Cuckoo	*Kedasi Laut*
Cuculidae	*Chrysococcyx xanthorhynchus* (Horsfield, 1821)	Violet Cuckoo	*Kedasi Ungu*
Cuculidae	*Rhinortha chlorophaeus* (Raffles, 1822)	Raffles's Malkoha	*Kadalan Selaya*
Cuculidae	*Phaenicophaeus curvirostris* (Shaw, 1810)	Chesnut-breasted Malkoha	*Kadalan Birah*
Cuculidae	*Phaenicophaeus sumatranus* (Raffles, 1822)	Chesnut-bellied Malkoha	*Kadalan Saweh*
Phasianidae	*Cortunix chinensis* (Linnaeus, 1766)	Blue-breasted Quail	*Puyuh batu*
Phasianidae	*Lophura ignita* (Shaw, 1797)	Crested Fireback	*Sempidan Biru*
Phasianidae	*Rollulus rouloul* (Scopoli, 1786)	Crested Partridge	*Puyuh Senggayan*
Rallidae	*Amaurornis phoenicurus* (Pennant, 1769)	White-breasted Waterhen	*Kareo Padi*
Rallidae	*Rallina fasciata* (Raffles, 1822)	Red-legged Crake	*Tikusan Celuring*
Stringidae	*Otus lempiji* (Horsfield, 1821)	Collared Scops-owl	*Celepuk Rebah*
Strigidae	*Otus rufescens* (Horsfield, 1821)	Reddish Scops-owl	*Celepuk Merah*
Turnicidae	*Turnix suscitator* (Gmelin, 1789)	Barred Buttonquail	*Gemak Loreng*

Note: Adapted from the Environmental Agency of Bangka Tengah District (2015)

Table 2. Biodiversity of flora in the Pelawan Biodiversity Park, Central Bangka District, Bangka Belitung Archipelago Province, Indonesia

Family	Species
Anacardiaceae	*Campnosperma auriculatum* (Blume) Hook.f.
Apocynaceae	*Alstonia scholaris* (L.) R.B.
Apocynaceae	*Alstonia angustifolia* Wall. ex A.DC.
Apocynaceae	*Alstonia spatulata* Blume
Aquifoliaceae	*Ilex cymosa* Blume
Aquifoliaceae	*Ilex* sp.
Araliaceae	*Polyscias diversifolia* (Blume) Lowry & G.M.Plunkett)
Arecaceae	*Korthalsia* sp
Arecaceae	*Caryota mitis* Lour.
Arecaceae	*Plectocomia elongata* Mart. ex Blume
Arecaceae	*Chamaedorea elegans* Mart.
Clusiaceae	*Garcinia parvifolia* (Miq.) Miq.
Clusiaceae	*Garcinia riedeliana* Pierre
Clusiaceae	*Calophyllum nodosum* Vesque
Clusiaceae	*Calophyllum pulcherrimum* Wall. ex Choisy
Clusiaceae	*Calophyllum soulattri* Burm.f.
Dilleniaceae	*Dillenia suffruticosa* (Griff.) Martelli
Dipterocarpaceae	*Shorea ovalis* (Korth.) Blume ssp. *ovalis*
Dipterocarpaceae	*Vatica rassak* (Korth.) Blume
Dipterocarpaceae	*Vatica chartacea* P.S. Ashton
Elaeocarpaceae	*Elaeocarpus floribundus* Blume
Euphorbiaceae	*Hevea brasiliensis* (Wild. ex A. Juss.) Müll.Arg
Euphorbiaceae	*Macaranga pruinosa* (Miq.) Müll.Arg
Phyllanthaceae	*Aporosa octandra* var. *malesiana* Schot.
Fagaceae	*Lithocarpus bancanus* (Scheff.) Rehder
Fagaceae	*Castanopsis argentea* (Blume) A.DC.
Hypericaceae	*Cratoxylum arborescen*s (Vahl) Blume
Hypericaceae	*Cratoxylum glaucum* Korth.
Lauraceae	*Dehaasia cuneata* (Blume) Blume
Lauraceae	*Litsea* sp
Lauraceae	*Cryptocarya crassinervia* Miq.
Leguminosae	*Adenanthera microsperma* Teijm. & Binn
Melastomataceae	*Pternandra azurea* (DC.) Burkill
Melastomataceae	*Pternandra caerulescens* Jack
Melastomataceae	*Pternandra rostrata* (Cogn.) M.P.Nayar
Moraceae	*Artocarpus integer* (Thunb.) Merr.
Moraceae	*Artocarpus nitidus* Trecul
Myrtaceae	*Leptospermum polygalifolium* Salisb.
Myrtaceae	*Rhodamnia cinerea* Jack
Myrtaceae	*Syzygium barringtonioides* (Ridl.) Masam.
Myrtaceae	*Syzygium bankense* (Hassk.) Merr. & L.M. Perry
Myrtaceae	*Syzygium decipiens* (Koord. & Valeton) Merr.& L.M. Perry
Myrtaceae	*Syzygium caudatilimbum* (Merr.) Merr. & L.M.Perry
Myrtaceae	*Syzygium kunstleri* (King) Bahadur & R.C.Gaur
Myrtaceae	*Syzygium grande* (Wight)Walp.
Myrtaceae	*Syzygium lineatum* (DC.) Merr. & L.M.Perry
Myrtaceae	*Syzygium muellerii* (Miq.) Miq.
Myrtaceae	*Syzygium pyrifolium* (Blume) DC.
Myrtaceae	*Syzygium rostratum* (Blume) DC.
Myrtaceae	*Syzygium zeylanicum* (L.) DC.
Myrtaceae	*Syzygium* sp
Myrtaceae	*Tristaniopsis merguensis* (Griff.) Peter G. Wilson & J.T.Waterh.
Olacaeae	*Ochanostachys amentacea* Mast.
Oleaceae	*Chionanthus ramiflorus* Roxb.
Pandanaceae	*Pandanus furcatus* Roxb.
Pandanaceae	*Pandanus dubius* Spreng.
Pentaphylaceae	*Adinandra dumosa* Jack.
Rhizoporaceae	*Gynotroches axillaris* Blume
Rubiaceae	*Gaertnera vaginans* (DC.) Merr.
Rubiaceae	*Jackiopsis ornata* (Wall).
Rubiaceae	*Timonius flavescens* (Jacq.) Baker
Rubiaceae	*Urophyllum* sp.
Sapotaceae	*Payena leerii* (Teijsm. & Binn.) Kurz
Sapotaceae	*Palaquium rostratum* (Miq.) Burck
Symplocaceae	*Symplocos adenophylla* Wall. ex G. Don
Symplocaceae	*Symplocos celatrifolia* Griff. ex C.B. Clarke
Theaceae	*Schima wallichii* Choisy
Theaceae	*Gordonia excelsa* (Blume) Blume

Note: Adapted from Wistaria et al. (2016)

The flowers of *Tristaniopsis* are known to be a feed source for honeybees in the area. The honey produced by the bees is commonly harvested by the local people, and the honey is well known as a popular and distinctive product of the province of Bangka Belitung Archipelago. It is popularly known as *madu pahit pelawan*. Unlike the sweet taste of honey in general, this honey produced by the bee *Apis dorsata* has a unique bitter taste, from which the local name 'bitter honey' derives.

Based on interviews with informants, there is local knowledge about the particular trees in *Taman Kehati* that are used as feed by *Apis dorsata* in the production of honey. These include not only the *Pelawan* Tree (*Tristaniopsis merguensis*), but also *Rempudung* Tree (*Elaecarpus* sp.), and *Kabal* Tree (*Lithocarpus bancanus* (Scheff.) Rehder) found predominantly in the area.

Two years ago, in 2015 the local people on Bangka Island began to produce a herbal tea from the leaves of *Tristaniopsis* sp. According to the local people's perceptions, the leaves of the *Tristaniopsis* can be used medicinally. Based on tradition and local knowledge inherited from their ancestors, the boiled leaves of *Tristaniopsis* have been traditionally used to cure high blood pressure and diabetes. Traditional healers (herbalists) in Namang havealso used the leaf of this species on an empirical basis as one of the herbs in the treatment of diseases for childhood illnesses. Laboratory tests, have shown that the dry powdered leaves of *Tristaniopsis* contain 0.03% flavonoid, 0.9% saponin,1.04% tannin, and 6 % protein (Titisari 2016).

Another example of the interesting flora of the Palawan Biodiversity Park is the pitcher plant *Nepenthes ampullaria* Jack. The IUCN Red List categorizes this species as of Least Concern in terms of its conservation status and currently it is listed in Appendix 2 of the CITES database. The species is is a detritivore and is recognized as having an important function in fixing carbon. For example, on Natuna Island, *Nepenthes ampullaria* has been estimated to absorb CO_2 at a rate of 9.96 µmol/$m^{2/}$s (Mansur 2012, pers. com). Based on tradition, local people in Namang village use the water contained in the closed pitchers as a cough medicine and in eye pain medications. Utilization of

Nepenthes as a remedy for eye ailments is also part of the traditional medicine of people in West Sumatra (Rismita 2009).

Disturbances to the Palawan Biodiversity Park

On the basis of field observation and interviews with informants, it is known that there have been disturbances to the conservation functions of Palawan Biodiversity Park, namely by some tourists/visitors. In general visitors are unaware of the need to conserve the environment of the Palawan Biodiversity Park. For example, they commonly exhibit inappropriate behaviors, such as littering, and writing and carving their names on the trees and facilities. Disturbance to the ecosystem of the Park also arises from activities of the local people. For example, some have harvested various non-timber forest products, such as mushrooms and honey, but have not yet participated in efforts to maintain the Biodiversity Park. They have sometimes secretly harvested timber wood at night time. Farmers also intensively use pesticides to control pests in their plantations located close to the Palawan Biodiversity Park area. Consequently, the biodiversity of fauna is probably affected by the toxic effects of these pesticides.

An additional issue concerning the management status of the Palawan Biodiversity Park is that the land is not fully owned by the local government. Consequently, the Palawan Biodiversity Park has not been optimally managed by the Managers of the Biodiversity Park. Another factor recorded as causing disturbance to the Park is the exploitation of various natural resources in the Park due to the increasing economic penetration by the people arising from their increasing economic needs (Suryawan et al. 2015).

As a result of these various deleterious impacts, the sustainability of the Palawan Biodiversity Park will require the development of a well-defined management model.

Management of the Pelawan Biodiversity Park

The formulation of a Biodiversity Park management model requires several activities to be carried out: (i) to completion of a data base of existing natural resources, such as the flora and fauna of the area; (ii) completion of a database of existing socio-economic and cultural conditions; (ii) establishment or improvement of management institutions; (iv) empowerment and organization of community participation; (v) preparation of the Pelawan Biodiversity Park management model; (vi) implementation of the plan; and (vii) monitoring and evaluation (RCOO-IPB 1998). Thus, in order to develop an effective management model, the following data are needed:

Natural resources

Data is required on natural resources including: inventories of flora and fauna; mapped details of the landscape; evaluation of the conservation status of natural resources, such as details of protected flora and fauna according to Indonesian and international (e.g. IUCN) standards; natural resource potential for ecotourism; level of utilization of natural resources by local community.

Economic and cultural aspect

This aspect includes the number and density of the population that exists around the Pelawan Biodiversity Park; the people's livelihoods; identification of the types of flora and fauna that the population uses; details of the frequency and forms of subsistence and commercial use of the area's resources; details of the people's product marketing system of natural resources; institutional and social organization of the community.

Empowerment and community organization

There is a need to identify existing community institutions, both formal and informal institutions. Participation by the community needs to be accommodated in order to strengthen the bargaining position of the community in making decisions about management of the Pelawan Biodiversity Park. If such community institutions do not already exist, then it will be necessary to encourage their grass-root development.

Modelling

A range of co-management planning procedures need to be implemented, such as (1) raising public awareness of the importance of natural resources of the Palawan Biodiversity Park to support the community; (2) improving the ability of the community to participate at every stage of the integrated management, and (3) increasing the income of the community by sustainable and environmentally sound utilization of resources.

Implementation of the plan

Implementation of the model management plan requires that activities involve the community, through education and training, and involving the community in detemining policy direction, in enforcement and in regulation.

Monitoring and evaluation

Monitoring involves assessing the effectiveness of the management process, as well as identifying deviations and conflicts in the implementation of the plan. Monitoring and evaluation is carried out in order to identify the weaknesses and advantages of the management system as implemented, and in order to improve the system in the future.

It is known that biodiversity provides direct economic benefits to communities in terms of food, medicine and industrial raw materials. It also supplies the ingredients for natural ecosystems to provide an array of essential environmental services (photosynthesis, absorption and breakdown of pollutants, and much more). Furthermore, like human beings, plants and animals, have inherent rights to existence. We have an ethical reponsibility to value and conserve their diversity (Smitinand 1997).

Because the Palawan Biodiversity Park of Central Bangka District has important ecological and socioeconomic functions for the local community, excellent management is essential to ensure the sustainability of the Park. Moreover, local people have a legitimate right to be allowed to participate in decision-making concerning the use of the local natural resources (Haugen 2011). Their forests need to be maintained

because public good arise from such forest resources (Adalina et al 2014). It is feared that if management does not take into account the conservation status of the area then the purposes in establishing the the Park will not be fulfilled.

Based on our preliminary field observations, some disruption and threats to the existence of the park have been documented. For example, visitors have caused littering, and have damaged trees with graffiti. Some visitors have also freely entered into areas of the Park and damaged some facilities due to lack of care by the visitors and lack of supervision by the Park managers. Defiencies in management are due in part to inadequete managerial resources. Moreover, the management system for the Palawan Biodiversity Park has not been established in accordance with standard operational procedures of the government. Management planning in the the Park has not yet been implemented along the lines of an integrated collaborative model. Engaging the community in collaborative planning must be integral to the management program in order to harmonize and optimize conservation of the environment with the economic development interests of the local community (Dahuri et al. 2013). Collaborative planning is an effective planning model that is more likely than other planning models to benefit the public interest (Gunton and Day 2003). To achieve this, it will be necessary to clarify the roles of the various participants in the collaborative process (Carr et al. 1998).

Based on the results of this study, it is concluded that management of Palawan Biodiversity Park should involve all relevant stakeholders in collaborative planning, implementation and monitoring of progress. These stakeholders, consisting of village government, community leaders, universities and researchers, non-governmental organisations and private organizations, should be invited to participate in planning the management system for the Park. Management of the Park should incorporate the conservation of existing flora and fauna in its precincts as a basic principle in the planning process, so that sustainability of the Park's natural ecosytem can be guaranteed for the future. In such a way the Park can achieve the goal of *in situ* conservation of the Kalung area for the benefit of the Namang Village community and the wider society, which would fulfil the original vision that inspired the establishment of the park.

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We would like to express our special appreciation and thanks to all those who generously provided information for this study — namely key representatives of the Environment Agency of the Central Bangka District (*Bappelitbangda*), the Forestry Service of the Provincial Government (*Dinas Kehutanan Provinsi*), the Namang Sub-district (*Kecamatan*), the Namang Village, the managers of the Pelawan Biodiversity Park management unit, and several local people. Without their willing participation, this research would not have been possible.

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Use of local Hydromacrophytes as phytoremediation agent in pond to improve irrigation water quality evaluated by Diatom Biotic Indices

CATUR RETNANINGDYAH♥, ENDANG ARISOESILANINGSIH, SETIJONO SAMINO
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, Jawa Timur, Indonesia.
Tel./Fax: +62-341-575840 Fax: +62-341-554403, ♥email: caturretnaningdyah@gmail.com

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Abstract. *Retnaningdyah C, Arisoesilaningsih E, Samino S. 2017. Use of local Hydromacrophytes as phytoremediation agent in pond to improve irrigation water quality evaluated by Diatom Biotic Indices. Biodiversitas 18: 1596-1602.* Irrigation water in Indonesia generally has been polluted because of agricultural, industrial and domestic activity. The aims of this research were to determine the effectiveness of phytoremediation models conducted by planting some local Hydromacrophytes in three phytoremediation ponds through a continuous culture system with water discharge about 0.3 L/seconds for improving the irrigation water quality. The quasi-experimental research was conducted in a phytoremediation pond located in Kepanjen District of Malang, East Java Indonesia. The phytoremediation pond was divided into four interconnected sections. Pond 1 and 2 were planted with some of floating leaf and emergent hydromacrophyte, pond 3 was planted by combinations of same plants added with submerged Hydromacrophytes, while pond 4 was container pond to collecting the water as a result of phytoremediation process. The success of phytoremediation process was known from some physico-chemical parameters of water and some of diatom biotic indices (trophic diatom index/TDI, percentage of pollution tolerant value (%PTV), and Shannon Wiener diversity Index) that was found from artificial substrate been installed in the early treatment. The water quality monitoring performed in each part of the ponds after the plants grew steadily. The results showed that planting of Hydromacrophytes can significantly improve the physico-chemical quality of water. This was reflected in the decline value of conductivity (from 188 µS/cm to 182 µS/cm), turbidity (30 NTU become 8 NTU), total suspended solid (TSS) from 31.3 mg/L to be 5.5 mg/L, nitrates (7.5 mg/L to 3.3 mg/L) and dissolved phosphates (from 0.16 mg./L become 0.04 mg/L) and increasing value of dissolved oxygen (DO) in waters from 2.6 mg/L become 2.9 mg/L. Based on biotic indices there were improvement of the water quality from heavily polluted (diversity index 0.91) become moderately polluted (diversity index 2.07), eutrophic (TDI 52) become meso-eutrophic (TDI 38), and from heavily organic pollution (PTV 93%) to be some organic pollution contribute to eutrophication (PTV 38%). Improvement of water quality was effectively occur after passing through the third pond.

Keywords: Diatom biotic indices, local Hydromacrophytes, phytoremediation pond, water quality

INTRODUCTION

Phytoremediation is an alternative new technology to clean up the pollution in the environment that utilizes abilities of plants. The plants can work together with microbes to convert pollutants in the media (soil, coral, water) into less or not harmful substances (EPA, 2000; Etim, 2012). Irrigation water in Indonesia has been polluted because of human activity especially agriculture. Some synthetic fertilizers have been used by farmers in agricultural practice that increase the nutrient element such as nitrogen (N), phosphorous (P) and potassium or kalium (K). This residue will cause a negative impact on the environment. Contamination of residual nitrogen and phosphorous from agricultural activity was higher than produced by industrial or domestic activities (Panagopoulos et al. 2007; Yang et al. 2007; Yin et al. 2007). It will trigger eutrophication in the water ecosystem.

The eutrophication process can be controlled by increasing the absorption and uptake by hydrophytes, microbes and denitrification process (Saunders and Kalff 2001; Sybil et al. 2002; Marcus and Kŏhler 2006; Chen et al. 2010; Retnaningdyah et al. 2009). Hydrophytes and riparian vegetation in the irrigation channels could play a significant role in reducing eutrophication risk and also could promote degradation of toxic residual pesticides accumulated from agriculture ecosystem (Chaudhry et al. 2002).

The result of previous research in laboratory experiments using batch culture systems have proved that some Hydromacrophytes such as *Azolla* sp., *Hydrilla verticillata*, *Limnocharis flava*, *Marsilea crenata, Equisetum ramosissium, Typha angustifolia, Scirpus grossus* and *Lymnocharis flava* in monoculture or polyculture showed high potential as a remediator agent of nitrate and phosphate in aquatic ecosystem (Retnaningdyah and Suharjono 2010; Vidayanti et al. 2012; Sundari et al. 2012; Prahardika et al. 2012). The success of phytoremediation process is determined by plant productivity. The productivity of Hydromacrophytes was influenced significantly by the type of Hydromacrophytes, availability of resources, environmental stress and its adaptability to the environment. Emergent Hydromacrophytes has highest productivity and respectively be followed by floating leaf and submerged plants. Environmental factors that affect species distribution and growth of plants in wetlands includes the depth and current velocity of water that correlates with the

supply of oxygen, light and nutrient (Guntenspergen et al. 1989; Barko et al. 1982).

Based on these data, in order to apply this phytoremediation technique in the irrigation channels, it is necessary to determine the effectiveness of phytoremediation models conducted by planting some of local floating leaf, emergent and submerged Hydromacrophytes in phytoremediation pond through a continuous culture system for improving the irrigation water quality. The success of phytoremediation process can be evaluated by monitoring the water quality.

The evaluation method of water quality can be done partially or in combination with physico-chemical and biological methods. The benefit of using physico-chemical parameters for monitoring the quality of water is because it has a simple value and can be determined at any time, while the disadvantage is that these measures merely describe a moment circumstances and can not give an idea of the whole condition of ecosystems (John 1995). The investigation of the periphyton community as bioindicator has become an important part of water quality monitoring. Periphytons, more commonly known as aufwuchs or attached benthic algae, are groups of various organisms that grow or live on the free surface of objects that float and or sink in water such as plants, wood, stones and so on. Periphytons have been widely recommended to be used as bioindicators of water quality (Herbst & Blinn, 2007; Mbao et al. 2013). The advantages of use of periphytons as a pollution indicators is due to their ubiquitous presence, relatively large number, ease of finding, collecting and identifying and also their rapid responses to environmental changes and degraded water quality (Tan et al. 2013). One of the periphyton groups often used as a bioindicator of water quality is the algae of the diatoms group (Roubeix et al. 2011). Indication of water quality using diatom as bioindicator can be seen from some biotic indices. Trophic diatom index (TDI), percentage pollution tolerant values (%PTV) and diversity index are some of biotic indices be use to indication the level of eutrophication, organic pollution and level of toxic pollution (Wu et al. 2014).

MATERIALS AND METHODS

Research design

The quasi-experimental research was conducted in situ in a phytoremediation pond located in Kepanjen District of Malang, East Java, Indonesia. Some of local Hydromacrophytes in the phytoremediation ponds were floating leaf macrophyte (*Nymphaea* sp.), emergent (*Ipomoea crassicaulis*, *Ipomoea aquatica, Acorus calamus*, *Fimbristylis globulosus*, *Limnocharis flava*, *Scirpus grossus*, *Typha angustifolia*, *Ludwigia* sp., *Cyperus alternatifolius*, *Colocasia esculenta, Alternanthera sessilis, Pandanus amaryllifolius* and *Coix lacrima-jobi*) and submerged Hydromacrophytes (*Hydrilla verticillata*, *Valisneria gigantea*, and *Cryptocoryne* sp.). The phytoremediation pond was divided into four interconnected sections restricted by partitions that served as dikes and were given a connecting pipe to drain water. Ponds 1 and 2 were planted with some of floating leaf and emergent Hydromacrophytes, while pond 3 was planted with combinations of the same plants which added with submerged Hydromacrophytes. Pond 4 was a container pond to collect the water as a result of phytoremediastion process (Figure 1). The planting of those Hydromacrophytes was done in each pond with area coverage started about 50% from the pond surface area. The Hydromacrophytes were maintained until about 3 months old, at which time plant coverage achieved 75-80% of the pond area. Then the experiment was done by using the continuous culture system methods with water discharge about 0.3 L/seconds.

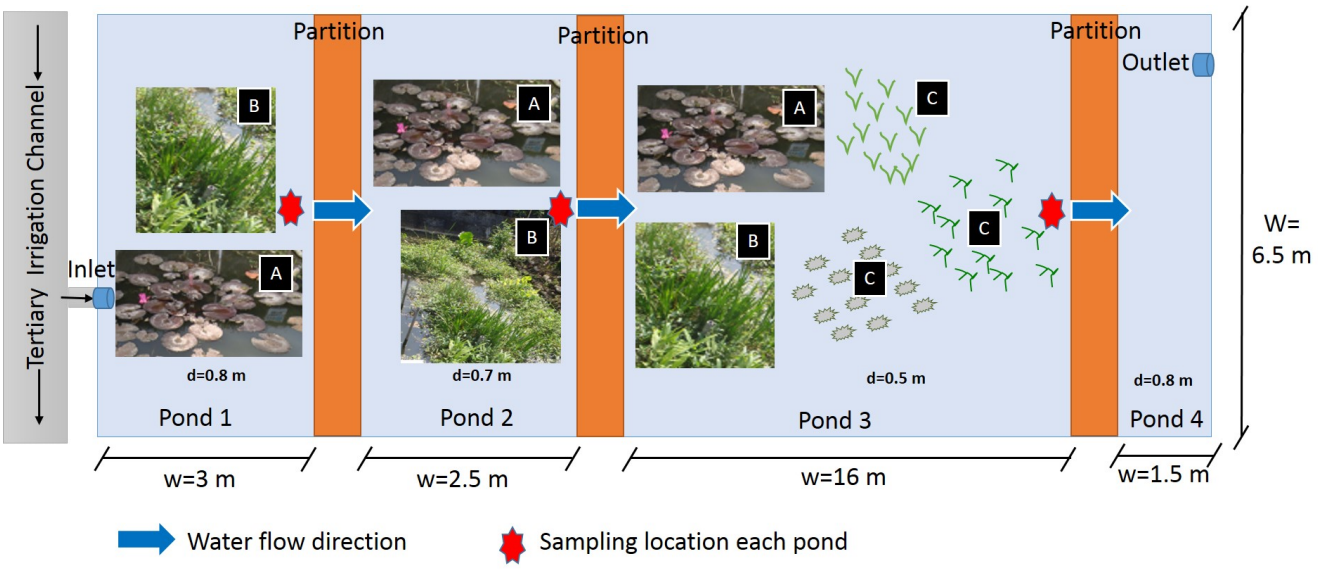


Figure 1. Pond phytoremediation model was used in this research. Note: A= floating leaf Hydromacrophytes; B= emergent Hydromacrophytes; C= submerged hydromarophytes; w= pond width; d = pond depth

Table 1. Water physico-chemical parameters were measure in this research

Parameters	Unit	Methods
Conductivity	µS/cm	Conductivitymeter
Dissolved Oxygen (DO)	mg/L	Digital Oxygenmeter
Turbidity	NTU	Turbidimeter
Nitrate	mg/L	Brucine methods
Total Suspended Solid (TSS)	mg/L	Gravimetric
Dissolved phosphates (Orthophosphate)	mg/L	Stannous chloride methods

Monitoring water quality

The success or effectiveness of the phytoremediation process was estimated from several physico-chemical parameters, diatom diversity index, trophic diatom index (TDI) and percentage pollution tolerant values (%PTV). Those parameters were measured at the end of ponds 1 to 3 compared to the water in the tertiary irrigation drain (Figure 1). The physico-chemical parameters measured in this research included conductivity, dissolved oxygen (DO), turbidity, total suspended solid (TSS), nitrates and dissolved phosphates. Those parameters were determined based on Clesceri et al. (1998) as can be seen in Table 1. Diatoms were obtained from artificial substrate (ceramics 20x20 cm^2) that had been installed in early treatment together with the time planting of Hydromacrophytes. The monitoring of water quality and community structure of diatoms were performed in each part of the ponds after the plants grew steadily for about 3 months after which plant coverage achieved 75-80% of the pond area. Diatom identification was based on Edmondson (1959) and Prescott (1978).

Data analyses

The data from diatoms (density and taxa richness) were used to determined the Shannon Wiener diversity index based on the formula of Wu et al. (2014). Trophic diatom index (TDI) and the percentage pollution tolerant values (%PTV) were based on Kelly and Whitton (1995) and Kelly (1998) as bioindicator of water quality in the ponds after phytoremediation process. The difference in water quality between the ponds were determined by Anova and followed by Tukey HSD test at the 0.05 level, performed with SPSS for Windows Release 16.

RESULTS AND DISCUSSION

Physico-chemical quality

Planting of some local floating leaf, submerged and emergent Hydromacrophytes over as much as 75-80% of the surface area in the phytoremediation ponds through a continuous culture system with discharge of irrigation water at aproximately 0.3 L/seconds was able to improve the water quality as reflected in the increase of dissolved oxygen (DO) and a decrease in the concentration of nitrate, dissolved phosphate, total suspended solid (TSS), conductivity, and turbidity significantly, especially in the pond 3 (Figure 2).

This model of phytoremediation process was able to increase the levels of DO from 2.6 mg/L or 32% to 2.9 mg/L or 3.4%. This concentration meets the quality standard of water class IV for irrigation based on the provision of Indonesia government regulation No. 82 of 2001 on water quality management and water pollution control. The phytoremediation process also significantly decreased the water conductivity from 188 µS/cm to 182 µS/cm. The range of conductivity values are equivalent to 0.188-0.182 dS/m which already meets the irrigation water quality standards of FAO in the amount of 0.0-3.0 dS/m.

The phytoremediation process was able to significantly reduce the value of turbidity in the irrigation channel from 30 NTU to 8 NTU in the outlet of pond 3. This showed that planting of Hydromacrophytes had an impact on the sedimentation process of suspended solid in irrigation water. Planting Hydromacrophytes has also reduced the levels of total suspended solid (TSS) significantly from 31.3 mg/L to 5.5 mg/L in the outlet of pond 3. The value of TSS is in compliance with the quality standards of Class I (raw water drinking water) and II (water for recreation, fishery, animal husbandry and irrigation) based on Indonesia government regulation with a maximum limit of level 50 mg/L.

The residue of synthetic fertilizers in irrigation water is reflected in the levels dissolved phosphate and nitrate. Planting of some local floating leaf, submerged and emergent Hydromacrophytes in phytoremediation ponds over as much as 75-80% of the pond surface area has been shown to significantly reduce levels of nitrates and phosphates dissolved in the water (Figure 2). Phytoremediation process has successfully decreased the levels of nitrates from 7.5 mg/L to 3.3 mg/L. Similarly, levels of dissolved phosphates or orthophosphate decreased from 0.16 mg/L to 0.04 mg/L. Concentration of nitrates between 5-30 mg/L indicates a slight to moderate degree of restriction on use, whereas the nitrate concentration of less than 5 mg/L indicated good quality and can be used for all agricultural crops. The quality standard of nitrate for recreation, fishery, animal husbandry and irrigation (Class II) based on Indonesia government regulation No. 82/2001 is 10 mg/L.

Diatom biotic indices

Improvement of water quality in the phytoremediation pond using some local of Hydromacrophytes can be seen from the decreasing value of some water physico-chemical parameters and it can also be seen from the diatom biotic indices as bioindicator of water quality. In this study, together with the planting of Hydromacrophytes, we were also installed the artificial substrate of diatoms from ceramics 20 x 20 cm^2. We then monitored those diatom diversity in the same time as water physico-chemical measurements.

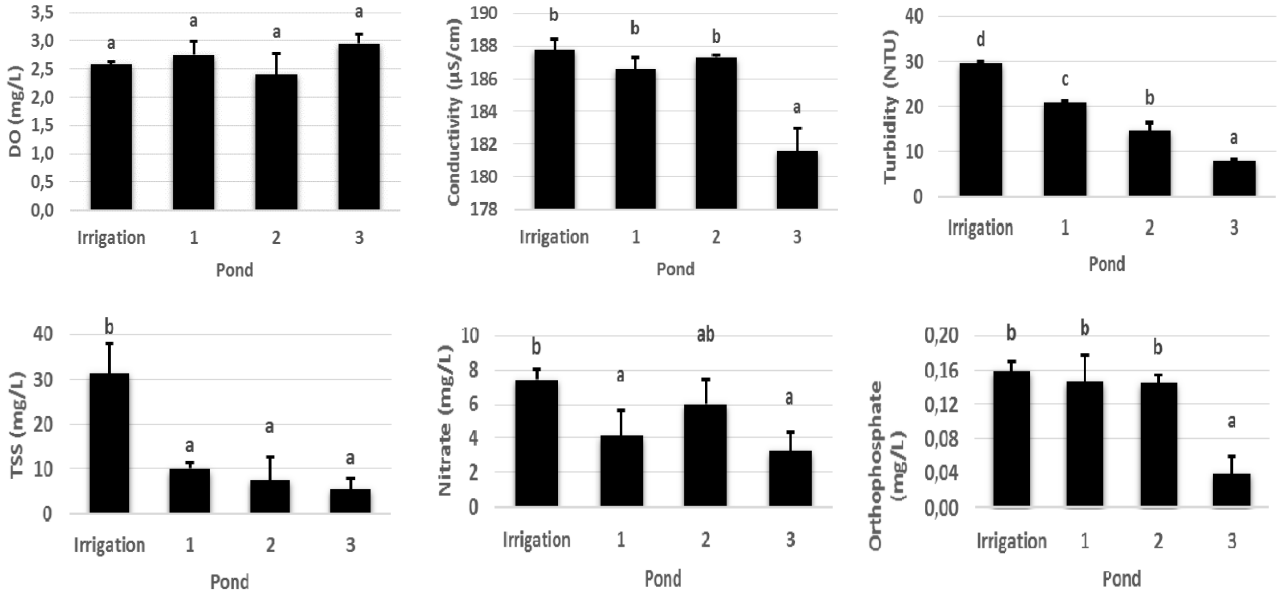


Figure 2. The average value of dissolved oxygen (DO), conductivity, turbidity, total suspended solid (TSS), nitrates and dissolved phosphates (orthophosphates) water in irrigation drain compared with three ponds after phytoremedation process through continuous culture system with discharge of irrigation water 0.3 L/seconds using some local floating leaf, submerged and emergent Hydromacrophytes over as much as 75-80% of the pond surface area. Note: The same notation (a,b,c,d) on each parameter showed no significant difference by ANOVA test followed by Tukey HSD α 0.05

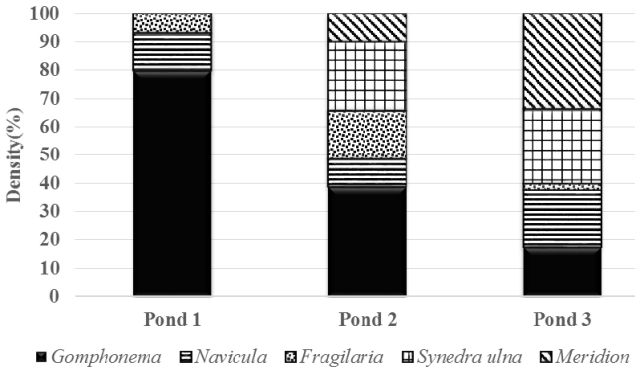


Figure 3. Taxa richness and relative density of diatom in ponds after phytoremedation process

Diatoms taxa richness tended to be higher at second and third ponds than in the pond 1. Taxa richness increased from three species in pond 1 to five species in the ponds 2 and 3 (Figure 3). The water quality improved after the phytoremediation process in all three ponds. This can be seen from the Shannon Wiener diversity index (Figure 4.A) that increased from 0.91 (heavily polluted) to 2.12 and 2.07 (moderately polluted). Similarly, based on the value of trophic diatom index/TDI (Figure 4.B) the status of water changed from eutrophic (TDI=52) to meso eutrophic (TDI=38) in pond 3. Based on the organic pollution level which can be indicated by the percentage of pollution tolerant values/%PTV (Figure 4.C), the process of phytoremediation increased the quality of water from heavily organic pollution (PTV=93.3%) to some organic pollution contribute to eutrophication (PTV = 37.8%).

The effectiveness of the phytoremediation process in three phytoremediation ponds through a continuous culture system planted by some local Hydromacrophytes can be seen from increasing the water quality in the outlet of third pond. This could be shown by differences of some parameters of physico-chemical and diatom biotic indices between pond 1 and 2 with pond 3. Based on cluster and biplot analyses (Figure 5), it can be concluded that the phytoremediation model used in this research was successfull in improving the irrigation water quality. This can be seen from the significant differences in water quality between outlet of first pond and outlet of second and third pond. Some parameters in pond 1 such as total suspended solid (TSS), turbidity, trophic diatom index (TDI) and percentage of pollution tolerant value (%PTV) significantly higher than in pond 2 and 3. This indicated that pond 1 had eutrophic water with heavily organic pollution. While all levels of physico-chemical parameters observed in pond 3 were significantly lower except dissolved oxygen (DO) and Shannon Wiener diversity index of diatom (H) which indicates the improvement status of the waters of this pond become meso-eutrophic with some organic pollution contribute to eutrophication.

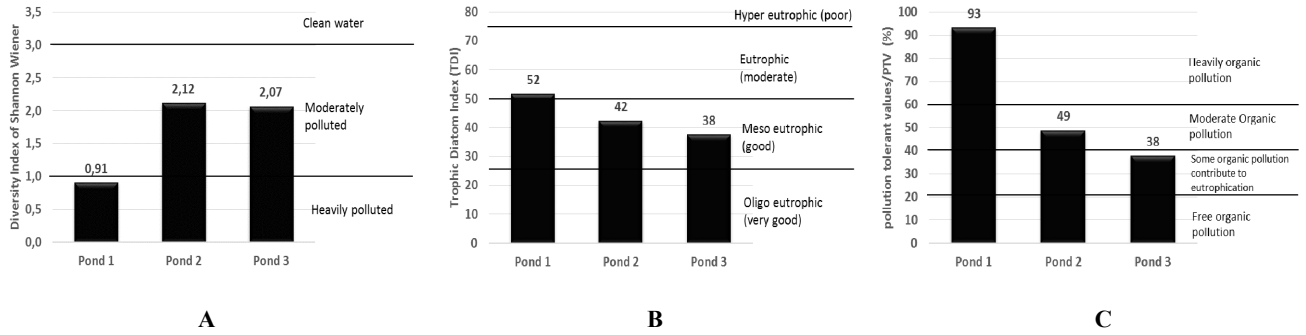


Figure 4. Shannon Wiener Diversity Index (A), Trophic Diatom Index/TDI (B) and Percentage of Pollution Tolerant Value/%PTV (C) of diatom be found in the three phytoremediation pond

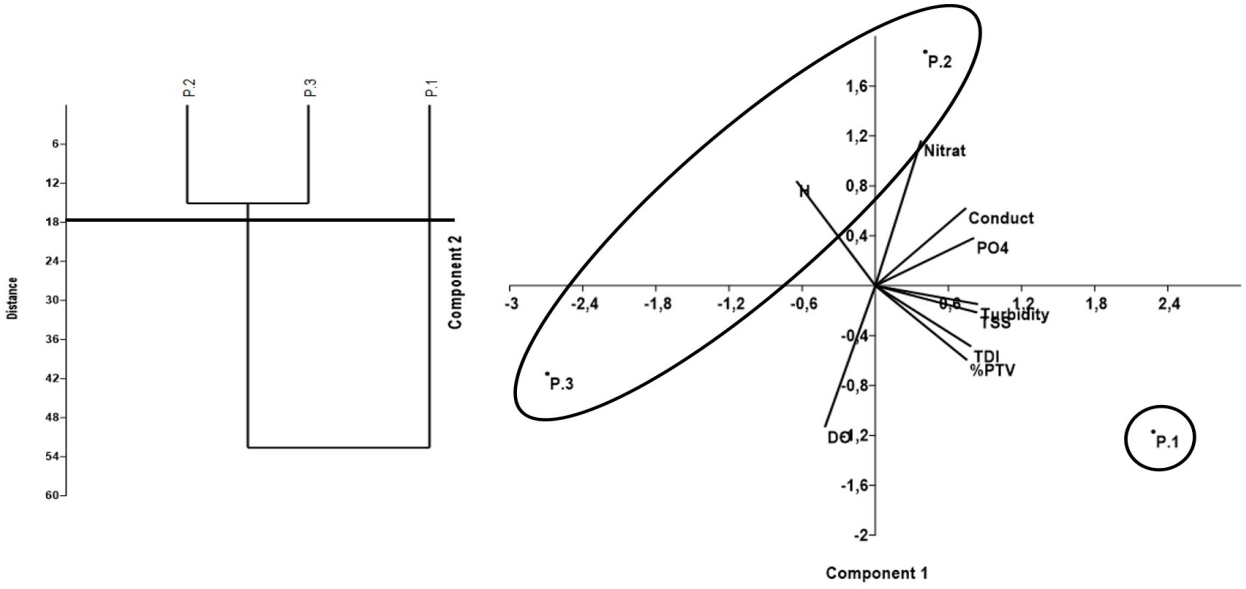


Figure 5. Grouping the quality of water in the pond (P) after phytoremedation process based on some physico-chemical parameters and diatom biotic indices using cluster analyses and biplot from principle component analyses (PCA). Note: P.1= pond 1; P.2= pond 2; P.3= pond 3; H= Shannon Wiener diversity index of diatom; TDI= Trophic Diatom Index; %PTV= Percentage of Pollution Tolerant Value; TSS=total suspended solid; PO4= dissolved phosphate/orthophosphate; Conduct= conductivity

Discussion

The results of this research showed that the water quality characteristics of irrigation drains have are contaminated, reflected by the high values of turbidity, conductivity, total suspended solid (TSS), nitrate and dissolved phosphates (orthophosphate) and low levels of dissolved oxygen (DO). Process of phytoremediation in three ponds were planted with various kinds of local floating leaf, submerged and emergent Hydromacrophytes over as much as 75-80% of the surface area in ponds through a continuous culture system with discharge of irrigation water of aproximately 0.3 L/seconds can significantly improve the water quality. Conductivity and dissolved phosphate levels in irrigation canals were high and were lower in third ponds after passing the first and second pond. The phytoremediation pond 1 and 2 were able to decrease the value of turbidity, total suspended solid (TSS) and nitrates in the irrigation water into a medium level and the levels of these parameters becomes significantly low after pass the third pond of phytoremediation (Figure 2).

The results of our research is in accordance with the results of others. Hydromacrophytes acts as a filter sediment, phosphorus and dissolved nitrogen so then can generally improve the quality of water (Jayaweera and Kasturiarachchi 2004; Xiang et al. 2009). Some Hydromacrophytes such as *Hydrilla verticillata*, *Marsilea crenata*, *Scirpus grossus, Limnocharis flava, Fimbristylis globulosa, Vetiveria zizanoides, Equisetum ramosissium, Typha angustifolia, Azolla* sp., *Monochoria vaginalis,*

Ludwigia hyssopifolia, and *Ludwigia adscendens* have also been proven to reduce pollutants of dissolved nirogen and dissolved phosphate derived from synthetic fertilizer and other materials on the scale of the greenhouse through a batch culture system (Jayaweera dan Kasturiarachchi 2004; Xiang et al. 2009; Retnaningdyah et al. 2012; Vidayanti et al. 2012; Retnaningdyah and Arisoesilaningsih 2013; Prahardika et al. 2013; Sundari et al 2013; Ivansyah and Retnaningdyah 2013).

Hydromacrophytes were used for phytoremediation process on third pond in this research is combination of floating leaf, emergent as in first and second pond with more abundance. Besides that, in the third pond was also enhanced with submerged Hydromacrophytes having advantages which is oxygen as a result of photosynthesis directly dissolved in water so that dissolved oxygen levels in the pond three is always the highest (averaged 2.9 mg/L) compared with the levels of oxygen of the others. This value has fulfilled the quality standards expected by Indonesia government regulation No. 82/2001 Class IV. However, the increasing value of dissolved oxygen still need to be done by determining a proper water retention time in the phytoremediation process of continuous culture system pond.

Planting of some local floating leaf, submerged and emergent Hydromacrophytes over as much as 75-80% of the surface area in phytoremediation ponds through a continuous culture system with discharge of irrigation water of aproximately 0.3 L/seconds in this study has successfully reduced water conductivity from 0.188 dS/m to 0.182 dS/m. Conductivity values can be used to determine the concentration of dissolved salt or salinity in the water which further will determine the availability of water for crops. Conductivity values may reflect the presence or absence of salinity problems or levels of dissolved salts conditions that affect agricultural crops (Ayers and Westcot 1994). Conductivity values less than 0.7 dS/m indicating no problems of salinity in irrigation water so that it can be used for all agricultural crops without restrictions.

Improvement of water quality after the phytoremediation process in three ponds using some local floating, emergent and submerged Hydromacrophytes besides may decrease some value of physico-chemical parameters, can also be seen from the diversity of diatoms. Results of monitoring the diatom diversity in an artificial substrate showed that the taxa richness increase from three taxa in the pond 1 to five taxa in the pond 3. Based on the calculations of density, there was a decreasing relative abundance of *Gomphonema* sp. from 80% in first pond to 18% in the third pond. According to Kelly and Whitton (1995), *Gomphonema* sp. is a tolerant species toward organic pollution, while based Onyema (2013) and Singh et al. (2013) *Gomphonema* sp. was a kind of bioindicator of high nutrient levels (eutrophic) and organic pollution in the water. Improvement of water quality as a result of phytoremediation process in this study also was reflected by Shannon Wiener diversity index (H) which categorized from heavily polluted in the first pond (H=0.91) become moderately polluted in the third pond (H=2.07). Besides, the value of trophic diatom index (TDI) shows the change in status of eutrophic waters (TDI=52) become into the meso eutrophic (TDI=38). Similarly, based on the percentage of pollution tolerant values (%PTV) there was decreasing the level of organic pollution from heavily organic pollution (PTV=93.3%) become some organic pollution contribute to eutrophication with PTV 37.8% (Kelly and Whitton 1995; Kelly 1998; Wu et al. 2014).

Based on the above illustration, it can be concluded that the planting of some local Hydromacrophytes in three phytoremediation ponds which plant coverage achieved 75-80% of the pond area through a continuous culture system with water discharge about 0.3 L/seconds has been able to improve the quality of water in irrigation canals. It was reflected from decreasing the concentration of water conductivity, TSS, nitrate and orthophosphate significantly in pond 3 and the change status from heavily polluted in the pond 1 to moderately polluted in the pond 3 (based on diatom Shannon Wiener diversity index), from eutrophic waters become meso-eutrophic (based on TDI) and from heavily organic pollution become some organic pollution contribute to eutrophication (based on %PTV).

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Short Communication: Biodiversity of the Gaga chicken from Pinrang, South Sulawesi, Indonesia based on the bioacoustic analysis and morphometric study

ABINAWANTO[1,♥], PIPIH SUNINGSIH EFFENDI[2]
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Jl. Lingkar UI, E Building UI Campus, Depok 16242, West Java, Indonesia Tel.: +62-21-7270163, Fax.: +62-21-78829010, Depok 16424, email: abinawanto@gmail.com

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Abstract. *Abinawanto, Effendi PS. 2017. Biodiversity of the Gaga chicken from Pinrang, South Sulawesi, Indonesia based on the bioacoustic analysis and morphometric study. Biodiversitas 18: 1618-1623.* Gaga chicken (*Gallus gallus domesticus*) is the local ornamental chicken originated from South Sulawesi, Indonesia, being kept for the crows. Gaga chicken which also called "*ayam ketawa*" ("laughing chicken") has the unique crowing song, which sounds like human laughing. Gaga chicken which has long and fast crowing song is called 'dangdut' type, while the short and slow crowing song is named 'slow' type. The objective of present study is to investigate the biodiversity of Gaga chicken from Pinrang, South Sulawesi, Indonesia based on morphometric and bioacoustic characters. Twenty samples of Gaga chicken have been collected, consisted of eight '*slow'* types and twelve '*dangdut'* types, respectively. Data were analyzed by t-test using SPSS ver. 22. The result showed that 'slow' type was bigger than 'dangdut' type. Morphometric characters could be applied to determine the biodiversity of Gaga chicken.

Keywords: Ayam ketawa, crowing song, Gaga chicken, morphometric

INTRODUCTION

Chickens (*Gallus gallus domesticus*) are estimated to have been domesticated since 8000 BC (Hirst 2017), originating from a single ancestor, the Red Jungle-Fowl (*Gallus gallus* (Linnaeus, 1758) in Southeast Asia (Fumihito et al. 1994; Hillel et al. 2003, 2007; Twito et al. 2007), but given the high diversity some researchers estimate it has multiple origins from Southeast Asia, South Asia and Southern China (Nishibori et al. 2005; Liu et al. 2006; Oka et al. 2007; Xiang et al. 2014; Peters et al. 2016). Now, Red Jungle-Fowl can still be found wildlife in India, China and Southeast Asia (BirdLife International. 2016).

Native chickens and local chicken breeds represent a diverse and unique genetic pool (Granevitze et al. 2007), but some of them are abandoned and endangered. Superior chickens breeds are used for cross breeding to produce new breeds according to market requirement and high production (Crawford 1990). New breeds continue to be generated, and old types that do not fit are removed.

The native or local chickens breeds are currently characterized by their geographic distribution (Suh et al. 2014). Today, Asia and Europe each have over 400 local chicken breeds. Africa and South America each have more than 100 local chicken breeds, and North America have less than 40 local chicken breeds (Hoffmann, 2009). Indonesia has at least 27 native chickens i.e. genuine chicken domesticated in Indonesia, and 11 local chickens i.e. cross-breeding between native and introduced chickens, that naturalized in Indonesia. In addition, there are also several types of introduced chickens (Ulfah 2016).

Chicken breeds are grouped by color, size, feathers, comb and body shape (Skinner 1978), even the rooster's sound (Childs et al 2016). There are more than 26 voice communications in chickens, and crowing is the most important to show social position in a population (Milius 2006).

Gaga chicken (in Buginese) or *ayam ketawa* (in Indonesian) is a type of local ornamental chicken that is popular among hobbyists due to its unique voice, namely slow and dangdut types (Bugiwati and Ashari 2013). Initially, this chicken was developed by the rulers of the old Ajatappareng region, which now includes Sidenreng Rappang (Sidrap), Pinrang, Enrekang, Parepare, and Barru of South Sulawesi Province, Indonesia. Currently, this chicken has been reared throughout Indonesia, but these areas remain the main population.

The crowing sound of Gaga chickens is different from crowing of other ornamental chickens of Indonesia, such as Balenggek from West Sumatra, Pelung from West Java, and Bekisar and Gaok from East Java, which has a longer sound of crowing (Rusfidra 2007), disjointed and resembles human laughter (Bugiwati and Ashari 2013).

Crowing sound behavior of this chicken is influenced by the dominance (status), morphology, and physiology (Leonard and Horn 1995). According to Shimmura and Yoshimura (2013), the chicken crowing behavior is influenced by circadian rhythms caused by the internal biological clock. Additionally, Yoshimura et al. (2000) stated that the internal biological clock between species

differs, so that the circadian system is different and lead to different behaviors of crowing sound. Yoshimura et al. (2000) also have mapped three genes which affect the internal biological clock, the clock gene is located on chromosome 4q1.6-Q2.1, while genes Per2 and Per3 respectively are in micro-chromosomes.

A limited number of individuals and the selection of a particular trait in the domestication process could increase the occurrence of inbreeding and influence number of genetic frequencies or genetic drift (Grasteau et al. 2005). Without proper control, this will affect on phenotypic diversity, such as changes in the external morphology, internal morphology, physiology, organ development, and behavior (Jensen 2006). Based on the Decree of the Minister of Agriculture No. 2920/Kpts/OT.140/6/2011, Gaga chicken has been established as one of the local chicken breeds of Indonesia which its genetic resources need to be protected and conserved. However, the problem is genetic diversity of Gaga chicken is still unknown.

The objective of this study was to investigate the biodiversity of Gaga chicken from Pinrang, South Sulawesi, Indonesia based on morphometric and bioacoustic characters. Therefore, this study is very important to increase the economic value of the chicken. The research activities were conducted, first, with an analysis of phenotypic form bioacoustics and morphometric analysis.

MATERIALS AND METHODS

Study area

This research was conducted in Pinrang Regency, South Sulawesi, Indonesia in 2014-2017. Pinrang Regency is located at 3° 19'13 " to 4° 10'30" S and 119° 26'30 " to 119° 47'20" E with an area of 1,961,77 km², and a population density of 171 ind./km² . This area is generally lowland (500 m, 60%), but there are also medium hills (500-1000 m, 20%) and highlands (> 1000 m, 10%). Field research was conducted in the lowland area.

Procedures

Twenty Gaga chicken (8 slow type and 12 dangdut type) is from several farms in Pinrang, South Sulawesi, Indonesia. Pinrang consists of the village of Malimpung, Tiroan, Mattiro Bulu, Lamrisang, and Sawito (Wattang Sawito). The morphometric and weight were used to measure and observe the behavior of crowing. The morphometric character measured is the length of neck bone (mm), weight (grams), femur length (mm), length of the tibia (mm), length of shank (mm) diameter shank (cm), the length of the maxilla above (mm), length wings (cm), the length of the third finger (mm), length of the Cestbone (mm) and length comb (mm) (Brahmantyo and Mulyono 2003).

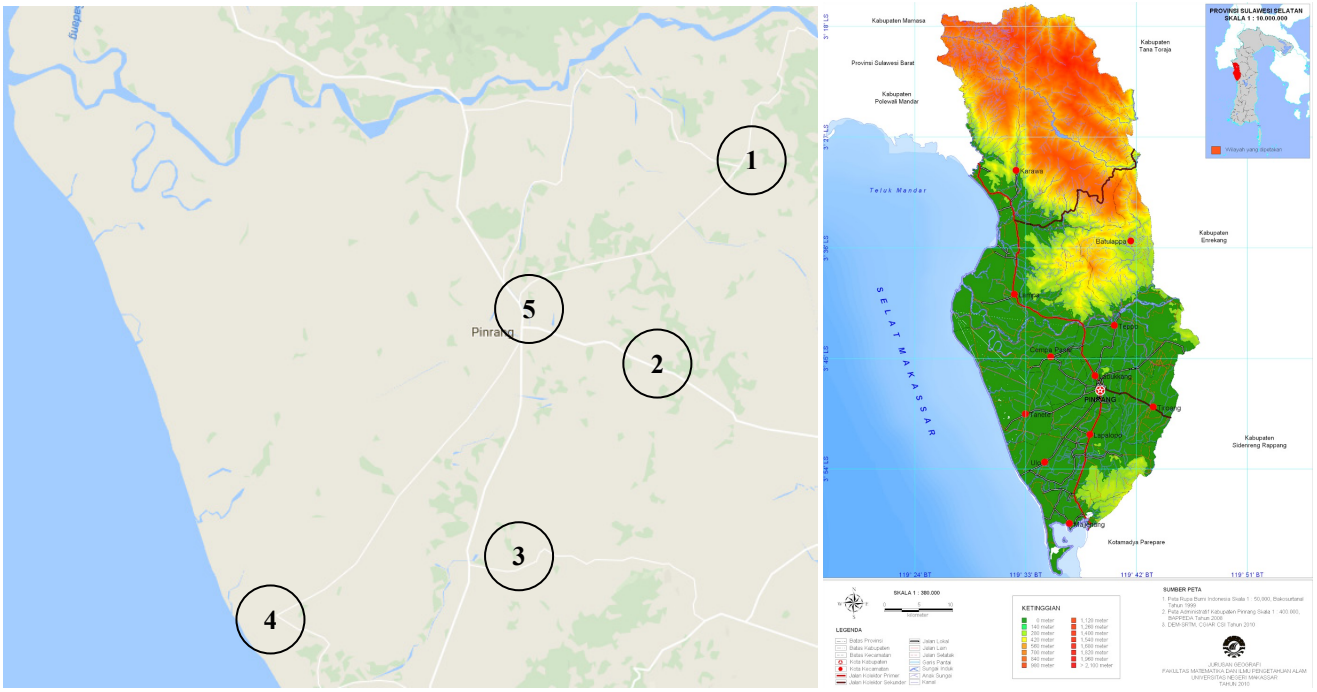


Figure 1. Study site in Pinrang District, South Sulawesi, Indonesia. A. Malimpung, B. Tiroan, C. Mattiro Bulu, D. Lamrisang, and E. Sawito (Wattang Sawito)

Morphometric characters were measured using a ruler. The total weight was weighed using a digital scale. Crowing behavior was observed using a video camera and voice recorder (Rachma et al. 2013). Number of beats that indicate the type of Gaga chicken as slow or dangdut were measured through software Cool Edit Pro 2.1 Portable (Bugiwati and Ashari 2013).

Data analysis

Data were analyzed by t-test using SPSS ver. 22, based on the average of each sample of observed morphometric characters. While, the crowing sound of Gaga chicken were analyzed descriptively using Cool Edit Pro 2.1 Portable.

RESULTS AND DISCUSSION

Bioacoustic analysis

Recording result of crowing sound from the two types of Gaga chicken was analyzed using Cool Edit Pro 2.1 Portable. Based on measurement of the software, crowing sound Gaga' chicken slow type has 3 - 5 times of beats and dangdut type has 4-7 times of beats. The result of bioacoustics analysis of the two types of Gaga chicken in a graphical display can be seen in Figure 1.

Based on the bioacoustics graphic, dangdut type of Gaga chicken has a longer crowing sound than slow type and it is influenced by genes factor in producing the crowing sound of both types of Gaga chicken. Bugiwati and Ashari (2013) stated that in the area of South Sulawesi, there are three types of Gaga chickens crowings, namely dangdut type, slow type, and crystal type. Slow type of Gaga chicken dominates the area of Sidrap, while dangdut type dominates Pinrang area. Gaga chicken that has a high value for sale is crystal type, it is a Gaga chicken of slow type which has a crisp and clear sound. It is rarely found.

Morphometric characters

Body size measurements were performed to eleven parameters of the two types of Gaga chicken. Based on the measurement, slow type of gaga chicken has a heavier weight of body than that of dangdut type, while the length of the neck bones of slow type is shorter than that of dangdut type. It causes the duration of the crowing sound of dangdut type longer than that of slow type. The average result of eleven morphometric parameters can be seen in Table 1 and Figure 2. While, the difference in morphometric parameters between slow and dangdut types can be seen in Table 2.

Discussion

All chickens breeds are descended from red jungle fowl in multilocation of Southeast Asia, South Asia, and southern China. Genetic selection over generations has developed a lot of chicken breeds that have specific characteristics. Gaga chicken is one of Indonesian local chicken, which has a unique physical shape and genetic composition, and good adaptability to environmental limitations. This breeds began to be widely farmed in Indonesia, but the continuous protection and conservation for them needs to be supported.

Evaluation of genetic diversity of native or local breeds is an important factor for the identification of unique and valuable genetic resources. Research on genetic structure and genetic diversity of these breeds is an important step to identify and conserve valuable genetic resources (Suh et al. 2014).

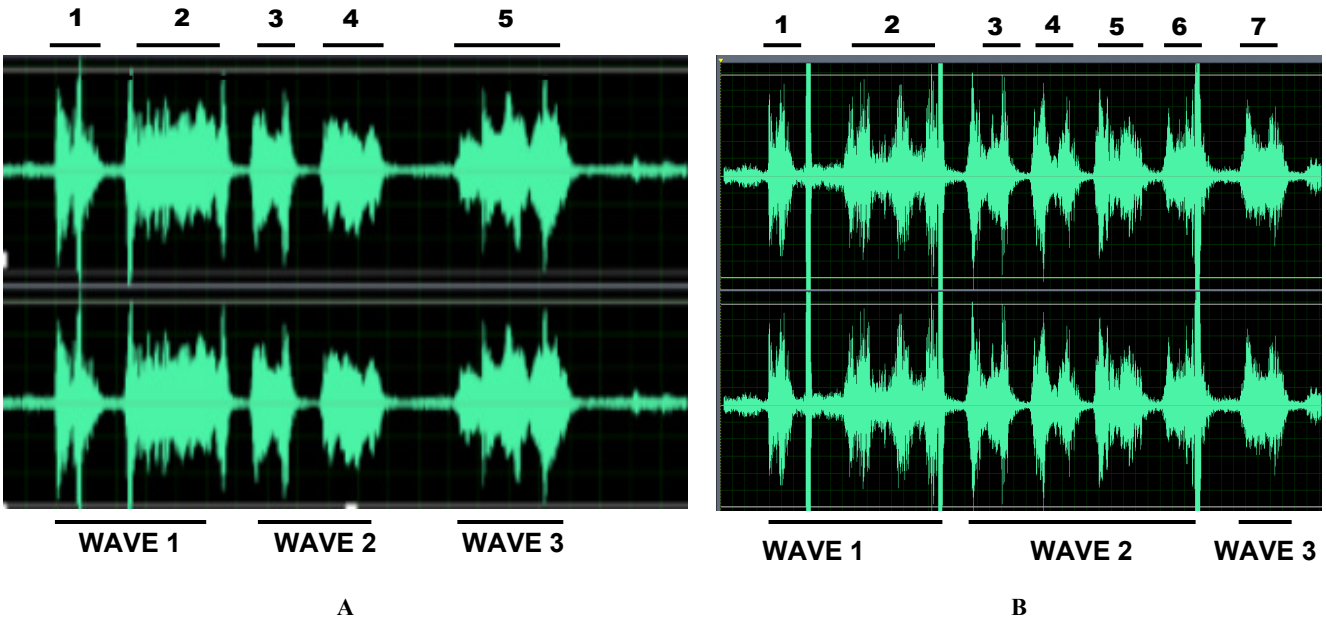


Figure 1. Bioacoustic analysis of Gaga chicken. A. Slow type, B. Dangdut type

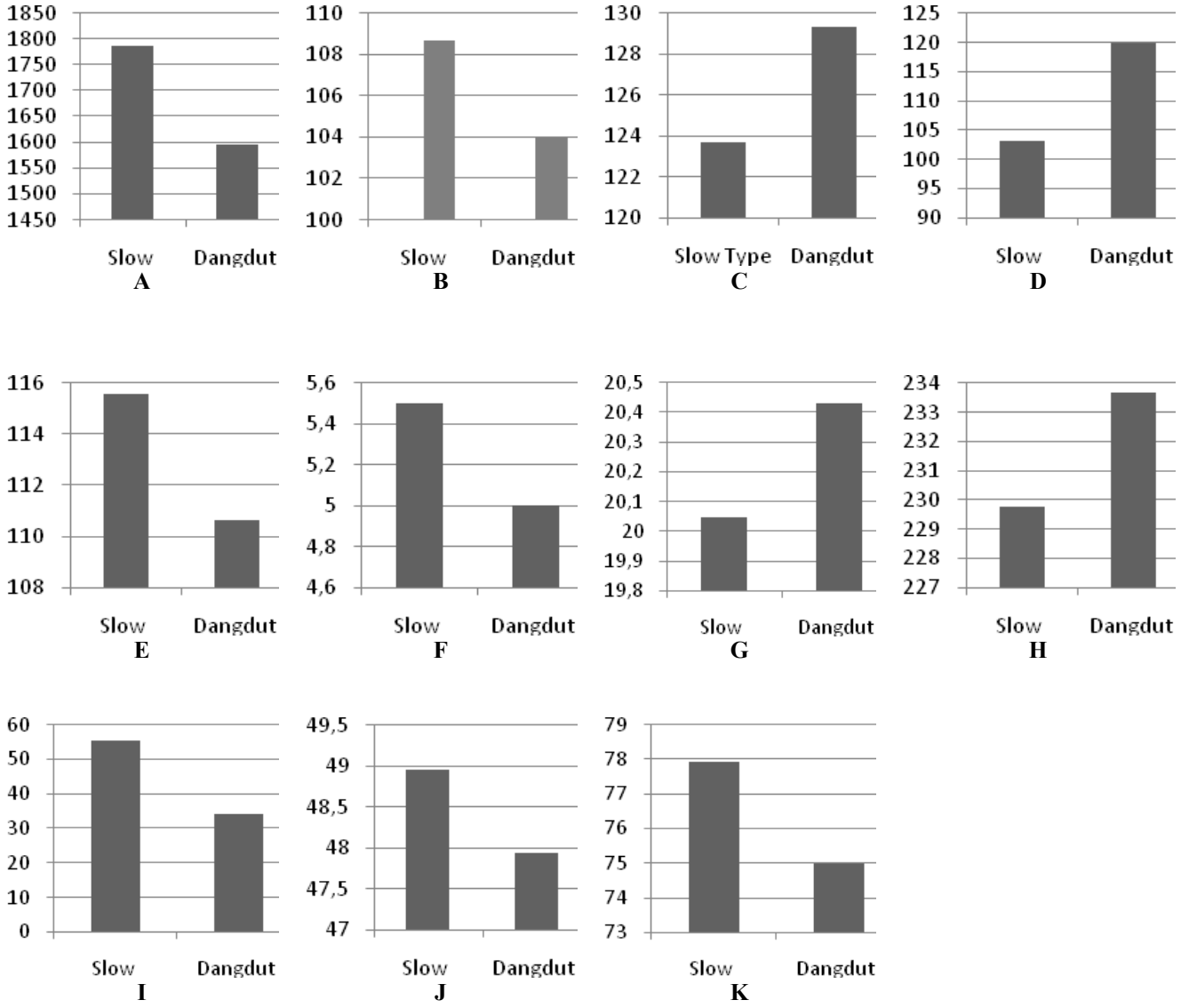


Figure 2. Graphic of Morphometric characteristic Analysis of Gaga chicken, Dangdut Type and Slow Type. A. A. Body weight (g), B. Neck bone length (mm), C. Femur length (mm), D. Tibia length (mm), E. Shank length (mm), F. Shank diameter (cm), G. Maxilla length (mm), H. Wings length (cm), I. Comb length (mm), J. Third finger length (mm), K. Cestbone length (mm)

Artificial selection and crossbreeding for superior chicken breeds have been conducted for a long time and cause commercial breed types to flourish and dominate the poultry industry. The preeminence of a highly productive of superior breed type threatens the existence of native or local breeds (FAO 2007), because superior breeds type is farmed more intensively and the low-performing of native or local chicken is neglected (Granevitze et al. 2007). Characterization of the genetic diversity of chickens can inform the development of conservation initiatives (Wilkinson et al. 2012)

On the other hand, local chickens often have the advantage of having better resistance to local environmental changes and adapting to limited food. It is passed from generation to generation genetically. With the extinction of local chickens, then this advantage is lost and not conserved to form a chicken breeding program (Blackburn 2006).

In addition, most genetic and phenotypic studies focus on commercial breeds stored in industrialized countries (FAO 2011). Due to poor commercial performance, native breed breeds in many countries are often overlooked and far less attention is paid to the genetic conservation of these resources (Wilkinson et al. 2011).

In conclusion, the results of bioacoustics analysis of Gaga chicken of slow and dangdut type show the difference beat at the end of the crowing sound. Slow type produces 3-5 beats while dangdut type produces 5-7 beats. Mean of morphometric data from both Gaga chicken types are still vary in results so it is necessary for the selection program to produce main breed.

Table 1. Morphometric characters of Gaga chicken from Pinrang, South Sulawesi, Indonesia.

No	Body weight (g)	Neck bone length (mm)	Femur length (mm)	Tibia length (mm)	Shank length (mm)	Shank diameter (cm)	Maxilla length (mm)	Wings length (cm)	Comb length (mm)	Third finger length (mm)	Cestbone length (mm)	Type
1	1400	98.56	114.69	91.6	106.72	50	18.26	218.11	51.05	50.64	77.59	Slow
2	1800	130.87	116.84	106.74	113.6	60	18.11	228.62	55.23	42.79	76.08	Slow
3	1300	97.48	75.76	79.04	114.14	50	19.3	202.64	39.22	49.83	82.69	Slow
4	2000	119.32	134.66	111.59	111.1	60	19.75	223.59	54.61	52.3	78.01	Slow
5	1500	105.41	134.13	73.8	99.34	50	16.58	165.09	50.77	47.22	68.01	Slow
6	2000	122.1	134.49	128.85	121.43	60	20.27	271.3	47.61	48.75	81.4	Slow
7	2200	108.21	144.47	119.86	114.54	60	21.58	225.1	38.35	43.63	112.92	Dangdut
8	2200	106.66	136.35	118.71	123.93	50	21.48	239.53	65.81	49.53	88.81	Slow
9	1400	90.42	136.85	95.53	113.94	50	15.42	224.93	42.56	49.02	65.99	Dangdut
10	2100	89.13	142.4	114.65	134.27	60	26.66	289.21	80.89	50.57	70.83	Slow
11	2300	109.87	137.72	107.99	138.97	60	26.01	260.37	51.74	63.35	92.4	Dangdut
12	1100	103.1	126.58	106.37	112.83	40	22.39	247.26	17.28	43.83	99.16	Dangdut
13	2400	105.28	150.72	108.51	125.38	60	19.79	258.1	19.82	56.57	68.83	Dangdut
14	1700	99.01	123.92	95.8	112.84	50	23.87	250.95	61.23	53.84	69.54	Dangdut
15	1400	116.9	131.7	102.93	93.36	50	23.41	247.48	28.72	49.28	73.26	Dangdut
16	600	65.53	93.91	67.73	83.93	40	16.76	135.09	10.66	46.1	60.67	Dangdut
17	1500	131.55	134.47	107.17	104.79	50	14.47	206.5	13.6	19.96	31.62	Dangdut
18	1800	106.07	112.4	98.78	107.47	50	14.98	236.95	63.06	45.88	74.17	Dangdut
19	1000	83.05	121.61	89.85	110.99	40	20.32	259.29	18.47	52.36	66.33	Dangdut
20	1750	128.91	138.13	121.5	108.37	50	26.1	252.11	44.42	51.35	84.59	Dangdut
∑	33450	2117.43	2541.8	2047	2251.94	1040	405.51	4642.22	855.1	966.8	1522.9	
x̄	1672.5	105.8715	127.09	102.35	112.597	52	20.2755	232.111	42.755	48.34	76.145	

Table 2. Morphometric characters of Gaga chicken from Pinrang, South Sulawesi, Indonesia based on crowing type

No.	Body weight (g)	Neck bone length (mm)	Femur length (mm)	Tibia length (mm)	Shank length (mm)	Shank diameter (cm)	Maxilla length (mm)	Wings length (cm)	Comb length (mm)	Third finger length (mm)	Cestbone length (mm)
	Slow type										
1	1400	98.56	114.69	91.6	106.72	50	18.26	218.11	51.05	50.64	77.59
2	1800	130.87	116.84	106.74	113.6	60	18.11	228.62	55.23	42.79	76.08
3	1300	97.48	75.76	79.04	114.14	50	19.3	202.64	39.22	49.83	82.69
4	2000	119.32	134.66	111.59	111.1	60	19.75	223.59	54.61	52.3	78.01
5	1500	105.41	134.13	73.8	99.34	50	16.58	165.09	50.77	47.22	68.01
6	2000	122.1	134.49	128.85	121.43	60	20.27	271.3	47.61	48.75	81.4
7	2200	106.66	136.35	118.71	123.93	50	21.48	239.53	65.81	49.53	88.81
8	2100	89.13	142.4	114.65	134.27	60	26.66	289.21	80.89	50.57	70.83
∑	14300	869.53	989.32	824.98	924.53	440	160.41	1838.09	445.19	391.63	623.42
x̄	1787.5	108.69	123.66	103.12	115.56	55	20.05	229.76	55.64	48.95	77.92
	Dangdut type										
1	2200	108.21	144.47	119.86	114.54	60	21.58	225.1	38.35	43.63	112.92
2	1400	90.42	136.85	95.53	113.94	50	15.42	224.93	42.56	49.02	65.99
3	2300	109.87	137.72	107.99	138.97	60	26.01	260.37	51.74	63.35	92.4
4	1100	103.1	126.58	106.37	112.83	40	22.39	247.26	17.28	43.83	99.16
5	2400	105.28	150.72	108.51	125.38	60	19.79	258.1	19.82	56.57	68.83
6	1700	99.01	123.92	95.8	112.84	50	23.87	250.95	61.23	53.84	69.54
7	1400	116.9	131.7	102.93	93.36	50	23.41	247.48	28.72	49.28	73.26
8	600	65.53	93.91	67.73	83.93	40	16.76	135.09	10.66	46.1	60.67
9	1500	131.55	134.47	107.17	104.79	50	14.47	206.5	13.6	19.96	31.62
10	1800	106.07	112.4	98.78	107.47	50	14.98	236.95	63.06	45.88	74.17
11	1000	83.05	121.61	89.85	110.99	40	20.32	259.29	18.47	52.36	66.33
12	1750	128.91	138.13	121.5	108.37	50	26.1	252.11	44.42	51.35	84.59
∑	19150	1247.9	1552.48	1222.02	1327.41	600	245.1	2804.13	409.91	575.17	899.48
x̄	1595.83	103.99	129.37	119.86	110.61	50	20.42	233.67	34.15	47.93	74.956

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Flower morphology, floral development and insect visitors to flowers of *Nepenthes mirabilis*

TRI HANDAYANI
Center for Plant Conservation, Indonesian Botanic Gardens (Bogor Botanic Garden), Indonesian Institute of Sciences. Jl. Ir. H. Juanda No. 13 Bogor 16122, West Java, Indonesia. Tel.: +62-251-8322187, Fax.: +62-251-8322187, email: irtri@yahoo.co.id

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Abstract. *Handayani T. 2017. Flower morphology, floral development and insect visitors to flowers of Nepenthes mirabilis. Biodiversitas 18: 1624-1631. Nepenthes mirabilis* Druce is a commercial ornamental pitcher plant belonging to the Nepenthaceae. This species is often used as a parent plant in artificial crossbreeding. The plant is also used in traditional medicine, rope-making, handicraft, and bouquets. Flower development and pollen maturity are important factors in pitcher plant crossbreeding. However, information about its flowering is still lacking. This study aimed to record the flower morphology, flower development, and faunal visitors to male inflorescences of *N. mirabilis* planted in Bogor Botanic Gardens, West Java, Indonesia. Twelve racemes of flowers were taken as a sample for observing the process of inflorescence development, while ten flowers on each raceme were observed for investigating the flowering pattern of individual flowers. The morphology of flowers, the process of inflorescence development, the flowering pattern for individual flowers, the number of open flowers, the longevity of anthesis, and the appearance of insect (and/or other faunal) visitors to flowers were observed and recorded, using naked eyes, a hand lens, and a camera. Six phases of inflorescence development were identified: inflorescence bud phase, raceme phase, the opening of the raceme-protecting sheath phase, inflorescence-stalk and flower-stalk growth phase, open flower phase and pollen maturity phase. Four phases of flower development were observed: growth of flower bud, the opening of tepals, pollen maturation, and flower senescence. The pattern of anthesis within an inflorescence was acropetal. The number of flowers per raceme was 56 to 163. The peak duration of anthesis of a flower was 11 days (30.7% of flowers). The length of the raceme-stalks was 17-31 cm. The length of the racemes was 23-38 cm. The most common visitors to the flowers were stingless bees, *Trigona apicalis.*

Keywords: Flower, *Nepenthes mirabilis*, pitcher plant, pollen, raceme

INTRODUCTION

Nepenthes L. (Nepenthaceae) are tropical pitcher plants. According to Jebb and Cheek (1997) and Cheek and Jebb (2001) there are 87 *Nepenthes* species in the world.. Most of them are grown and distributed in Malesian Region. The species *Nepenthes mirabilis* Druce is a popular ornamental plant and has commercial value (Plachno 2007). The species is often used as a parent plant in artificial crossbreeding. This plant is also used in traditional medicine, rope-making, handicraft, and bouquets (Handayani et al. 2005, 2012). It is one of the most widely known species in the genus. It has a broad distribution in China, Vietnam, Thailand, Peninsular Malaysia, Indonesia (Sumatra, Java, Borneo), Philippines, Sulawesi, Maluku, New Guinea, Palau Island and Australia (Cheek and Jebb 2001; Hua and Li 2005).

Nepenthes mirabilis is classified as lower risk (least concern) based on IUCN and as not threatened based on WCMC (Cheek and Jebb 2001). In Indonesia this species is protected by Peraturan Pemerintah (Government Law) No.7, 1999 about protection and conservation of plant and wildlife (Mogea et al. 2001). Therefore the trade of this plant is controlled internationally under CITES (D'amato 1998).

In nature, this species is most abundant in slightly seasonal habitats, such as swamps. It is equally common on alkaline and acidic soils. It does not compete well with other *Nepenthes*, so rarely is found in places where other *Nepenthes* species are abundant (Clarke 2001). Plants are dioecious (Phillipps and Lamb 1996; D'amato 1998) with each individual plant producing male flowers or female flowers only (Cheek and Jebb 2001; Clarke 2001).

The flowering patterns in lowland species are seasonal, while the mountain species flowered continuously throughout the year (Clarke 2001). *Nepenthes mirabilis* is one of the pitcher plants that is easily cultivated (Handayani et al. 2012). It is a climbing plant that blooms quite frequently, with individual plants producing a significant quantity of flowers. Plants generally bloom after climbing to a height where pitchers are produced (Clarke 2001; Cheek and Jebb 2001). Flowers are borne on peduncles which have simple pedicels. Male flower buds are rounded, while the female flowers have ovaries that are elliptical in shape. In general, the number of male flowers in a raceme of a male plant is more than the number of female flowers in the racemes of a female plant (Clarke 2001). D'amato (1998) reported about 70% of *Nepenthes* plants in forests produce male flowers, and only 30% produce female flowers. Male flowering plants are also more often found among cultivated *Nepenthes mirabilis*.

Pollination occurs when pollinators transfer pollen from male flowers to the stigma of a female flower (Clarke 2001). The olfactory signals from flowers play an important role in the specific attraction of pollinators. Many pollinators learn the particular scent signals of different species to recognize those flowers offering the highest quality rewards. However, not all floral rewards are available for all the potential visitors in a community (Farre-Armengol 2015). Pitcher plants attract insects by providing nectar throughout the year (Pavlovic et al. 2007; Giusto et al. 2008), by the scent emitted by the flowers (Pavlovic et al. 2007; Giusto et al. 2008), and by the attractiveness of the pitchers and brightly colored flowers (Hua and Li 2005). Flowers attract visitors specifically by the presentation of nectar and pollen. Nectaries and pollen are a reward for the insects and animals visitors pitcher (Hua and Li 2005; Bouer et al. 2007, Plachno 2007, Farre-Armengol 2015). Nectar is a sugar-rich liquid whose production in flowers is highly related to the energy requirements of pollinator species, especially when flowers are pollinated by only one or a few species (Clarke 2001; Farre-Armengol 2015). Kato (1993) and Owen et al. (1999) reported that the nectar is particularly attractive to ants. The tepals of the flowers are equipped with glands to produce nectar (Phillipps and Lamb, 1996). The shape of the tepals is rather cupped, intended for nectar containers. Nectar is a reward, enticing insects and animals to visit the flowers (Hua and Li 2005). The flowers can be located on any above-ground part of the plant (Plachno 2007).

Information regarding the biology of flowering in pitcher plants is still very limited. Kato (1993) conducted research into the flower biology of *Nepenthes gracilis*. Its flowers are pollinated by moths and flies. Moran (1993) cited in Clarke (2001) reported that insect visitors to *N. rafflesiana* are beetles, ants, moths, and flies. The flowering biology of *N. ampullaria, N. gracilis* and *N. reinwardtiana* in West Sumatra (Indonesia) has also been reported on by Meriko (2012). The flowers of these species bloom one by one or in groups, starting from the base toward the tip.

On the other hand, information on the flowering biology of *N. mirabilis* is lacking. The purpose of this current study was to identify the morphology of flowers, the process of inflorescence development, the flowering pattern for individual flowers, the number of flowers, the longevity of anthesis, and the appearance of insect (and/or other faunal) visitors to flowers. Understanding the flower morphology, flower development, and the kinds and numbers of insect visitors to the flowers will be useful in developing crossbreeding and propagation programs for this plant.

MATERIALS AND METHODS

Time and place

This research was carried out at Bogor Botanic Gardens, West Java, Indonesia from January 2015 to June 2015. The altitude is about 260 m asl, relative humidity was 34-80%, and the temperature was 25-39^{o}C.

Plant materials

Nepenthes mirabilis plants studied in the Gardens were part of a collection from Kalimantan that has been grown in the Bogor Botanic Gardens since 2004. Twelve staminate inflorescences were observed. Ten individual flowers from each inflorescence were investigated for flowers pattern. Observations of flower morphology, flower development, and flower visitors were made every day.

Observation of flower morphology

Observations of raceme and flower morphology were made on male flowering plants. Flowers were examined in terms of the type of inflorescence and the features of individual flowers, including their peduncles, pedicels, tepals, and stamens. Determination of the morphological characters of flowers was made by naked eye. The peduncle length, pedicel length, tepal length, tepal width, and stamen length were measured with a ruler.

Observation of floral development

These observations focused on four aspects: the inflorescence development process; the pattern of anthesis of individual flowers; the number of flowers; and the longevity of individual flowers . Twelve racemes of flowers were taken as a sample for observing the process of inflorescence development, while ten flowers on each raceme were observed for investigating the flowering pattern of individual flowers. Observation of the process of inflorescence development started from inflorescence bud enlargement until all individual flower had opened. Data were collected from when the inflorescence emerged from out of its inflorescence-protecting leaf (sheath) until when all individual flowers had opened. The stages of the process of inflorescence development were photographed using a Camera Canon Power Shot SX160IS. The flowering patterns of individual flowers were observed from the time of opening of the tips of the tepals until the time when the flowers withered and dried down. The number of flowers at anthesis (when the tepals of the flowers were open) were counted every day. The longevity of anthesis was calculated from the first opening of a flower until the time when the flower withered and dried.

Observation of the appearance of insects and other fauna

Insects and/or faunal visitors to the flowers were generally recorded at 06.00 to 10.00 h; 10.00 to 14.00 h, and 14.00 to 18.00 h. Visitors were identified through direct observation and grouped into bees, ants, beetles, moths, flies, wasps, butterflies or spiders. Determination of insects and/or faunas both as pollinator or not were recorded by using the foraging and feeding behaviors of flower visitors. Photograph taken during the visits.

RESULTS AND DISCUSSION

The flowering morphology

Nepenthes are dioecious plants. Most of the *Nepenthes* collection in Bogor Botanical Gardens only produce male flowers. D'amato (1998) reported that it is male flowers

that are most often produced in *Nepenthes* raised by *ex situ* cultivation. The inflorescence of *N. mirabilis* generally appears at the tip of the stem or branches. The flowering season for the *Nepenthes* collection in Bogor Botanical Gardens usually occurs between March and June. The growth of inflorescence buds was observed in January-March, the flowers bloomed in March-May, and then the flowers withered and dried in May-June.

The male inflorescence is a raceme, with a main axis, and with partial peduncles branching off, bearing 1-2 flowers, each with its own pedicel (Figure 1). The length of the inflorescence stalk (peduncle) is 17.0-31.0 cm. It has a brownish green color. The length of the raceme is 23.0-38.0 cm, with a brownish color. The individual pedicels are green in color and 1.0-2.0 cm in length. The form of the flower is simple with four tepals. The shape of the tepals is orbicular-elliptical and rather cupped. The color of tepals is reddish-purple. The length of tepals is 0.4-0.7 cm, while the width is 0.3-0.6 cm. The length of the staminal column is 0.3- 0.6 cm. It has a dark green-dark red color. At the tip of the staminal column are yellow-colored anthers. The inflorescence-stalks, peduncles, and tepals are all covered with soft hairs.

The development of flowering

The process of inflorescence development

The process of inflorescence development starts with the enlargement of an inflorescence bud and continues until all individual flowers have opened. The inflorescence bud is easily distinguished from vegetative shoots because of the enlargement of the buds. From the time of enlargement of the inflorescent bud until the full opening of all individual flowers, there are five phases: growing of inflorescence bud phase, growing of raceme phase, the opening of the inflorescence-protecting sheath phase, inflorescence-stalk and flower-stalk growth phase, anthesis phase, and pollen maturity phase (Figure 2).

Phase 1. Growing of inflorescence bud phase

This phase is first characterized by a reduction in the size of the leaves growing at the apex of a stem or branch. One leaf, the sheath that will protect the developing inflorescence, then begins to expand in contrast to the other leaves. This enlargement of the sheath is indicative of the initiation of the inflorescence within. The color of this encircling leaf is light green, covered by glaucous hairs.

Phase 2. Growing of raceme phase

This phase is characterized by the full enlargement of the inflorescence-protecting leaf. The size of the raceme enlarges towards the tip of this sheath. The middle part of the sheath bulges the widest, compared to the base and the tip. The color of the leaf is light green, covered by glaucous hairs. The inflorescence-stalk (peduncle) located inside the base of the sheath, while the tip of the raceme is inside the apex of the sheath.

Phase 3. Opening of the inflorescence sheath phase

This phase is characterized by the raceme emerging from its protecting leaf. After the raceme reaches full size, the edges of the sheath open gradually until fully open. After that, the raceme will be emergent and freed from the inflorescence sheath. The individual flowers at this point are still clustered together. The color of the flowers is silvery, silvery brown or silver-green. In our experiment, this phase occurred one month after the inflorescence shoot was first apparent in its protective leaf.

Phase 4. Growing of raceme-stalk and flower-stalk phase

This phase is characterized by the elongation of the raceme-stalk and individual flower-stalks. After the sheath opens, both raceme-stalk and the flower-stalks grow continuously. Finally, the individual flowers that are still clustered together, become separated one from the other. The length of the raceme-stalk emerging from the inflorescence sheath is 6-8 cm. The raceme-stalk elongates until the time when the individual flowers bloom. The longest raceme-stalk observed was 31 cm. Early on, the length of the raceme is only 5 cm, but after the individual flowers bloom the raceme reaches 38 cm in length.

At the time when the raceme is just emerging from the sheath, the flowers are still clustered together. With subsequent growth, the individual flower-stalks (pedicels) grow longer, which means ultimately that the flowers are no longer clustered together. The pattern of a flower blooming within an inflorescence is from the base toward the tip. From 1 to 5 days, the flowers on the lower third of the raceme separate from each other. By 5-8 days, two-thirds of the raceme has its flowers separated from one another, while by days 8-11 all the individual flowers are separate.. The separation of individual flowers starts from the base and progresses towards the tip of the raceme In our experiment, it occurred 2-12 days after the young sheath opened. The color of the raceme at this point was silver or silver-green.

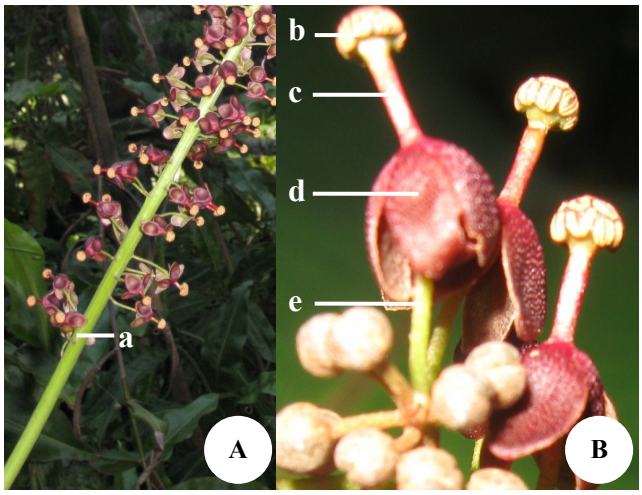


Figure 1. Parts of male inflorescense. A. Male inflorescense, B. Male flowers, a. Inflorescense stalk, b. Anters, c. Column, d. Tepal, e. Pedicle



Figure 2. The process of inflorescence development. A. First phase , W1, B. Second phase,W 1-3, C. Third phase, W 3-4, D. Fourth phase, W 5-6, E. Five-six phase, W 6-8. W: week

Phase 5. Opening of flowers phase

This phase is characterized by the opening of tepals of the individual flowers. This phase starts with the opening of the tepals at their apices until the flower tepals are fully opened. After the tepal tips open, the light yellow anthers appear in the opening space between the tepal apices. Tepals open further until fully apart. The tips of the sepals then curve outwards and downwards towards the pedicel. In our experiment, this occurred 2-12 days after the leaf-wrapper opened. The duration of the opening of the raceme until it dries was from 22-49 days.

Phase 6. Pollen maturity phase

In our observations, the maturation of pollen generally occurred a day after the opening of a flower. The maturation of the stamens was followed by the rupture of the anthers and the appearance of pollen on the anthers. The time of stamen maturation began in the afternoon. The amount of visible pollen was greater at night than in the daytime. This. would suggest that the flowers are pollinated by nocturnal insects? It should be observed and experiment further.). This phase occurred 1-2 days after the blooming of flower (i.e. after the opening of the tepals).

The pattern of individual flower blooming

Nepenthes mirabilis flowers are borne on partial peduncles that have 1-2 flowers each with its own pedicel. A male flower of *Nepenthes* generally consists of flower-stalk, tepals, staminal column, and anthers. Flowers open throughout the day and the pollen is shed some hours later. Male *N. mirabilis* flowering starts from the raceme base with a total set of 56-163 flowers per inflorescence. A variable number of flowers (1-40 flowers) open each day, starting from the base of the inflorescence. The anther cluster releases pollen after the tepals open.

Hua and Li (2005) reported that when *Nepenthes mirabilis* is flowering in field, the odor is almost undetectable. It is unknown where the odor is released from, but they explained that it was from nectaries on tepals. They suggested making further observation and experiment of this species to prove that the odor emanating from the flower. From my observation, the odor release from the flower, if the flower tip opened a fragrant smell like the odor of pandanus leaves release from the flower. Then, the yellow pollen is often shed directly into the tepals. As soon as the flower tip opened, the insects visit the flower.

In this research, there were four phases detected in flower development: growing of the flower bud, opening of tepals, pollen maturation, and flower senescence.

Phase 1. Growing of the flower bud

Initial growth of flower buds occurs in the inflorescence, protected by the inflorescence sheath. Each raceme consists of 56-163 flowers and the growing of the individual flowers is in line with the growing of the whole inflorescence. After the opening of the protecting leaf wrapper, the growth of the flowers appears faster. The flower buds are ovoid. Their color is green covered by brownish hairs. The length of each pedicel is 1.0-2.0 cm. The pedicel is greenish-yellow and is covered by brownish hairs.

Phase 2. Opening of the tepals

Tepals are 0.4-0.7 cm long and 0.3-0.6 cm wide. The process of a flower blooming is presented in Figure 3. The process starts with the opening of the tips of the tepals. The tepals open wide until their positions are almost perpendicular to the stamens. With increasing age of the flowers, the tepals reflex backward arching down towards the flower-stalk to almost cover the flower-stalk. The tepals gradually dry out during flower blooming. Their inner, adaxial surfaces are usually reddish-green, reddish-brown, or yellowish-green, when they open on first day, turning reddish-purple post full blooming. On the other hand, their outer, abaxial surfaces are yellowish-green or reddish-brown when they open, turning greenish-red, reddish-purple or greenish-red.

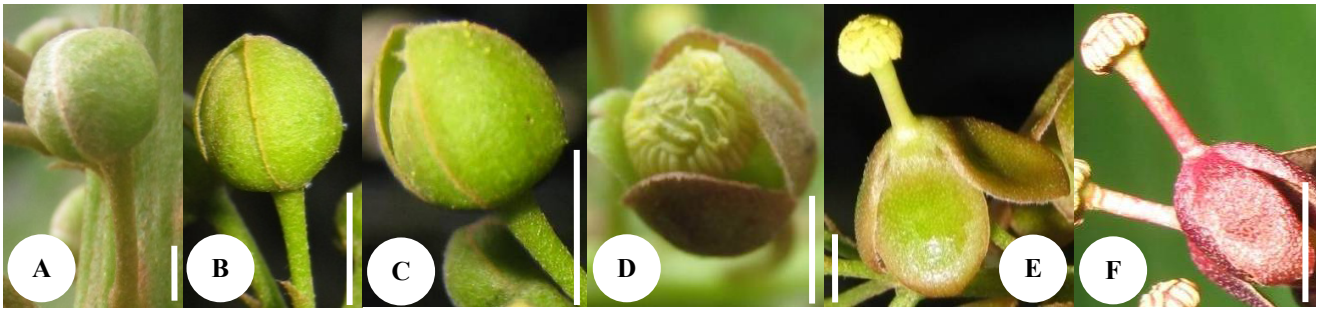


Figure 3. The process of a flower blooming. .A-B. Closed flower bud. C-D. Opening tip tepals. E. Fully opening tepals. F. Post blooming flower. Bar: 0.5 cm



Figure 4. A. Pollen maturation phase. B. Senescense phase

Phase 3. Pollen maturation

Flowers that will bloom are characterized by the stretching of the tepals and the opening of their tips. One to two days after these signs, the flower usually bloom. The yellow anthers usually appear when the tips of the tepals are opening. Stamens are more apparent as the tepals open out.

The anther cluster releases pollen after the tepals open (Figure 4.A) The release of pollen from the anthers lasts for 2 days. The flower-stalk, tepals, and stamens change during the progress from blooming to post-blooming. The flower-stalk is usually brownish-green on the first day of a flower blooming, turning reddish-green or brownish-green, 2-7 days later. After the seventh day, the flower-stalk turns dark green. The color of the staminal column and anthers change after the maturation of pollen. The column is usually light yellow when in full bloom, turning light red or purplish-yellow a day later. Two days later the column turns dark red to purplish-red, while the anthers are light yellow or yellow during blooming, turning dark yellow a day later. Two days later, the anthers turn dark yellow-purplish (Figure 4.B).

Phase 4. Senescence of the flower

This phase is characterized by the drying out of the anthers. The flower-stalk, tepals, and stamens have reached their maximum size and so do not grow again. Next, these parts wither and dry up, starting from the anthers and progressing to the flower-stalk. In our observations, anthers began to dry out 9-13 days after the start of blooming of the flower. All parts of the flower had dried down 49 days after blooming in the quickest maturing flowers.

The number of flowers in bloom

Flowers bloom uniformly, starting from the base to the tip. The number of flowers that bloom in a raceme each day varies significantly, from 2 to 40 flowers per day (Figure 5). In general, only a few flowers bloom initially, with the number per day peaking at about day 3 to 4. Later, the blooming tends to be stretched out with fewer open flowers per day. The accumulated number of flowers that open per raceme ranges from 56 to 163 flowers (Figure 6). The total flower number per raceme is related to its length. The results of my observation indicate that for racemes less than 30 cm in length, less than 100 flowers are produced. Racemes more than 30 cm long produce more than 100 flowers.

The type and amount of insect visitors to the flowers were observed to be related to the number of blooming flowers per raceme. The greater the number of flowers that bloom in a raceme the greater the quantity of pollen and nectar released, and the more favorable are the conditions for visitors to forage for nectar or pollen. When a flower is in full bloom, the color of the four tepals is bright (reddish-brown), so attracting insects to visit the flower. The sweet smell of the flowers is also an attractant for insects. Tepals are one of the sources of nectar in *Nepenthes* The main visitors in *Nepenthes* flowers are insects. Visual signals are important in attracting diurnal insects to flowers. High-contrast color pattern help pollinators orient themselves to reach the nectar source. Among the common floral pigments are antocyanins (blue, red) and carotenoids (Chittka and Menzel 1992; Moran et al. 1999; Clarke 2001).

The longevity of blooming in individual flowers

The longevity of blooming in flower is the time from the beginning of its tepals opening until the time when the flower dries. This research found that the longevity of flower blooming was from 10-15 days. The mode for the distribution of longevity was at 11 days, with 30.7% of all flowers observed having a longevity of 11 days (Figure 7). Although the duration of the blooming process for an individual flower is quite long, the duration of pollen maturity is only about 1-2 days. This means that potential

pollinators have only a relatively short time in which mature pollen can be obtained and can contribute to the pollination of flowers on female plants.

Flower visitors

Nepenthes is a dioecious (Chua 2000), obligate cross-pollinated species that depends on insect pollinators. It is well known that olfactory signals from flowers play an important role in the specific attraction of pollinators. *Nepenthes* flowers attract visitors using color, odor, nectaries and pollen. These species seem to be mainly pollinated by flies (Calliphoridae, Muscidae and Syrphidae) and moths. However, Jurgen et al. (2001) found that Formicidae was the dominant prey category present in the pitchers, accounting for between 50-90% in the ground pitchers. Several insects visit *Nepenthes* flowers, but the most effective pollinators are moths, flies, wasps, and butterflies (Plachno 2007). Kato (1993) reported that flowers of *N. gracilis* are pollinated by moths and flies. Clarke (2001) reported that insect visitors on *N. rafflesiana* are beetles, ants, moths, and flies. In my observations, from the time that the tips of flower tepals opened, numerous insects visited *Nepenthes* flowers between 06.00 a.m and 18.00 p.m. The staminate inflorescences were visited by a variety of flower visitors, ranging from stingless bees (*Trigona* spp), ants, wasps, flies (hoverflies and green bottles), moths and mosquitoes (Table 1, Figure 8).

Although many of these types of insects visited the flowers, some of those visitors appeared not necessarily to be effective pollinators; feeding on pollen but not necessarily transferring it to other flowers. My observations suggest that stingless bees were effective gatherers of pollen, but not necessarily effective pollinators, with their hind legs often carrying away the yellow pollen (Figure 9A). Ants and flies were common flower visitors (Figure 9B) . They ate nectar, disturbing other flower visitors, sometimes damaging pollen. Ashmand and King (2005) reported that ants could be effective pollinators especially in plant species with a low, dense, flowering stature. The positive relationship between ant and plant can be seen in ant-plant associations the ants defend their resources and thus protect their plant partners against herbivores (Merbach et al. 2007). In other situation, ants can act as antagonists, by removing nectar, disrupting the visitation of effective pollinators, and reducing pollination. According to Clarke (2001), several visitors visit *Nepenthes* flowers during both day and night for nectar and pollen.

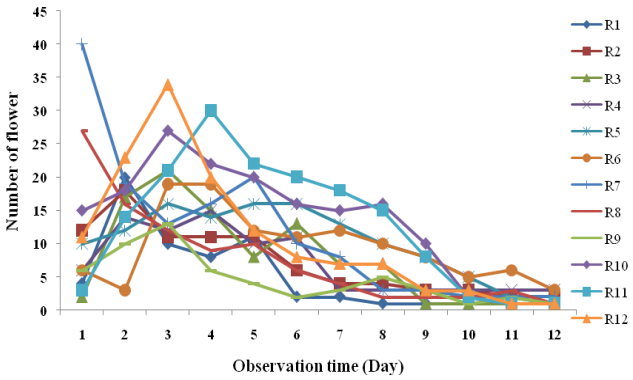


Figure 5. The number of flowers in bloom per day in twelve racemes observed (R1-12= Raceme 1-12).

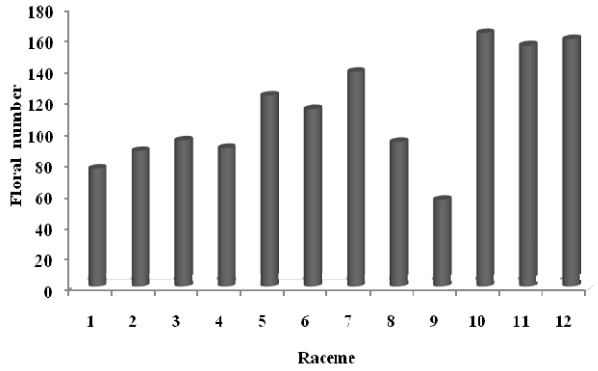


Figure 6. The accumulated number of flower that bloomed in each of 12 racemes

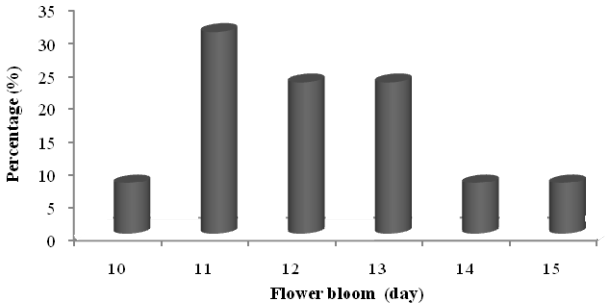


Figure 7. Distribution for longevity of a flower in bloom

Table 1. The common visitors to the male flowers of *N.mirabilis*

06.00 to 10.00 h	10.00 to 14.00 h	14.00 to 18.00
Whitish-brown spider	White spider	*Trigona* spp. (stingless bees)
Yellow spider	Yellow spider	Ants
Ants	Green spider	Yellow spider
Trigona spp (stingless bees)	Ants	Hoverflies
	Hoverflies	Green bottles (fly)
	Green bottles (fly)	Mosquitoes
	Trigona spp (stingless bees)	
	Aphis dorsata (bee)	
	Wasps	

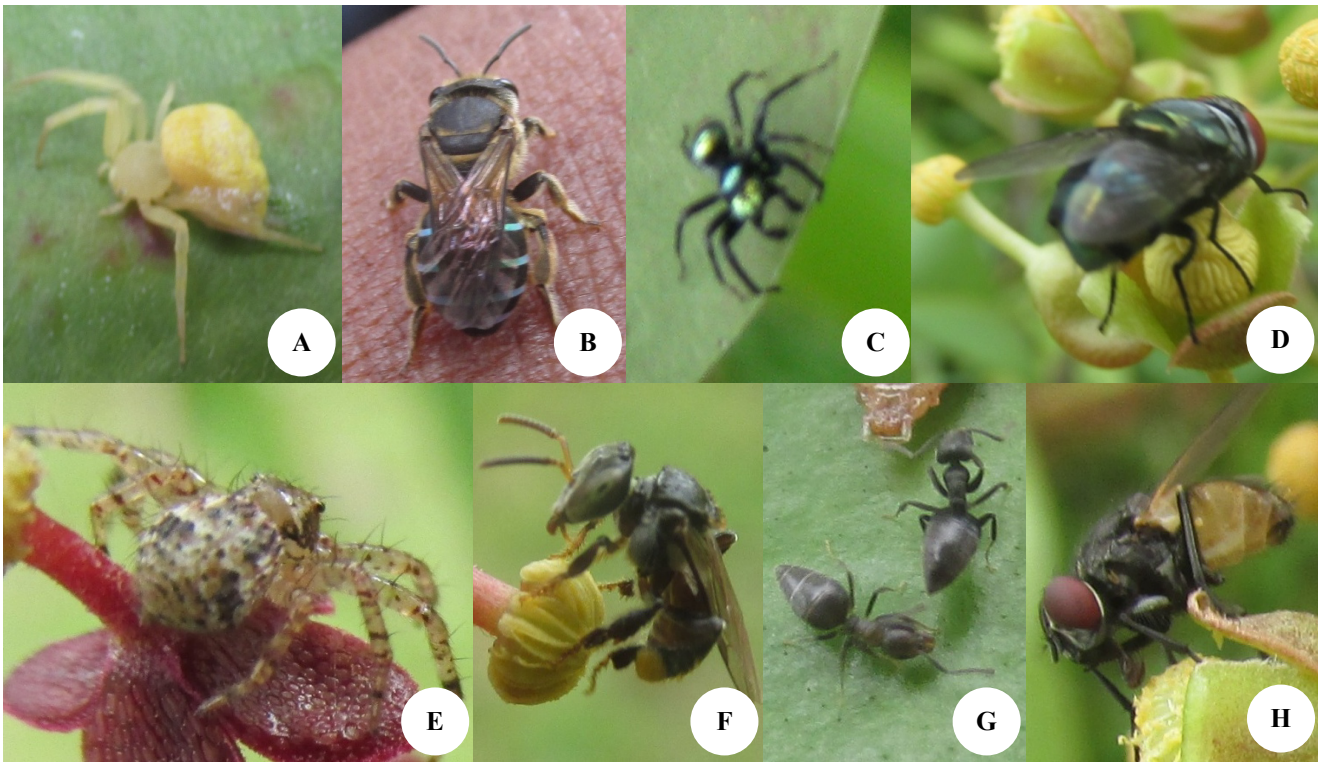


Figure 8. The common visitors to the male flowers of *N. mirabilis,* A. Yellow spider, B. *Aphis dorsata*, C. Green spider, D. Green bottles, E. Whitish spider, F. *Trigona* sp., H. Hoverfly



Figure 9.A. Stingless bees with yellow pollen in it hind leg. B. Green bottles on male flowers

In summary, the present study has shown that the process of inflorescence development can be described in six phases while the blooming pattern for individual flowers is most usefully described in four phases. The number of flowers blooming within the flowering period of a raceme was found to vary from 2-40 per day, while the total number of flowers that bloomed over the lifetime of a raceme varied from 56 to 163. The duration of a flower from beginning to the end of blooming was 10-15 days. The staminate inflorescences were visited by a variety of flower visitors, ranging from stingless bees (*Trigona* spp.), ants, wasps, flies (hoverflies and green bottles), moths and mosquitoes.The possible pollinators are stingless bees.

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Short Communication: Using RAPD technique to evaluate genetic diversity of longan (*Dimocarpus longan*) population in Vietnam

VIET THE HO[1,♥], QUOC NGUYEN NGO[2]

[1]Faculty of Biotechnology and Environmental Technology, Ho Chi Minh City University of Food Industry. 140 Le Trong Tan Street, Tan Phu District, Ho Chi Minh City, Vietnam, Tel.: +84-933-025-523, ♥email: thehv@cntp.edu.vn

[2]Ho Chi Minh City University of Science, 227 Nguyen Van Cu, District 5, Ho Chi Minh City, Vietnam

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Abstract. *Ho VT, Ngo QNg. 2017. Short Communication: Using RAPD technique to evaluate genetic diversity of longan (*Dimocarpus longan*) population in Vietnam. Biodiversitas 18: xxxx.* Longan (*Dimocarpus longan* L.) is an important fruit plant in Vietnam where several cultivars and landraces are being cultivated. Presently, the identification of longan cultivars and landraces is mainly based on personal experience relying on morphological traits leading the difficulty of genetic conservation and longan breeding. In this study, genetic richness and relativeness of 36 longan accessions collected from different provinces in Vietnam were evaluated by using 30 Random Amplified Polymorphic DNA (RAPD) primers. Our results reveal that there is the large variation of genetic background of studied longan accessions with separate groups in the obtained dendrogram. We also identified the combination of different RAPD markers could help to identify 15 longan genotypes. The results from this project could provide valuable information which is necessary for classifying, identifying plant origins, breeding and conserving programs of longan in Vietnam.

Keywords: *Dimocarpus longan*, genetic diversity, molecular markers, RAPD

INTRODUCTION

Longan (*Dimocarpus longan* L.) is an important fruit tree in Vietnam with a total area of 75,200 ha and the annual production is around 620,000 tons. Despite its importance, breeding and conserving activities of the plant in this country is limited and the origin of this plant in the country is uncertain (FAO 2004). Longan varieties in this area are mostly identified by their local names mostly based on where plants are grown or notable plant features without clear clue about their origin or genetic characteristics. Vernacular names of longan varieties are just based on plant or fruit features which are not accurate because these characteristics can change with environment and culture (Qiu and Zhang 1996). Additionally, studies on longan is mainly depended on agronomical and morphological characteristics (FAO 2004; Hong and Chau 2004; Hoang 2011). Thus, historical identification of plant is challenged because morphological observations are highly dependent on environmental conditions and developmental stages of a plant (Yonemoto et al. 2006).

Presently, RAPD was shown as highly potential technique to genetic study of plant without adequate prior genetic information such as longan. In 2001, by using RAPD Chen and Liu (2001) re-identified new longan cultivar "Reying 17" which was previously considered belong to "Shixia" cultivar. Also in this study, they also detected several cultivar names are replicated because of the identical genetic composition such as "Dazao" and Zaohong", "Feiziziao" and "Zimai", or "Baiye" and "Guahong" in 62 lichi and longan accessions studied. More recently, Yang and colleagues (2013) were able to generate specific loci which are later used to distinguish cultivated longan *D. longan* from its wild and poisonous relative *D. confinis*. Moreover, Mei's research group used successfully this technique to distinguish different longan accessions from geographical origins in China (Mei et al. 2014). The obtained results in this study will be useful for genetic conservation and breeding purposes. The accuracy of genotype identification will be crucial help for farmers to set up new longan orchards because they can significantly reduce the maintaining of undesirable plants. Furthermore, the finding markers tightly linking to favorable cultivars among accessions will also pave the way for breeding programs to produce new longan varieties with better yield and higher fruit quality of longan in Vietnam.

MATERIALS AND METHODS

Sample collection and DNA extraction

Total of 36 leaf samples was collected from North to South of Vietnam (Figure 1 and Table 1). Leaf samples were dried and stored in silica gel until use. DNA was extracted with CTAB method (Cetyl Trimethyl Ammonium Bromide) as Madhou et al. (2013). PCR reactions were

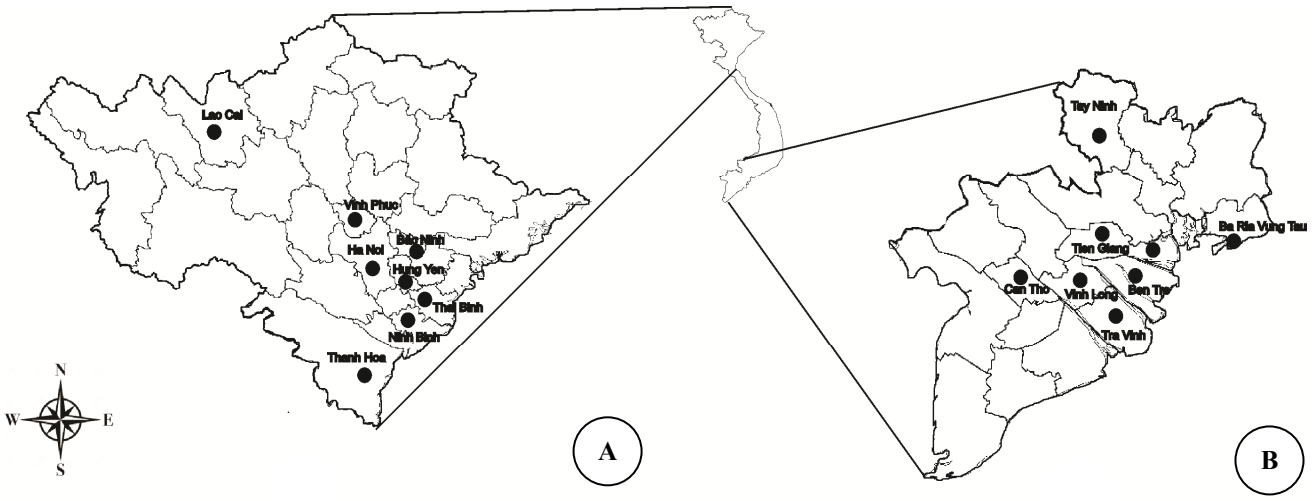


Figure 1. Targeted areas for sample collection of *Dimocarpus longan* in Vietnam. (A: Northern region; B: Southern region; black dots indicate the sample collection sites in this study)

Table 1. Samples collected for genetic characterization in present study

Code	Name of cultivar/variety/local name	Collected location
1	Nhan To Chua Hien	Hung Yen
2	Nhan Long Chin Som	Plant Genetic Resources of Vietnam
3	Nhan Xuong Com Vang	Tay Ninh
4	Nhan Chin Muon	Hung Yen
5	Nhan Chin Muon	Ha Noi Agriculture University
6	Nhan Thuan Thanh Bac Ninh	Bac Ninh
7	Nhan Co Bac Ninh	Bac Ninh
8	Nhan Gia Vien Ninh Binh	Ninh Binh
9	Nhan dai Quang Xuong	Thanh Hoa
10	Nhan dai TP Ninh Binh	Ninh Binh
11	Nhan Duong Phen	Fruit and Vegetable Research Insitute
12	Nhan Xuong Com Vang	Vung Tau
13	Nhan Cui Thai Binh	Thai Binh
14	Nhan dai Lao Cai	Lao Cai
15	Nhan Duong Phen Chua Hien	Hung Yen
16	Nhan Thai Tay Ninh	Tay Ninh
17	Nhan Tieu Hue Tay Ninh	Tay Ninh
18	Nhan Ha Tay	Fruit and Vegetable Research Insitute
19	Nhan Xuong Com Vang Ben Tre	Ben Tre
20	Nhan Xuong Com Vang Vinh Long	Vinh Long
21	Nhan Idor Can Tho	Can Tho
22	Nhan My Ben Tre	Ben Tre
23	Nhan Huong Chi	Ha Noi University of Agriculture
24	Nhan Da Bo Long Huong	Ba Ria
25	Nhan dai TP Ninh Binh	Vinh Phuc
26	Nhan Long Chin Som Thanh Hoa	Thanh Hoa
27	Nhan dai Cau Lim Ninh Binh	Ninh Binh
28	Nhan Mien Thiet	Fruit and Vegetable Research Insitute
29	Nhan Huong Chi	Fruit and Vegetable Research Insitute (Ha Noi)
30	Nhan Xuong Com Vang	Southern Fruit Research Institute
31	Nhan dai Quoc Tu Giam	Ha Noi
32	Nhan dai Ben Tre	Long An
33	Nhan tieu da bo	Tra Vinh
34	Thanh Nhan Ben Tre	Ben Tre
35	Nhan dai Ba Dinh Ha Noi	Ha Noi
36	Nhan dai But Thap	Bac Ninh

performed in a total volume of 20 µL containing 30 ng DNA, 1X reaction buffer, 2 mM $MgCl_2$, 0.3 µM of each primer, 200 µM of each dNTP, 1 unit of *Taq* polymerase and sterile water to the final volume. PCR amplification was carried out as follows: initial denaturation at 95°C for 2 minutes; after that followed by 40 cycles of 30 s at 95°C, 30 s at 35°C, 54 s at 72°C and final extension for 5 min at 72°C. PCR amplification was then separated by electrophoresis in 1.5% agarose gel in 1X TAE buffer, and stained with 0.5 µg/mL Gelred TM loading buffer then visualized under ultraviolet light. The primers for PCR reactions were chosen as described by Yonemoto et al. (2006) and Mei et al. (2014) shown in Table 2.

RAPD reactions and data analysis

After electrophoresis, only clear and reproducible bands with size from 200 to 2,500 bp among two repeated PCR were considered for data analysis (Figure 2). The quality of RAPD markers is evaluated through polymorphism information content (PIC) value described by Chesnokov and Artemyeva (2015) as the formula:

$$PIC_j = 1 - \sum_{i=1}^{n} P i^2 .$$

Where i is i-th allele of the j-th marker, n is the number of the j-th marker's alleles, P is allele frequency.

PCR products were scored as "1" for the presence and "0" for absence in specific position. The dendrogram was built based on the unweighted pair group method with arithmetic mean and the algorithm (UPGMA) was produced by using SAHN module in NTSYSpc 2.1 package (Rohlf 2000). Principal Coordinate Analysis (PCoA) was performed based on RAPD data to have better understanding about similarity among accessions by using PCoA package in NTSYS-pc 2.1 (Ibrahim et al. 2017).

Table 2. List of RAPD primers to analyze genetic diversity of longan in Vietnam

Primer	Primer sequence
SBS-A15	TTCCGAACCC
SBS-Q19	CCCCCTATCA
SBS-M1	GTTGGTGGCT
SBS-Q12	AGTAGGGCAC
A02	GCCAGCTGTACG
A03	TGCCTCGCACCA
A13	CTCAGCGATACG
A18	GACCTGCGATCT
A19	AAGGCGCGAACG
A37	AGCGCGGCAAAA
A39a	CCTGAGGTAGCT
A58	GTCATGCCTGGA
A62	TCGTCCGGAGAT
C11	AGGTACGCCCGA
C16	CGCCCTGCAGTA
C31	GAGTTGCCCGGA
C34	TACCCAGGAGCG
C59a	CGCGTTCGTGGA
C62a	CCATCCGCACGA
C82	ATCGTCACCCCG
D12	CTGGTCTCTGGG
D29	GACCCGGAACGA
D38	AAGCTCGACGGG
D41	GAGACCCGTCGA
D50	GACTCGCGGTCT
D53	GCCGCGGAACTA
D84	AGACACACGGGC
F10a	CAGGCCGAAGTC
F44a	GGTGTCTTGCGG
Z1	CCGGTGCCTTCT

Table 3. Test results of RAPD primers used in present study

Primer	Band number	Polymorphism (%)	PIC value
SBS-A15	12	58.3	0.43
SBS-Q19	11	27.3	0.27
SBS-M1	9	55.6	0.51
SBS-Q12	9	44.4	0.47
A02	10	30.0	0.42
A03	8	50.0	0.57
A13	11	45.5	0.43
A18	8	25.0	0.41
A19	10	70.0	0.58
A37	11	18.2	0.47
A39a	9	44.4	0.37
A58	13	38.5	0.56
A62	12	66.7	0.75
C11	10	40.0	0.51
C16	14	21.4	0.69
C31	4	25.0	0.39
C34	6	33.3	0.63
C59a	8	37.5	0.40
C62a	7	42.9	0.45
C82	12	41.7	0.40
D12	10	70.0	0.39
D29	7	57.1	0.72
D38	8	37.5	0.68
D41	10	20.0	0.36
D50	12	16. 7	0.34
D53	7	57.1	0.43
D84	9	77.8	0.33
F10a	9	66.7	0.39
F44a	6	50,0	0.52
Z1	12	41.7	0.35

RESULTS AND DISCUSSION

Total of 36 longan accessions was characterized with 30 RAPD primers. We found that all primers used in this study generated high polymorphism among different accessions (Table 3) with clear amplification and high reproducibility after two replications. Tested primer generated from 4 to 14 amplifications ranging from 200 to 1500 bp with a large variation of total and polymorphic bands from 18.2 to 77.8%. All primers show PIC value from 0.27 to 0.75 (Table 3), meaning that all of these used primers are suitable for genetic characterization of longan collected in research areas. As PIC classification of Botstein et al. (1980) as following: highly informative if PIC > 0.5; reasonably informative if 0.5 > PIC > 0.25 and slightly informative if PIC < 0.25.

The combination of different amplicons from this study shows the high discrimination capacity of RAPD markers, relying on RAPD data, we could successfully identify several important longan cultivars by using specific amplification from either single or combined different primers. For example, by using primer D38, there is a candidate allele at 600 bp which could be used to identify Nhan Xuong Com Vang Tay Ninh province as shown in line 3 (Figure 2). This is a high-value cultivar because of its superior feature as high resistance to diseases and high fruit quality. Additional bands with size of 1000 bp and 1100 bp could also be potential for recognizing two longan landraces from Thuan Thanh district, Bac Ninh provine (line 6) and Gia Vien district, Ninh Binh province (line 8), respectively. The total RAPD amplification can be utilized to different 15 longan genotypes are presented in Table 4. The ability of RAPD method to differentiate longan genotypes early reported in the previous study of Yenomoto and colleagues (Yenomoto et al. 2006) because RAPD is able to produce a higher number of genetic loci than morphological and biochemical markers. Moreover, RAPD markers were also successfully applied in testing the fidelity of micropropagation of different plants (Rani et al. 1995). Thus, the specific bands obtained in our study could be used to identify the origin of materials for longan breeding programs at least in studied areas. In spite of numerous genetic information was generated, the RAPD makers used in this study is not able to distinguish completely all longan accessions. We suggest that the estimation of genetic diversity by RAPD marker is highly influenced by specific genome of selected accession and by the specific RAPD primer examined. In order to get more accurate result from RAPD markers, future studies need use higher number of marker to cover higher density on genome of plant to measure the genetic variation more exactly. Another reason should be noted that the most samples in this study are from cross-pollinated plants, consequently, the obtained genetic variability is highly depended on individual plants.

Based on obtained RAPD data, the relatedness of 36 longan genotypes was analyzed with NTSYSpc 2.1 amplification profiles were compared to generate a similarity matrix and showed in Table 5. Overall, the genetic similarity varied from 0.46 to 0.95. The lowest

similarity value (0.46) was observed between Nhan Dai Ben Tre (32) and Nhan Dai Thanh Pho Ninh Binh (10) and the highest similarity value (0.95) was observed between Nhan Xuong Com Vang Tay Ninh (3) and Nhan Xuong Com Vang Ben Tre (19). This result is reasonable because Nhan Dai Ben Tre (32) and Nhan Dai Thanh Pho Ninh Binh (10) were collected from South and North regions, respectively. Whereas the close relationship between Nhan Xuong Com Vang Tay Ninh (3) and Nhan Xuong Com Vang Ben Tre (19) is not surprised because both of them are from the southern region and belong to Nhan Xuong cultivar suggesting these two accessions have a common ancestor.

The dendrogram was then built based on similarity matrix which showed clear three main groups. The first group consists of 16 genotypes, the second group consists of 16 genotypes and the last group includes 4 genotypes. We found that there is the difference of genetic composition of longans in the North and the South of Vietnam. It is interesting that all group I consists only accessions collected from North of Vietnam. Group III includes only longan from the Southern area with four accessions namely Nhan Dai Ben Tre (32), Nhan Tieu Da Bo Tra Vinh (33), Thanh Nhan Ben Tre (34) and Thanh Nhan Bac Lieu (35), whereas there is the mixture among accessions collected from both areas. This could explain that longan breeders might have used materials from same desirable plants and grafted to several different plants or multiplied and sell to different places. Interestingly, we also found that some accessions from the North and the South showing high similarities such as Nhan My Ben Tre (22) and Nhan Bac Ninh (7). This could be the exchange longan seedlings from different places because in Vietnam farmer is relatively willing to accept new cultivars from different places to grow in their gardens because this will increase income due to the new product in market. It is also common when in the market or seedling nursery, there are several longan accessions with origin from foreign countries is sold.

Our results are in agreement with the previous publication of Yonemoto et al. (2006) where they found the high genetic diversity of longan population in Thailand. Interestingly, among 22 longan genotypes, their study also included four longan accessions from Vietnam consisting of two well-known accessions: Xuong Com Vang and Tieu Da Bo, together with two unknown identified accessions namely Ishigaki-2 and Ishigaki- 2. Using RAPD marker and built dendrogram with NTSYSpc 2.2, they also reported that two unknown identity longan accessions from Vietnam belong to separate group and have far genetic distance with other accessions from Vietnam, Thailand, China, United State, Taiwan, Malaysia, and Japan. On the other hand, Xuong Com Vang and Tieu Da Bo cultivars were also grouped together and processing genetic similarity with their nearest neighbor just as low as 0.824.

The relationship among different longan accession was also evaluated by PCoA, the first two coordinates were shown in Figure 4. On the basis of the first coordinate, up to 83% was explained by the first two axes accounting for 50 and 33% of dimension 1 and dimension 2, respectively.

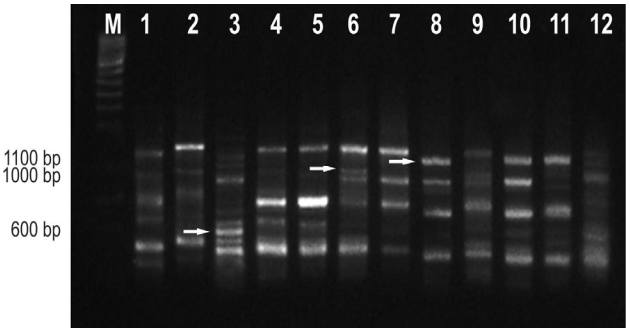


Figure 2. Representative RAPD result with D38 primer. (The arrow shows the candidate amplification marker to distinguish specific longan cultivars with other longan accessions; the number is corresponding to sample code in Table 1; M: DNA ladder)

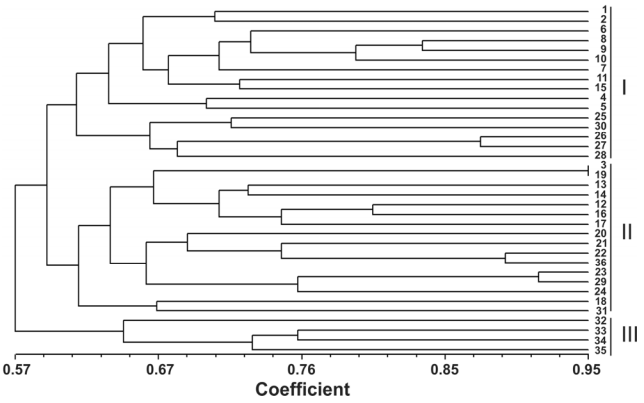


Figure 3. Dendrogram generated by using 30 RAPD markers to show the genetic relativeness of 36 longan accessions. This dendrogram was developed using UPGMA cluster procedure of NTSYSpc 2.2. The scale shown at the bottom is the measure of genetic similarity. The scale shown at the bottom is the measure of genetic similarity. (The number is corresponding to sample code in Table 1).

Table 4. Longan accessions-specific amplified bands produced by selected RAPD primers

Accession name, collected place	Primer	Size of candidate amplification (bp)
Nhan Duong Phen, Hung Yen	D29	900
Nhan Mien Thiet, Khoai Chau	C59a	1000
Nhan My, Ben Tre	C82	2500
Nhan Thai, Tay Ninh	A58	1200
	C16	500
Nhan Tieu Hue	A58	1200
	D29	400
	C16	800; 1000
Nhan Xuong Com Vang, Tay Ninh	D38	600
	C31	850
	C82	300
Nhan Xuong Com Vang, Vinh Long	D12	500; 2000
Nhan Xuong Com Vang, SOFRI	C34	900
	D38	400; 1000; 1200
Nhan To Chua Hien, Hung Yen	A62	900
Nhan Long Chin Som, Hung Yen	A62	1200
Nhan Ha Tay	D29	300
Nhan Huong Chi, Hung Yen	C34	500
Nhan Ben Tre	D38	1200
Nhan Thuan Thanh, Bac Ninh	D38	1000
Nhan Gia Vien, Ninh Binh	D38	1100

Table 5. Simple matching coefficients of similarity among 34 genotypes of longan (*Dimocarpus longan*)

	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	1.00																																			
2	0.70	1.00																																		
3	0.54	0.53	1.00																																	
4	0.70	0.56	0.56	1.00																																
5	0.68	0.62	0.56	0.70	1.00																															
6	0.67	0.71	0.58	0.69	0.63	1.00																														
7	0.63	0.66	0.56	0.62	0.54	0.68	1.00																													
8	0.65	0.65	0.59	0.73	0.65	0.77	0.74	1.00																												
9	0.66	0.72	0.53	0.69	0.62	0.74	0.76	0.84	1.00																											
10	0.61	0.66	0.52	0.68	0.63	0.68	0.66	0.79	0.80	1.00																										
11	0.65	0.70	0.56	0.65	0.58	0.68	0.68	0.73	0.71	0.74	1.00																									
12	0.62	0.64	0.57	0.57	0.60	0.64	0.61	0.65	0.65	0.66	0.72	1.00																								
13	0.68	0.59	0.61	0.65	0.65	0.65	0.58	0.64	0.56	0.61	0.56	0.60	1.00																							
14	0.61	0.53	0.65	0.71	0.66	0.62	0.59	0.67	0.62	0.61	0.58	0.58	0.73	1.00																						
15	0.62	0.67	0.66	0.58	0.60	0.63	0.61	0.65	0.63	0.63	0.61	0.61	0.73	0.73	1.00																					
16	0.65	0.60	0.68	0.59	0.61	0.60	0.59	0.60	0.58	0.56	0.55	0.54	0.71	0.75	0.81	1.00																				
17	0.59	0.58	0.67	0.55	0.53	0.59	0.61	0.58	0.59	0.57	0.58	0.58	0.68	0.65	0.75	0.74	1.00																			
18	0.58	0.56	0.61	0.58	0.54	0.60	0.55	0.58	0.54	0.54	0.50	0.59	0.63	0.53	0.63	0.60	0.61	1.00																		
19	0.55	0.53	0.95	0.58	0.56	0.60	0.56	0.62	0.56	0.53	0.57	0.57	0.62	0.68	0.69	0.69	0.69	0.61	1.00																	
20	0.61	0.55	0.66	0.60	0.59	0.61	0.60	0.62	0.62	0.59	0.61	0.67	0.62	0.63	0.65	0.64	0.69	0.64	0.68	1.00																
21	0.61	0.58	0.62	0.59	0.60	0.56	0.52	0.60	0.55	0.57	0.61	0.65	0.65	0.62	0.59	0.65	0.61	0.59	0.59	0.73	1.00															
22	0.64	0.56	0.60	0.61	0.62	0.62	0.48	0.60	0.54	0.56	0.59	0.63	0.68	0.63	0.63	0.63	0.65	0.68	0.62	0.68	0.76	1.00														
23	0.58	0.56	0.62	0.60	0.57	0.56	0.56	0.61	0.58	0.64	0.62	0.60	0.65	0.63	0.68	0.65	0.70	0.63	0.66	0.65	0.64	0.68	1.00													
24	0.62	0.63	0.59	0.63	0.63	0.62	0.57	0.70	0.65	0.68	0.64	0.61	0.68	0.66	0.70	0.67	0.71	0.60	0.63	0.68	0.66	0.73	0.78	1.00												
25	0.60	0.60	0.57	0.65	0.62	0.66	0.58	0.71	0.66	0.64	0.58	0.58	0.60	0.58	0.58	0.58	0.56	0.58	0.61	0.62	0.58	0.68	0.60	0.68	1.00											
26	0.66	0.62	0.56	0.61	0.65	0.63	0.58	0.62	0.60	0.59	0.64	0.55	0.63	0.62	0.59	0.60	0.56	0.56	0.56	0.59	0.62	0.68	0.54	0.61	0.66	1.00										
27	0.68	0.63	0.57	0.62	0.66	0.62	0.63	0.65	0.65	0.62	0.68	0.54	0.63	0.57	0.62	0.60	0.60	0.54	0.57	0.62	0.62	0.63	0.56	0.58	0.66	0.88	1.00									
28	0.59	0.56	0.59	0.62	0.58	0.60	0.52	0.66	0.59	0.61	0.59	0.53	0.63	0.62	0.63	0.61	0.62	0.61	0.62	0.61	0.61	0.69	0.66	0.69	0.67	0.68	0.68	1.00								
29	0.57	0.55	0.58	0.57	0.55	0.56	0.59	0.59	0.58	0.60	0.61	0.60	0.63	0.63	0.63	0.62	0.66	0.62	0.60	0.62	0.63	0.64	0.91	0.74	0.56	0.54	0.53	0.59	1.00							
30	0.64	0.60	0.58	0.56	0.62	0.67	0.57	0.65	0.64	0.62	0.61	0.59	0.59	0.58	0.61	0.61	0.59	0.62	0.59	0.63	0.60	0.68	0.59	0.67	0.72	0.68	0.64	0.67	0.53	1.00						
31	0.62	0.59	0.64	0.67	0.62	0.62	0.57	0.64	0.60	0.58	0.59	0.59	0.62	0.63	0.65	0.60	0.61	0.67	0.65	0.68	0.57	0.61	0.58	0.61	0.62	0.68	0.66	0.62	0.55	0.66	1.00					
32	0.54	0.51	0.54	0.53	0.53	0.59	0.52	0.53	0.49	0.46	0.53	0.55	0.52	0.51	0.52	0.53	0.57	0.59	0.56	0.55	0.49	0.59	0.55	0.56	0.64	0.51	0.51	0.61	0.52	0.66	0.58	1.00				
33	0.58	0.61	0.55	0.55	0.55	0.59	0.53	0.55	0.55	0.48	0.60	0.53	0.61	0.56	0.59	0.58	0.62	0.56	0.59	0.55	0.58	0.63	0.60	0.60	0.62	0.59	0.56	0.60	0.60	0.59	0.57	0.63	1.00			
34	0.63	0.62	0.53	0.60	0.59	0.55	0.54	0.59	0.56	0.60	0.63	0.58	0.59	0.59	0.64	0.63	0.58	0.55	0.55	0.61	0.61	0.62	0.59	0.61	0.60	0.59	0.62	0.63	0.58	0.63	0.62	0.65	0.76	1.00		
35	0.55	0.55	0.55	0.55	0.53	0.55	0.53	0.58	0.55	0.56	0.59	0.57	0.56	0.53	0.60	0.58	0.65	0.59	0.59	0.62	0.56	0.62	0.62	0.64	0.60	0.56	0.56	0.61	0.61	0.56	0.60	0.65	0.74	0.72	1.00	
36	0.60	0.55	0.61	0.55	0.60	0.58	0.51	0.59	0.53	0.55	0.59	0.62	0.63	0.60	0.60	0.61	0.62	0.65	0.61	0.65	0.73	0.89	0.66	0.69	0.62	0.62	0.59	0.65	0.64	0.62	0.60	0.57	0.62	0.61	0.58	1.00

Note: * The number is corresponding to sample code in Table 1.

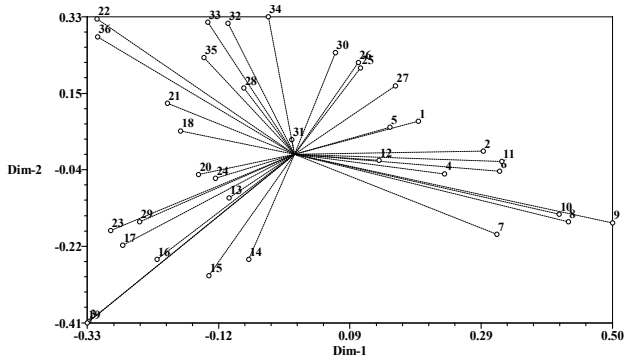


Figure 4. Two-dimensional plot of principle coordinates analysis depicting the genetic relatedness of thirty-six longan genotypes (The number is corresponding to sample code in Table 1)

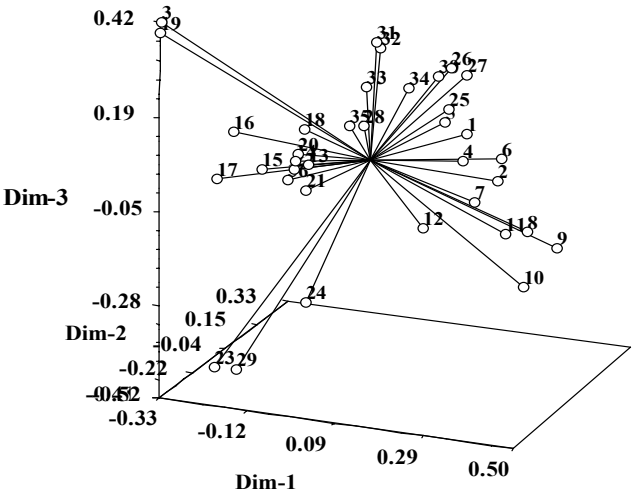


Figure 5. Principle coordinate plot for the first three principal coordinates estimated for RAPD markers of the thirty-six longan genotypes (The number is corresponding to sample code in Table 1)

The result of this analysis is relatively corresponding with dendrogram analyzed by UMPMA (Figure 3). Among total accessions, Nhan Xuong Com Vang Tay Ninh (3) and Nhan Xuong Com Vang Ben Tre (19) were grouped at distant position of dendrogram and which is also repeated after PCoA analysis at 2 dimensional and 3-dimensional plot as shown in Figure 4 and Figure 5, respectively. Thus, PCoA can be used for further confirmation of genetic diversity by using UPGMA method as described previously by Johar et al. (2017).

RAPD is considered as a frontline technique to study organism with limited prior genetic knowledge such as longan. In this project, we identified the usefulness of this technique in investigating genetic diversity of longan in Vietnam. By using 30 RAPD primers to analyze 36 longan samples, the collected samples were divided into 3 main groups showing the close relativeness of accessions in different places. We also found several specific RAPD bands which could be further analyzed to use as specific markers to identify 15 specific longan varieties. The obtained results in this study will be important information which will be useful for plant breeders to use for several purposes such as classification, conservation and longan breeding programs in Vietnam.

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Phenotypic, morphometric characterization and population structure of Pasundan cattle at West Java, Indonesia

SYAHRUDDIN SAID[1,♥], WIDYA PINTAKA BAYU PUTRA[1], SAIFUL ANWAR[1], PASKAH PARTOGI AGUNG[1], HANI YUHANI[2]

1Research Center for Biotechnology-Indonesian Institute of Sciences. Cibinong Science Center, Jl. Raya Bogor KM. 46, Cibinong 16911, West Java - Indonesia, ♥email: syahruddinsaid01@gmail.com.

2 Center for Research and Development of West Java Province, Citarum Rd No. 8, Bandung 40115, West Java - Indonesia.

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Abstract. *Said S, Putra WPB, Anwar S, Agung PP, Yuhani H. 2017. Phenotypic, morphometric characterization and population structure of Pasundan cattle at West Java, Indonesia. Biodiversitas 18: 1638-1645.* Identification of phenotypic and morphometric characteristics in indigenous cattle is important in characterizing breeds of animals. This study was carried out to identify the phenotypic and morphometric characteristics of 813 indigenous Pasundan cattle from West Java Province of Indonesia. The coat color of Pasundan cattle is mainly solid reddish brown (>90%) with black color on hoof (>90%), switch of tail (>80%), eyelid (>90%), muzzle (>90%), horn (>90%) and whitish color on mouth lash (>80%) and reddish brown color on legs (>60%) and rump patch (>60%). The physical characteristic of Pasundan cattle mainly is the hump in males (65.23%) or humpless in female (74.91%), small dewlap size (>60%), presence of back line (>60%) and horned (>80%) with upper curved horn (>90%). The average of withers height (WH), body length (BL), chest girth (CG) and scrotal circumference (SC) of adult male Pasundan cattle were 128.94+16.69 cm; 128.72+15.98 cm; 159.36+15.84 cm and 17.68+3.51cm respectively. Therefore, the average of WH, BL, and CG of adult female Pasundan cattle was 122.56+11.40 cm; 115.63+19.06 cm and 140.13+12.16 cm respectively. Natural Increase (NI) and calf crop values were 18.46% (low category) and 20.40% respectively. Net Replacement Rate (NRR) values were 300% (male) and 507% (female) and indicated that West Java province was still capable to produce seed stock per year.

Keywords: Body measurement, Indonesian indigenous cattle, physical characteristics

INTRODUCTION

Pasundan cattle are one of Indonesian indigenous cattle which are well adapted to some regions of West Java province. The cattle were originally came from crossbreeding between *Bos javanicus* (Bali cattle) and *Bos indicus* (Ongole and Madura cattle) established in 1904 (Hardjosubroto, 1994) and were declared as an indigenous Indonesian cattle based on Ministerial Decree no. 1051/Kpts/SR.120/10/2014 (Ministry of Agriculture of the Republic of Indonesia, 2014).

The reproductive traits of Pasundan cows for first calving is at 30-40 months, sex maturity at (25-30 months), gestation length (8.5-10.0 months), first estrus (18-24 months), estrus periods (18-24 days) and calving interval (1.1-1.3 years). The slaughter weight, hot carcass and dressing percentage of Pasundan bull weights are 240.40 kg, 127.20 kg and 53.02%, respectively (Department of Animal Husbandry, 2013). The carcass traits of Pasundan cattle such as water holding capacity (23-30%), cooking loss (25-45%), tenderness (35-96 mm/10s/g) as reported by Department of Animal Husbandry (2013). Despite their well-known quality and adaptive capacity to harsh environment, this breed remains unrecorded making *in situ* conservation action and breeding program difficult. Due to its vital role in future, conservation program and breeding program for Pasundan cattle have recently been initiated by the Indonesian government. However such programs for the conservation and breeding of Pasundan cattle remain ineffective due to lack of scientific information on phenotypic and morphometric characteristics. As an indigenous crossbred cattle, the phenotypic and morphometric characteristics of Pasundan cattle are varied and need to be described.

There are few studies on morphometric characteristic in Pasundan cattle with a large number of animals. Morphometric characterization of Pasundan cattle has been reported by Nugraha et al. (2016) and Akbar et al. (2016).

The base line data will provide crucial information for establishment the standard breeds of Pasundan cattle. Furthermore, analysis of population structure in Pasundan cattle is important to identify the capability of some breeding tracts for supply a livestock. The present study was carried out to investigate the phenotypic and morphometric characteristics of Pasundan cattle and also population structure analysis to identify the potency of West Java province as breeding center of Pasundan cattle.

MATERIALS AND METHODS

Study area

The present study was carried out from June until December 2016 at four regencies of West Java Province, Indonesia, Ciamis, Majalengka, Tasikmalaya, and Pangandaran (Figure 1). The West Java province is situated

at latitude 50°54' to 70°45'S and longitude 106°022' to 108°050'E. The humidity 65% to 90% with temperature 16°C to 28°C and rainfall 2000 - 5000 mm/year.

Procedures

A total of 813 animals consisting of 256 males and 557 females were used in this study. Data of animals consisted of age, qualitative traits, and body measurements. Identification of age was carried out based on a number of pairs of permanent incisors (PPI). Qualitative traits data were physical coloration (including coat, hoof, switch of tail, eyelid, mouth lash, muzzle, horn, legs, and rump patch color), presence of hump, dewlap size, strip along backbone, presence of horns and horn tips orientation. A measuring tape and vernier caliper were used for measuring animals. The body measurements consisted of withers height (WH: taken from the ground level to the highest point of withers), body length (BL: taken from the point of the shoulder to the pin bone), chest girth (CG: the circumference around the chest at the fourth rib) and scrotal circumference (SC: the circumference around the scrotum) especially for male animals. The chest girth of pregnant's animals was not included for analysis.

Population structure was calculated and analyzed according to Samberi et al. (2010), as follows:

$$\text{Number of adult cattle (\%)} = \frac{\text{Number of adult cattle}}{\text{Number of population}} \times 100\%$$

$$\text{Calving rate based on female adult female cattle or calf crop (\%)} = \frac{\text{Number of calves}}{\text{Number of adult female cattle}} \times 100\%$$

$$\text{Calving rate based on population (\%)} = \frac{\text{Number of calves}}{\text{Number of population}} \times 100\%$$

$$\text{Mortality (\%)} = \frac{\text{Number of death cattle}}{\text{Number of population}} \times 100\%$$

Natural increase (%) = Calving rate based on population (%) - Mortality (%)

$$\text{Number of young cattle (\%)} = \frac{\text{Number of young cattle}}{\text{Number of population}} \times 100\%$$

Requirement of cattle replacement (%) = Number of adult cattle (%) / Breeding length (years)

Remains of young cattle (%) = Percent of young cattle (%) - Requirement of cattle replacement (%)

Number of culled cattle (%) = Requirement of cattle replacement (%)

Output (%) = Number of culled cattle (%) + Remains of young cattle (%)

$$\text{Net Replacement Rate (\%)} = \frac{\text{Number of young cattle (heads)}}{\text{Remains of young cattle (heads)}} \times 100\%$$

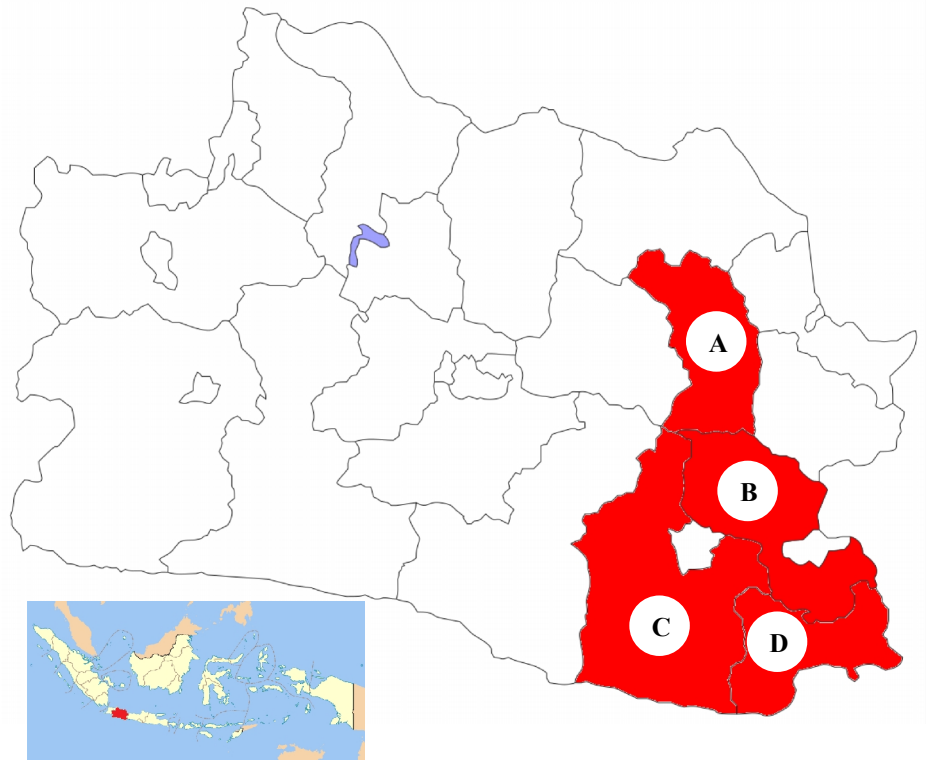


Figure 1. Location of the research site at four regencies at West Java Province, Indonesia consisted of: A. Majalengka, B. Ciamis, C. Tasikmalaya, and D. Pangandaran

Data analysis

Data were analyzed using Microsoft Office Excel 2007 computer program to obtain mean, standard deviation, coefficient of variation, minimum/maximum value and data percentage. All data were analyzed separately for male and female to avoid bias doe to sex effect. The NI value consisted of three categories such as low (NI<50%), moderate (51%<NI<80%) and high (NI>50%) as reported by Samberi et al. (2010).

RESULTS AND DISCUSSION

Colour characteristics

The color characteristics of Pasundan cattle are shown in Table 1. The dominant (more than 95%) coat color of Pasundan cattle found both in males and females to be solid reddish brown. The most common color of hoof, switch of tail, eyelid, horn, and muzzle both in males and females was found to be black. Whereas the whitish color was found on mouth lash, legs (stocking) and rump patch. The percentage of whitish color on legs and rump patch for male Pasundan was about 60%, lower than female Pasundan, more than 90%. (Figures 2 to 4).

Table 1. Proportion of color characteristics in Pasundan cattle

Variable	Colour	%	
		Male	Female
Coat	Solid reddish brown	95.31	96.59
	Black	4.69	1.97
	White	0.00	1.44
Hoof	Black	91.80	97.31
	Gray	8.20	2.69
Switch of tail	Black	85.94	92.80
	Reddish brown	11.33	5.22
	White	2.73	1.98
Eyelid	Black	91.37	95.51
	Reddish	8.63	4.49
Mouth lash	Whitish	85.94	80.25
	Reddish brown	14.06	19.75
Muzzle	Black	94.14	98.20
	White	3.91	0.90
	Spotted black-white	1.95	0.90
Horn	Black	95.20	90.28
	White	4.80	9.72
Legs (stocking)	Light brown	68.36	90.84
	Whitish	29.30	8.62
	Black	2.34	0.54
Rump patch	Light brown	67.19	92.10
	Whitish	30.08	7.90
	Black	2.73	0.00

Note: N = Number of observation

Based on phenotypic appearance, the coloration of Pasundan cattle was similar to some *Bos indicus* breeds such as Ponwar, Bachaur Dhesi (Gaur et al. 2003; Sarkar et al. 2007; Chandran et al. 2014) and Bali cattle (*Bos javanicus*). Panchung and Roden (1996) reported that the coat color of Siri cattle (*Bos indicus*) was black (69%), red (14%), spotted black-white (12%) and black colors (100%) on eyelids and muzzle.

The solid reddish brown coat color was the major color in several found in Indonesian local cattle such as Aceh (*Bos indicus*), Katingan (*Bos indicus* x *Bos javanicus*), Madura (*Bos indicus*) breeds and Pesisir breeds (Abdullah et al. 2007; Hartatik et al. 2009; Utomo et al. 2012; Hendri 2013). Two color types of hoof (black and gray) were found in Katingan cattle (Utomo et al. 2012) , similar to Pasundan cattle. Hartatik et al. (2009) reported that the black colour in Madura cows were found on muzzle (96.61%), absence of back line (77.97%), black color on hoof (100%) and reddish color on legs (50.85%) and whitish color on rump patch (91.53%).

Most of coat colors in non-descript cattle in some region of India were white with dominant black color on muzzle, eyelid, and hoof (Khirari et al. 2014; Biswas et al. 2015). Kayastha et al. (2011) reported that the coat colors of local cattle of Assam, India were brown (31.18%), white (28.53%), fawn/light grayish (15.29%), gray (13.53%), black (4.41%) and mixed (7.06%). The majority, the black colors in local cattle of Assam were found on muzzle (86.47%), switch of tail (90.88%), hoof (84.71%), horn (100%) and similar to Pasundan cattle. The coat color of local cow at Chhattisgarh Plains (India) according to Dahariya (2011) were red (60.80%), white (33.00%) and black (6.20%). Therefore, the major black color (88.70%) characteristics were found on muzzle and switch of tail in local cow at Chhattisgarh Plains and similar to Pasundan cattle. However, there is no information for their genetic distance. It may that the black color on horn, eyelids, hoof, muzzle, horn and tail switch in Pasundan originated from *Bos indicus* (Madura or Ongole). The coat color of solid reddish brown that found in the Pasundan cattle was derived from Madura or Bali cattle. The light brown colors on legs and rump patch in the Pasundan cattle were derived from Bali cattle or Madura (Indrijani et al., 2012).

Physical characteristics

The physical characteristics of Pasundan cattle are shown in Table 2. The characterization is given separately for males and females. The majority, the humped animal were found in male (65.23%), and humpless animal was found in female (74.91%). The dewlap size in Pasundan cattle was small in male (62.89%) and female (91.56%) with horned status (>80%) of both sexes. Commonly, the back line was observed in male (60.55%) and female (81.96%). Most of Pasundan cattle were horned (>80%) with horn tips orientation of upper curved/stumpy (>90%). The major physical characteristics of back line and horn tips orientation are shown in Figure 5.



Figure 2. The major coat color (solid reddish brown) of Pasundan bull (A) and cow (B)

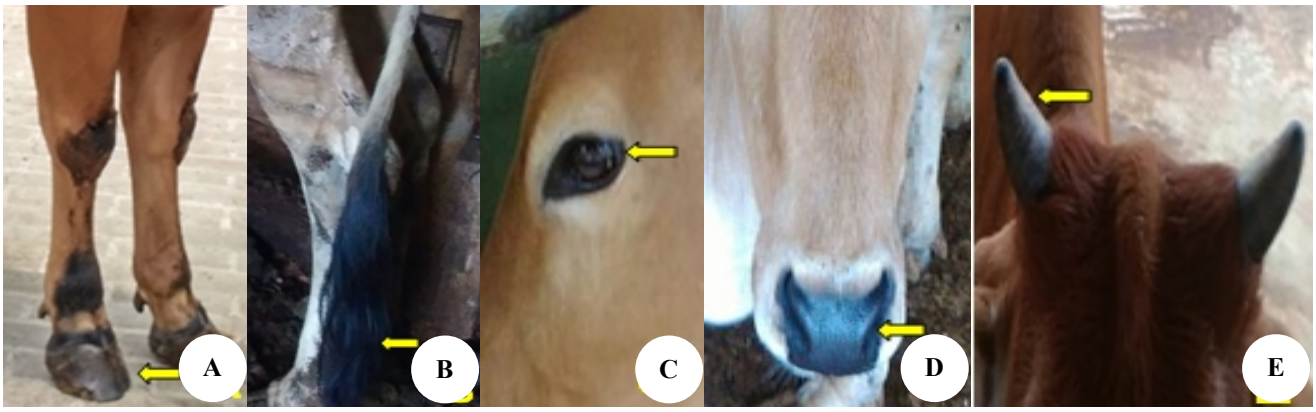


Figure 3. The major black colors of Pasundan cattle were found on hoof (A), switch of tail (B), eyelid (C), muzzle (D) and horn (E)

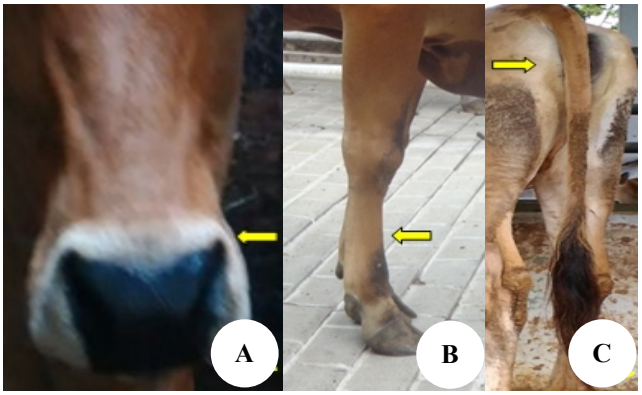


Figure 4. The major colors characteristics on several body parts of Pasundan cattle such as: whitish color on mouth lash (A); light brown color on legs (B) and rump patch (C)

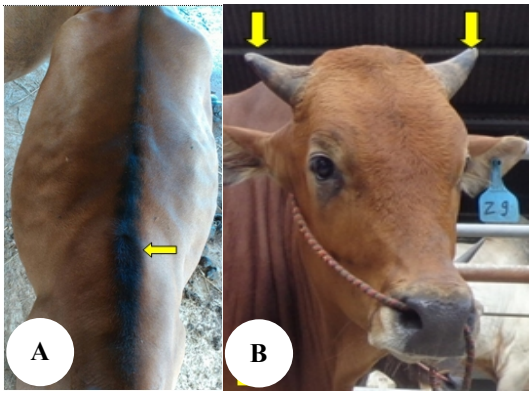


Figure 5. The major physical characteristics of back line (A) and stumpy horn (B) of Pasundan cattle

Table 2. Frequencies (%) of each level of the five physical characteristics assessed in Pasundan cattle

Variable	Characteristics	Percent (%)	
		Male	Female
Hump	Humpless	34.77	74.91
	Humped	65.23	25.09
Dewlap size	Small	62.89	91.56
	Large	37.11	8.44
Backline	Presence	60.55	81.96
	Absence	39.45	18.04
Horn status	Horned	87.11	87.07
	Hornless	12.89	12.93
Horn tips orientation	Upper curved (stumpy)	93.73	93.44
	Forward curved	4.71	0.36
	Downward curved	1.56	6.20

Note: N = Number of observation

In this study, the stumpy horn in Pasundan cattle is similar to many Indonesian indigenous cattle breeds such as Aceh (20%), Ongole grade (32.30%) and Madura (69.49%) (Abdullah et al. 2007; Hartatik et al. 2009; Sudrajad et al. 2011). Therefore, the horn tips orientation of forwarding curved was the major characteristic (78.40%) in Katingan cow and the stumpy horn was the major characteristics (98.30%) in Katingan bull (Utomo et al. 2012). The small of dewlap size was the major characteristic (78%) in Madura cattle (Setiadi and Diwyanto, 1997) and similar to Pasundan cattle. The large of dewlap size was found in Kenana cattle (97%) and 55.38% for Deshi cattle (Sarkar et al. 2007; Aamir et al. 2010).

Morphometric characteristics

Data of morphometric traits in Pasundan cattle were shown in Table 3 (for male) and Table 4 (for female). These data shown that morphometric traits on male were higher than female in each category. Gunawan and Jakaria (2011) reported that sex factor had significant effect on yearling weight of Bali cattle ($p<0.01$) which male was higher than female. According to Table 5, the WH of Pasundan cattle (4 PPI) were higher than some adult Indonesian indigenous cattle such as Aceh, Katingan, Madura, Pesisir, and Bali. The BL in Pasundan cattle (4 PPI) were similar to Katingan cattle. The BL of Pasundan bulls (4 PPI) were highest than Aceh, Madura, Pesisir and Bali bulls. Therefore, the BL of Pasundan cows (4 PPI) were highest than Aceh, Pesisir, and Bali cows. Thus, The BL of Pasundan cows were lower than Madura cow. The CG of Pasundan bulls (4 PPI) were highest than Aceh, Katingan, Madura, Pesisir and Bali bulls. Therefore, the CG of Pasundan cows (4 PPI) were highest than Aceh, Katingan, Madura and Pesisir cows. Thus, the CG of Pasundan cows were similar to Bali cows. The WH, BL, and CG of Pasundan cattle were lower than Ongole grade cattle as presented in Table 5.

Table 3. Mean, standard deviation, and the coefficient of variance for withers height, body length and chest girth of Pasundan bulls with different ages

Measurement	N	Mean (cm)	SD	CV (%)
PPI				
Withers height	23	111.13	11.34	10.20
Body length	23	113.39	13.45	11.86
Chest girth	21	132.33	9.56	7.22
Scrotal circumference	31	14.72	2.90	19.71
PPI				
Withers height	63	120.95	12.11	10.01
Body length	67	121.73	12.39	10.18
Chest girth	99	138.22	13.63	9.86
Scrotal circumference	95	15.45	2.80	18.15
PPI				
Withers height	23	124.87	8.90	7.13
Body length	20	126.40	7.40	5.86
Chest girth	36	146.86	14.20	9.67
Scrotal circumference	37	17.09	3.67	21.45
PPI				
Withers height	53	128.94	16.69	12.94
Body length	53	128.72	15.98	12.42
Chest girth	53	159.36	15.84	9.94
Scrotal circumference	50	17.68	3.51	19.84

Note: Ages of cattle were determined by the appearance of pairs of permanent incisors (PPI); PPI: pairs of permanent incisors; N: number of observation; SD: standard deviation; CV: coefficient of variation; Min: minimum value; max: maximum value

Table 4. Mean, standard deviation, and the coefficient of variance for withers height, body length and chest girth of Pasundan cows with different ages

Measurement	N	Mean (cm)	SD	CV (%)	Min.	Max.
1 PPI						
Withers height	40	106.93	11.72	10.96	75.00	125.00
Body length	40	109.63	14.65	13.36	70.00	131.00
Chest girth	40	130.28	11.76	9.03	100.00	145.00
2 PPI						
Withers height	71	116.07	14.82	12.77	89.00	160.00
Body length	71	115.73	15.75	13.61	85.00	175.00
Chest girth	71	136.59	12.18	8.92	100.00	163.00
3 PPI						
Withers height	97	120.90	14.33	11.86	81.00	185.00
Body length	95	125.45	25.67	20.46	86.00	185.00
Chest girth	97	140.75	10.11	7.18	118.00	164.00
4 PPI						
Withers height	251	122.56	11.40	9.30	100.00	197.00
Body length	227	115.63	19.06	16.49	85.00	189.00
Chest girth	250	140.13	12.16	8.68	102.00	195.00

Note: Ages of cattle were determined by the appearance of pairs of permanent incisors (PPI); PPI: pairs of permanent incisors; N: number of observation; SD: standard deviation; CV: coefficient of variation; Min: minimum value; Max: maximum value

Tabel 5. The morphometric measurements (cm) of some adult indigenous cattle in Asia and Africa

Breed	Group	Origin	Bull			Cow		
			WH	BL	CG	WH	BL	CG
Aceh[1]	*Bos indicus*	Indonesia	105.56±4.76	107.69±5.68	138.69±2.28	99.32±4.59	103.95±6.98	129.09±6.62
Katingan[2]	-	Indonesia	121.10±13.30	128.40±9.90	157.10±18.10	100.60±5.00	115.60±7.40	137.00±6.60
Madura[3]	*Bos indicus*	Indonesia	113.50±16.26	111.00±8.48	144.50±8.06	114.50±6.14	116.75±10.24	142.50±7.00
Pesisir[4]	*Bos indicus*	Indonesia	98.20±5.20	107.00±3.10	124.20±9.10	97.80±4.00	106.70±6.10	122.60±6.50
Bali[5]	*Bos javanicus*	Indonesia	107.59±6.11	103.84±13.49	139.06±12.39	107.29±6.32	106.00±11.31	140.65±15.19
Ongole grade[6]	*Bos indicus*	Indonesia	132.90±5.50	132.10±7.60	163.30±11.00	125.70±5.10	134.30±7.60	157.10±12.50
Begait[7]	*Bos indicus*	Ethiopia	136.99±0.10	135.96±0.09	168.91±0.10	131.48±0.25	128.13±0.16	159.55±0.24
Ankole[8]	*Bos taurus*	Uganda	144.60±3.40	210.30±5.70	182.60±2.80	139.50±1.70	201.40±3.00	171.30±1.30
Oulmes-Zaer[9]	*Bos taurus*	Marocco	-	-	-	116.80±0.76	139.60±1.37	161.20±1.28
Tidili[9]	*Bos taurus*	Marocco	-	-	-	108.20±0.84	127.50±1.50	147.70±1.41
White Fulani[10]	*Bos indicus*	Nigeria	101.11±2.19	152.29±4.63	125.62±4.35	100.48±2.70	154.34±7.52	113.71±3.04
Kenana[11]	*Bos indicus*	Sudan	-	-	-	122.69±0.35	131.50±0.28	151.97±0.69
Native cattle of Sylhet[12]	-	Bangladesh	85.26±0.94	114.01±1.51	106.66±1.60	93.36±0.49	126.41±0.72	118.97±0.59
Red Chittagong[13]	*Bos indicus*	Bangladesh	-	-	-	107.41±0.19	100.08±0.72	128.91±0.42
Siri[14]	*Bos indicus*	Bhutan	138.00±2.60	108.00±2.40	170.00±4.80	121.00±2.10	98.00±2.20	152.00±1.50
Achai[15]	*Bos indicus*	Pakistan	-	-	-	-	112.00±4.78	142.00±0.98
Bachaur[16]	*Bos indicus*	India	119.23±0.47	116.99±0.48	150.88±0.55	112.53±0.25	109.71±0.25	140.46±0.32
Deoni[17]	*Bos indicus*	India	134.36±2.03	129.59±2.28	163.55±1.55	122.22±1.23	120.11±2.16	151.82±1.92
Native cattle of Assam[18]	-	India	98.09±0.76	91.20±0.82	121.69±1.02	85.79±0.70	76.14±0.75	104.60±0.94
Ponwar[19]	*Bos indicus*	India	115.60±0.40	102.50±0.50	158.80±0.90	108.90±0.40	97.10±0.50	140.60±0.50
Native cow of Manipur[20]	-	India	-	-	-	111.34±0.92	103.92±0.33	135.34±0.47
Kankrej[21]	*Bos indicus*	India	-	-	-	124.49±0.28	123.44±0.37	162.56±0.56
Native cow of Chhattisgarh[22]	-	India	-	-	-	103.09±0.24	102.70±0.26	125.30±0.62

Note: [1)] Abdullah et al. (2007); [2)]Utomo et al. (2012); [3)]Setiadi and Diwyanto (1997); [4)]Adrial (2010); [5)]Tonbesi et al. (2009); [6)]Hartati et al. (2009); [7)]Ftiwi and Tamir (2015); [8)]Kugonza et al (2011); [9)]Boujenane (2015); [10)]Yakubu et al. (2009); [11)]Aamir et al. (2010); [12)]Koirala et al. (2011); [13)]Hadiuzzaman et al. (2010); [14)]Phancung and Roden (1996); [15)] Khan et al. (2015); [16)]Chandran et al. (2014); [17)]Singh et al. (2002); [18)]Kayastha et al. (2011); [19)]Gaur et al. (2003); [20)]Tolenkhomba et al. (2012); [21)]Pundir et al. (2011); [22]Dahariya (2011)

Table 6. Technical coefficient for output estimation of Pasundan cattle at West Java Province of Indonesia

Component	Value
Number of adult females (%)	10.46
Calving rate based on adult female cattle (%)	20.40
Calving rate based on population (%)	10.46
Mortality (%)	2.00
Natural increase (%)	18.46
Percentage of adult cattle (%)	
Male	13.25
Female	51.25
First mating age (years)	
Male	3
Female	2
Breeding length (years)	
Male	3
Female	5
Sex ratio (male/female)	32/68
Number of population observed (heads)	679

Table 7. The output estimation of Pasundan cattle at West Java Province of Indonesia

Component	Heads	Percent (%)
Number of young cattle (2 years age)		
Male	99	14.58
Female	71	10.46
Total	170	25.04
Requirement of cattle replacement		
Male	33	4.86
Female	14	2.09
Total	47	6.95
Remains of young cattle (2 years age)		
Male	66	9.72
Female	57	8.37
Total	123	18.09
Number of culled cattle		
Male	33	4.86
Female	14	2.09
Total	47	6.95
Net Replacement Rate		
Male	-	300
Female	-	507
Total	-	807
Output estimation	99	14.58
Male	73	10.82
Female	172	25.40
Total	99	14.58

The comparison of morphometric measurements between breeds cattle of Asia and Africa are shown in Table 5. The morphometric measurements (WH, BL, and CG) of Pasundan bulls (4 PPI) in this study were lowest than Begait bulls, Ankole bulls and Deoni bulls (Table 5). Therefore, the morphometric measurements of Pasundan bulls (4 PPI) were higher than native bulls of Sylhet, Bachaur bulls, native bulls of Assam and Ponwar bulls. The WH of Pasundan cows (4 PPI) were similar to Kenana and Deoni cows. The CG of Pasundan cows were similar to Bachaur and Ponwar cows. Commonly, the morphometric measurements of Pasundan cows were higher than Red Chittagong cows and native cows of Assam and Chhattisgarh Plain. The variation of morphometric measurements between indigenous cattle breeds in Asia and Africa are caused by the difference of genetic, climate, management systems and feeds quality (Falconer and Mackay 1996).

Population structure

In this study, the Natural Increase (NI) value was in low category (18.46%) as presented in Table 6. The NI value consisted of three categories such as low (NI<50%), moderate (51%<NI<80%) and high (NI>50%) as reported by Samberi et al. (2010). Low NI value was reported in the several Indonesian native cattle in 2015 such as Bali (16,35%), Pesisir (27.80%) and 16.22% for Ongole grade (Putra et al. 2015; Susanti et al. 2015). The NI value depends on the calf crop and mortality values. Increasing calf crop and decreasing mortality values will cause the increasing of NI value. Calf crop in the present study was 20.40%. This value was very different to those reported by Chase et al. (2004), that calf crop value in Senepol x Angus, Brahman Angus and TulixAngus were 76.9%, 89.0%, and 94.7%, respectively. Putra et al. (2015) reported that the calf crop at 2015 years in native cattle at West Sumatera Province was Bali 51.27% (Bali), 62.29% (Pesisir) and 25.00% (Ongole grade). Moreover, the calf crop at 2015 years in Madura and Madura cross cattle at Tambelangan District were 85.60% and 82.20% respectively (Umam et al. 2015). Calf crop value can be increased with reducing mortality rate and increasing conception rate. Net Replacement Rate (NRR) in male and female at West Java Province were more than 100% (showed in Table 7) and indicated that the surplus population occurred in West Java province. The NRR value in bull was 300% and indicated the number of bull was three folds from total bull needs. According to Table 7, total ideal livestock output per year were 99 heads (14.58%) for male and 73 heads (10.82%) for female.

We conclude that the appearance of cattle Pasundan cattle is a combination of *Bos indicus* and *Bos javanicus*. However, phylogenetic investigation of Pasundan cattle is necessary to describe their origins. The population structure in Pasundan cattle in West Java showed that the calf crop and natural increase values were low but itwas still capable to produce the seedstock per year.

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Tree growth, mortality and recruitment in four inland tropical dry evergreen forest sites of Peninsular India

ELUMALAI PANDIAN♥, NARAYANASWAMY PARTHASARATHY
Department of Ecology and Environmental Sciences, Pondicherry University, Puducherry-605014, India. ♥email: pandianmaha11@gmail.com

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Abstract. *Pandian E, Parthasarathy N. 2017. Tree growth, mortality and recruitment in four inland tropical dry evergreen forest sites of Peninsular India. Biodiversitas 18: 1646-1656.* Tree diversity was re-inventoried after a decade (2003-2013) for assessing growth, mortality and recruitment rates in four inland tropical dry evergreen forest sites on the Coromandel Cost of India. Four 1-ha square plots (100 m x 100 m) were established in 2003 in four tropical dry evergreen forest sites, i.e. Araiyapatti (AP), Karisakkadu (KR), Maramadakki (MM) and Shanmuganathapuram (SP). These four plots were re-inventoried in 2013 to determine decadal changes in tree diversity. All trees ≥10 cm girth at breast height (gbh) were measured at 1.3 m from the ground level. The initial inventories in 2003 recorded 57 tree species, whereas, in 2013, 56 species were recorded from 46 genera and 26 families. Tree basal area declined by 6.2 % and 3.4 % in sites KR and SP respectively, whereas in the other two sites it increased; AP (1.6 %) and MM (16.8 %). The mean growth rate of trees (≥ 10 cm gbh) in four tropical dry evergreen forest sites ranged from 0.68 to 1.52 cm yr^{-1}. High recruitment rate was recorded in less disturbed sites KR (21.8 % yr^{-1}) and MM (11.5 % yr^{-1}), while the moderately disturbed sites showed 9.1% yr^{-1}in site AP and 5.1 % yr^{-1} in site SP. Mortality rates were 23.5 and 45.7% yr^{-1} in highly disturbed sites AP and SP respectively.

Keywords: Forest dynamics, mortality, recruitment, tree growth, tropical dry evergreen forest

INTRODUCTION

Long-term studies are important for assessing the forest dynamics (Pandian and Parthasarathy 2016). The understanding of the tree growth is important in many ecological and management applications, not only is it a basic demographic process that profoundly influences tree population dynamics (Harcombe 1987; Metcalf et al. 2009), but it is also one of the primary means of evaluating forest management goals (Chojnacky 2001). Several ecological studies examined population changes by counting and later recounting a defined sample and assessing survivors, losses, and gains (Sheil and May 1996). Such information is commonly summarized as rates of mortality, recruitment, or turnover in tropical forest studies (e.g., Phillips et al. 1994; Sheil et al. 1995).

Repeated measurements of permanent forest plots across the tropics have shown increased tree growth over the past decades in many cases (Lewis et al. 2004; 2009), but decelerating or stable growth rates in others (Feeley et al. 2007; Clark et al. 2010). Permanent tree plots have been established in Mudumalai Game Reserve (Sukumar et al. 1992, 1998), Uppangala reserve forest (Pascal and Pelissier 1996) and in Varagalaiar, Anamalasis (Ayyappan and Parthasarathy 1999). Population density and competition is a fundamentally limiting factor (Lines et al. 2010; Kunstler et al. 2011) in closed-canopy conditions In addition, the supply of energy, water, and nutrients (typically measured by proxies such as insolation, water deficit, and soil type) can strongly limit tree growth and can mask density dependence (He and Duncan 2000). There is strong evidence that the growth rates of tropical forest trees have increased over the last few decades (Laurance et al. 2004; Phillips et al. 2008), probably as a result of increased atmospheric CO_2 concentration (Lloyd and Farquhar 2008). Our study was conducted in tropical dry evergreen forest sites which are disappearing at an alarming rate due to anthropogenic activities and are replaced by forests composed of different species or their land use pattern is changed (Parthasarathy 1999; Chittibabu and Parthasarathy 2000). Our study was undertaken with an aim to investigate decadal changes (2003-2013) in tree species richness, abundance, growth, mortality and recruitment rate in four inland Indian tropical dry evergreen forests on the Coromandel Coast of India.

MATERIALS AND METHODS

Study area

The study was conducted in four inland tropical dry evergreen forest sites (Araiyapatti/AP, Karisakkadu/KR, Maramadakki/MM and Shanmuganathapuram/SP) which are located around 40 km west of the eastern coast in Pudukottai District (9°50" and 10°40", 78°25" and 79°15") of Tamil Nadu, southern India (Figure 1).

All the four study sites are sacred groves or temple forests and are protected based on the religious belief systems of local people. Sites MM and SP were located near to human settlement, while in sites AP and KR were nearly 300 m from habitation. Agricultural fields of paddy, maize, millet, *Casuarina*, sugarcane and vegetable plantations surround all the study sites. Distance between the four studied sites ranged from 4 to 25 km. The four

study sites experience various levels of anthropogenic disturbance (Pandian and Parthasarathy 2016). Anthropogenic activities are high in highly disturbed sites AP and SP, where selectively logged (tree felling for poles, timber, and fuelwood extraction) by local people, whereas MM is a least disturbed site while KR is a moderately disturbed site (Table S1). The climate data available for 1992-2012 from the nearest station (located 15 km away from our study sites) reveal mean annual rainfall of 1033 mm per year received largely during the northeast monsoon (October–December) with a mean annual temperature of 29.5°C. The mean annual maximum and minimum temperature ranges from 33.4°C to 25.4°C for the same period (Pandian and Parthasarathy 2016). The vegetation of all our study sites is described as tropical dry evergreen forests (Type 7/CI of Champion and Seth (1968); Mani and Parthasarathy (2005). Soils are basically lateritic and sandy to sandy loam in texture in all the sites.

Methods

Four 1-hectare permanent plots (100 m x 100 m) were established a decade ago (2003) in four tropical dry evergreen forest sites, AP, MM, KR and SP. All trees ≥10 cm girth at breast height (gbh) was measured at 1.3 m from the ground level (Mani and Parthasarathy 2005). These four plots were re-inventoried in 2013 to determine the tree diversity changes over a decade. We follow the same methodology for each 1 ha plot and each was subdivided into one-hundred 10 m x 10 m quadrats. For multi-stemmed trees, bole girths were measured separately. The recensus (2013) results were compared with initial inventories (2003) to assesses the changes in tree growth, mortality, and recruitment rate over a decade (2003-2013) at species and stand levels.

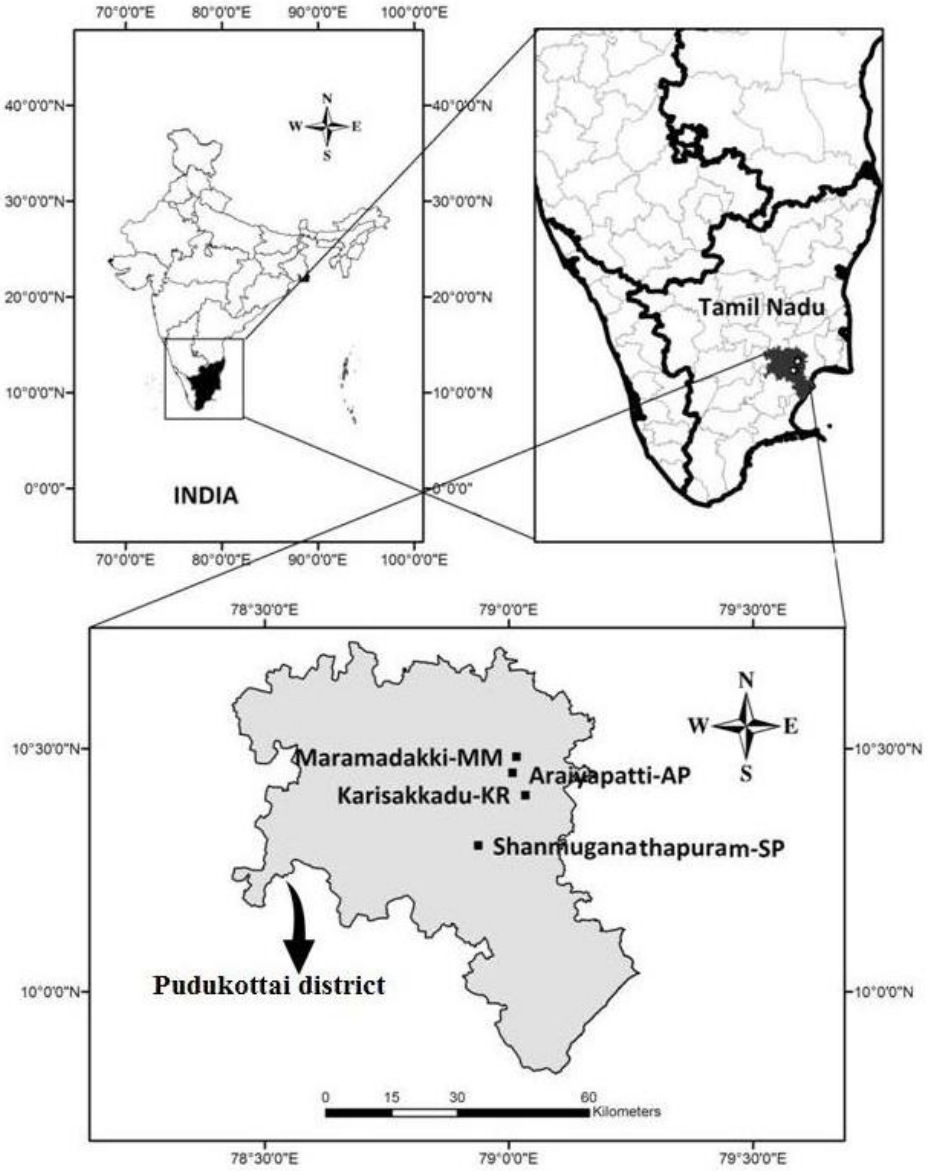


Figure 1. Map showing the location of four tropical dry evergreen forest sites on the Coromandel Coast of India (Maramadakki (MM), Karisakkadu (KR), Araiyapatti (AP) and Shanmuganathapuram (SP) in Pudukottai District of Tamil Nadu state, India)

Table 1. Decadal changes (2003 & 2013) in tree diversity in four tropical dry evergreen forest sites - Araiyapatti (AP), Karisakkadu (KR), Maramadakki (MM) and Shanmuganathapuram (SP) on the Coromandel Coast of peninsular India

Variable	AP			KR			MM			SP		
	2003	2013	Net change	2003	2013	Net change	2003	2013	Net change	2003	2013	Net change
Species richness	35	37	2	30	31	1	28	27	-1	26	29	3
Stand density (stems ha-^{1})	807	705	-102	596	678	82	724	750	26	1663	1182	-481
Stand basal area (m^2 ha^{-1})	19.1	19.43	0.42	21.6	20.26	-1.34	15.5	18.63	3.13	22.1	20.38	-1.72

Each tree tag number was verified and its species reconfirmed by collecting a voucher specimen. In the case of trees that had lost their tags, the species and its girth was compared to previous data and also to its nearest neighbors to ensure measurement of all trees. Original inventory trees that were dead or had disappeared were classified as dead. Recruits were appended with aluminum tags (number distinguishable from those of original inventory) and voucher specimens were collected. All trees were identified from their vegetative and reproductive features using the regional floras (Gamble and Fischer 1915-1935; Henry et al. 1987, 1989; Matthew 1991; Nair and Henry 1983) and the field key of Pascal and Ramesh (1987). The differences in diameter distribution of trees between the two inventories (2003-2013) were tested using Kolmogorov-Smirnov two-sample test (Zarr 2006), and we used paired t-tests to check for the significant differences in tree variables across four forest sites using SPSS software. A six-letter code was assigned to all species (for floristic structure) with the first three letters denoting the generic name and the next three letters, the specific epithet. The mean growth rates were calculated for all species. Mean girth was taken in each species and its growth was calculated from 2003. Annual rates of mortality and recruitment were calculated from the equation of Newbery et al. (1999).

$$\text{Mortality rate (m)} = 1 - [1 - (n_d/n_{03})]^{l/t}$$

$$\text{Recruitment rate (r)} = [1 + (n_r/n_{03})]^{l/t} - 1$$

Where, t = relevant time interval, n_d = number of death, n_r = number of recruits and n_{03} = population size in 2003.

RESULTS AND DISCUSSION

The re-inventory of trees in the four sites showed substantial changes in species richness, density, basal area and tree growth over a decade. There was significant change in species richness (df=99, $p<0.05$; except in SP), density (df=99, $p<0.05$) and basal area (df=99, $p<0.05$; except in KR) in the ten-year interval (2003-2013). The initial inventory was carried out in 2003 and yielded 57 tree species, whereas in 2013 a total of 56 tree species were recorded, with an overall loss of three species and two species as a new addition in the ten-year interval. Among the four studied sites, the species richness in 2013 ranged from 27 species in site MM to a high of 37 species in AP, through intermediate values of 30 and 29 species KR and SP respectively (Table 1). This could be attributed either to natural cause of tree death or due to human interference, during the ten-year gap. Sheil et al. (2000) also reported increased species richness over a time period (1944-1992) in Budongo rain forest, Uganda. In contrast, Fashing et al. (2004) reported that tree species richness declined by 21% over an 18-year gap in Kakamega forest, Kenya.

The stand density decreased in ten-year interval by 480 and 102 stems in SP and AP respectively, but increased moderately by 82 stems in KR and 26 stems in MM. This could be attributed to the removal of stems by local residents, whereas in moderately disturbed sites density increased by 82 stems in KR and 26 stems in MM. Over decades, stem density showed sharp reduction in coastal tropical dry evergreen forest sites (Baithalu et al. 2012, 2013; Mani and Parthasarathy 2009. Felfili (1995) also reported 2% of density decreased over a six-year period in the gallery forest of Central Brazil. In another Brazilian site, Felfili et al. (2000) reported that the stem density declined by 4.5% over a nine-year period. The tree basal area per hectare declined in site KR from 21.6 m^2 to 20.26 m^2 and in site SP from 22.1m^2 to 20.38 m^2, but increased from 19.1m^2 to 19.43 m^2 in AP and from 15.5m^2 to 18.63 m^2 in MM (Table 1). Tree basal area increased in all the girth classes (except 121-150 cm) in site MM, while in site KR, basal area increased only in highest girth class (151-180 and >210 cm) but in other girth classes it decreased. Whereas in site AP, it decreased in lower and higher girth classes but moderately increased in middle girth class. In site SP, basal area decreased in lower and middle girth classes, but its increased in higher girth classes (Figure 2). This could be due to anthropogenic pressure, (by the way of cutting tree). Bhat et al. (2000) also found decreased basal area with increasing human disturbance in tropical forests of Western Ghats, India. Laurance et al. (2009) reported tree basal area increased by 4% in central Amazonian forests and Krisnawati et al. (2011) also reported an increase in basal area over a 4.3-year time period in Samboja research forest, east Kalimantan, while Hitimana et al. (2004) reported that the basal area in larger diameter classes has become reduced in western Kenya.

Tree girth increment by species

Overall mean girth increment rate by species ranged from 0.05±0.18 to 3.53±1.17 cm yr^{-1} across the four study sites. Among the 56 tree species greatest girth increment was shown by *Ficus benghalensis* (3.53±1.17 cm yr^{-1}), its density increased just by one individual (1stem), followed

by *Syzygium cumini* (2.03±1.73 cm yr^{-1}) and its density (3 stem) declined and *Ficus religiosa* (1.70±0.28cm yr^{-1}) no change occurred in density and above mensioned tree species basal area was increased by 0.173 m^2, 0.776 m^2 and 0.059 m^2 respectively. Whereas *Benkara malabarica* (0.06±0.01 cm yr^{-1}), *Clausena dentata* (0.06±0.02 cm yr^{-1}) and *Diospyros ferrea* (0.05±0.18 cm yr^{-1}) showed lowest girth increment, but its density increased (by 7, 5 and 2 stems respectively) and basal area by 0.012 m^2, 0.009 m^2 and 0.006 m^2 respectively over a decade (Table 2). These values, when compared to other forests, are greater than those of 0.25 cm yr^{-1} in central Brazil (Felfili 1995), 0.11 to 0.65 cm yr^{-1} in Puerto Rico (Schmidt and Weaver 1981), 0.26 to 0.78 cm yr^{-1} in northwestern Costa Rica (Chapman and Chapman 1990), 0.44 cm yr^{-1} in Uganda (Taylor et al. 1996); but it is well within the range of 0.08 to 1.15 cm yr^{-1} reported in Panama (Lang and Knight 1983) and 0.04 to 1.30 cm yr^{-1} in Costa Rica (Lieberman et al. 1985). Increments of tree diameter may vary widely between measurement periods over the short-term, thus biasing estimates of plant species-specific growth rates (Clark and Clark 1994). Our results are closer to those reported by Dong et al. (2012) that a five-year mean growth rate at Huai Kha Khaeng (HKK) was 0.23 cm yr^{-1} during the first census (1994-1999) interval, but declined to 0.21 cm yr^{-1} during the second census (1999-2004) interval. In contrast, at Lambir, the five-year stand-level mean tree growth rate has increased from 0.065 cm to 0.067 cm yr^{-1} between the two census intervals (1992-1997 and 1997-2002). In terms of direction, this corresponds to a decrease by 0.02 cm yr^{-1} at HKK and increase by 0.002 cm yr^{-1} for Lambir.

Mean tree girth increment by size class

In the four sites, the mean girth increment varied considerably across the girth classes. Maximum girth increment was found in the larger size classes at site MM (4.3 cm yr^{-1}), followed by AP (3.1 cm yr^{-1}), SP (2.9 cm yr^{-1}) and KR (0.92 cm yr^{-1}). Whereas the lowest and middle girth class showed low girth increment in all the study sites (Figure 3). Mean girth increment rate was calculated by girth class for all the species (Table S2).

Girth increment in dominant species by girth class

The mean girth increment of four abundant species varied considerably (Figure 4); particularly *Albizia amara* showed greater girth increment in higher girth class in sites MM (3.8 cm yr^{-1}) and AP (2.9 cm yr^{-1}), but in site SP it increased in all the girth classes; while in site KR showed a moderate increase, except in higher girth class. Girth increment in *Memecylon umbellatum* was low in lowest girth class in all the sites, whereas it was greater in middle girth classes in all sites (except in KR). In *Drypetes sepiaria*, the girth increment was greater in higher girth classes in sites MM (1.55 cm yr^{-1}), KR (1.2 cm yr^{-1}) and SP (1.6 cm yr^{-1}), but in site AP (1.7 cm yr^{-1}) it was greater in lower girth class. In *Glycosmis mauritiana* girth increment is high only in lower girth class. This result is accordance with Sundaram and Parthasarathy (2002) at Kolli hills wherein the six species showed a > 3.5 cm yr^{-1} difference between their maximum and minimum growth rates and such fast-growing species are ideal for afforesting degraded sites. Elouard et al. (1997) reported a similar inter and intra-specific variability in individual diameter increment of trees ≥ 30 cm gbh in the forest of Kadamakal, Western Ghats, India. The repeated measurements of permanent forest plots across the tropics have shown increase the tree growth over the past decades in many cases (Lewis et al. 2009, 2004), but decelerating or stable growth rates in others (Fleeley et al. 2007; Clark et al. 2010). One of the major factors suggested causing an increased tree growth is the rise in atmospheric CO_2 concentration, as it can increase plant photosynthetic rates and enhance water-use efficiency (Holtum et al. 2010; Keenan et al. 2013). However, in a study at La Selva in Costa Rica, researchers found that growth rates declined in six canopy species during 1984-2000 (Clark et al. 2003); and the same trend of decelerating growth was also found from large forest dynamics plots in Panama and Malaysia (Feeley et al. 2007).

Changes in mortality, recruitment, and turnover over a decade

The mean growth rate of trees (≥ 10 cm gbh) in four tropical dry evergreen forest sites ranged from 0.68 to 1.52 cm yr^{-1} (Table 3). Taylor et al. (1996) reported a mean annual increment of 0.44 cm yr^{-1} for trees ≥ 9.5 cm dbh in Mpanga, Uganda. Sundaram and Parthasarathy (2002) reported a mean annual girth increment for trees ≥ 30 cm gbh that ranged from 1.42 cm yr^{-1} in site Perumakkai Shola (PS) to 2.60 cm yr^{-1} in site Mottukadu Shola (MS) in Kolli hills, Eastern Ghats. These values are greater than that of 0.27 cm yr^{-1} exhibited by trees ≥ 30 cm dbh in Mount Bellenden-Ker, Queensland (Herwitz and Young 1994). Amazon forests had been observed to be experiencing accelerated dynamics over the past several decades, including significantly increased rates of tree growth, mortality, recruitment and aboveground biomass (Lewis et al. 2004b; Phillips et al. 2004; Baker et al. 2004). Stimulation of individual diameter increment due to logging in forest sites has been reported for trees ≥ 30 cm gbh in a dense moist evergreen forest in Kadamakal, Indian Western Ghats (Elouard et al. 1997).

The maximum diameter increment rate was recorded in site AP (1.19 cm yr^{-1}) followed by KR (1.15 cm yr^{-1}), MM (1.52 cm yr^{-1}) and SP (0.68 cm yr^{-1}). The mortality rate was high in the highly disturbed sites SP (45.7 % yr^{-1}) and AP (23.5% yr^{-1}), while in the least disturbed sites mortality rate was less (9.7 % yr^{-1} in site KR and 8 % yr^{-1} in site MM).

Table 3. Mean tree growth rate, mortality, recruitment and turnover rate in four tropical dry evergreen forest sites - (AP, KR, MM and SP) on the Coromandel Coast of peninsular India

Variable	Site				Mean (±SD)
	AP	KR	MM	SP	
Mean girth increment (cm yr^{-1})	1.196	1.15	1.52	0.68	1.14±0.35
Mortality (% yr^{-1})	23.5	9.7	8	45.7	21.7±17.43
Recruitment (% yr^{-1})	9.1	21.8	11.5	5.1	11.8±7.12
Turnover rate (%)	16.3	15.7	9.7	25.4	16.7±6.48

Table 2. Summary of density (stems ha^{-1}) and basal area (m^2 ha^{-1}) of all tree species (≥10 gbh) during 2003 and 2013 inventories and number of stems surviving, dead, recruited and mean girth increment (cm yr^{-1}) in four inland tropical dry evergreen forest sites AP, KR MM and SP. Data were pooled.

Species	Family	Density		Basal area		Surviving	Died	Recruitment	Mean girth increment rate (cm yr^{-1} and ±SD)
		2003	2013	2003	2013				
Acacia leucophloea (Roxb.) Willd.	Mimosaceae	3	4	0.08641	0.08837	3	-	1	0.74±0.32
Albizia amara (Roxb.) Boivin	Mimosaceae	164	144	8.55742	8.67098	164	20	-	1.50±0.37
Albizia lebbeck (L.) Benth.	Mimosaceae	6	2	1.12198	0.68454	6	4	-	0.99±0.85
Albizia odoratissima (L.f.) Benth.	Mimosaceae	3	2	0.12709	0.13359	3	1	-	0.85±0.73
Allophylus serratus (Roxb.) Kurz	Sapindaceae	1	0	0.00127	-	1	1	-	0.00±0.00
Atalantia monophylla (L.) Correa	Rutaceae	10	10	0.09026	0.1235	10	-	-	0.36±0.08
Azadirachta indica A. Juss.	Meliaceae	17	11	0.43842	0.37163	17	6	-	0.50±0.04
Benkara malabarica (Lam.) Tirven.	Rubiaceae	3	10	0.02571	0.03713	3	-	7	0.06±0.01
Borassus flabellifer L.	Arecaceae	11	8	1.00083	0.71477	11	3	-	0.09±0.06
Cadaba trifoliata (Roxb.) Wight & Arn.	Capparaceae	22	38	0.17873	0.27522	22	-	16	0.41±0.31
Canthium coromandelicum (Burm.f.) Alston	Rubiaceae	1	1	0.00385	0.00093	1	-	-	0.00±0.00
Canthium dicoccum (Gaertn.) Teijsm. & Binn.	Rubiaceae	80	100	0.75788	1.0442	80	-	20	0.48±0.04
Cassia fistula L.	Ceasalpiniaceae	20	20	0.28415	0.35116	20	-	-	0.66±0.01
Cassia roxburghii DC.	Caesalpiniaceae	4	3	0.04635	0.06781	4	1	-	0.81±0.18
Cassia siamea Lam.	Caesalpiniaceae	4	3	0.06457	0.04633	4	1	-	0.57±0.04
Chloroxylon swietenia DC.	Flindersiaceae	92	93	1.39999	1.43448	92	-	1	0.69±0.08
Clausena dentata (Willd.) Roemer	Rutaceae	9	14	0.01247	0.02238	9	-	5	0.06±0.02
Commiphora caudata (Wight & Arn.) Engle.	Burseraceae	2	2	0.00839	0.00739	2	-	-	0.55±0.50
Cordia obliqua Willd.	Cordiaceae	18	11	0.70557	0.48407	18	7	-	1.10±0.44
Crateva magna (Lour.) DC.	Capparaceae	4	3	0.06613	0.08149	4	1	-	1.02±0.47
Dalbergia paniculata Roxb.	Papilionaceae	1	6	0.14296	0.16361	1	-	5	0.00±0.00
Diospyros ebenum Koen.	Ebenaceae	14	17	0.64463	0.71072	14	-	3	0.43±0.55
Diospyros ferrea (Willd.) Bakh.	Ebenaceae	2	4	0.00349	0.01021	2	-	2	0.05±0.18
Diospyros montana Roxb.	Ebenaceae	9	8	0.16394	0.27317	9	1	-	0.82±0.54
Drypetes sepiaria (Wight & Arn.) Pax. & Hoffm.	Euphorbiaceae	380	322	13.9698	12.4757	380	58	-	0.59±0.01
Ehretia pubescens Benth.	Cordiaceae	2	0	0.02987	-	2	2	-	0.00±0.00
Euphorbia antiquorum L.	Euphorbiaceae	97	81	1.7242	1.36972	97	16	-	0.50±0.27
Ficus amplissima J.E.Smith	Moraceae	3	4	0.56223	0.70999	3	-	1	0.82±0.07
Ficus benghalensis L.	Moraceae	5	6	1.38598	1.5592	5	-	1	3.53±1.17
Ficus microcarpa L.f.	Moraceae	3	3	0.94686	1.26031	3	-	0	1.60±0.87
Ficus religiosa L.	Moraceae	2	2	0.34854	0.408	2	-	0	1.70±0.28
Flacourtia indica (Burm.f.) Merr.	Flacourtiaceae	2	6	0.00596	0.01798	2	-	4	0.09±0.13
Gardenia resinifera Roth	Rubiaceae	19	26	0.13825	0.17562	19	-	7	0.46±0.03
Glycosmis mauritiana (Lam.) Yuich. Tanaka	Rutaceae	165	352	0.19654	0.55221	165	-	187	0.48±0.06
Gmelina asiatica L.	Verbenaceae	6	7	0.12699	0.14683	6	-	1	0.27±0.09
Jatropha gossypiifolia L.	Euphorbiaceae	0	1	-	0.00093	0	-	1	0.00±0.00
Lannea coromandelica (Houtt.) Merr.	Anacardiaceae	23	26	1.06847	1.56611	23	-	3	1.06±1.83
Lepisanthes tetraphylla (Vahl.) Radlk.	Sapindaceae	129	106	1.54835	1.7129	129	23	-	0.76±0.23
Manilkara hexandra (Roxb.) Dubard	Sapotaceae	71	57	8.49337	7.94218	71	14	-	1.02±0.10
Maytenus emarginata (Willd.) Ding Hou	Celastraceae	1	0	0.00161	-	1	1	-	0.00±0.00
Memecylon umbellatum Burm.f.	Melastomataceae	1633	1171	4.68979	4.34299	1633	462	-	0.62±0.13
Morinda pubescens J.E. Smith	Rubiaceae	14	16	0.338	0.20655	14	-	2	0.70±0.82
Phyllanthus polyphyllus Willd.	Euphorbiaceae	2	2	0.01592	0.0215	2	-	-	0.62±0.09
Pleiospermium alatum (Wall. ex Wight. & Arn.) Sw.	Rutaceae	5	5	0.01625	0.01678	5	-	-	0.25±0.14
Pongamia pinnata (L.) Pierre	Papilionaceae	72	51	2.05516	2.27028	72	-21	-	1.61±0.36
Premna serratifolia L.	Verbenaceae	4	4	0.38394	0.40718	4	-	-	0.97±0.21
Prosopis juliflora (Sw.) DC.	Mimosaceae	19	18	0.21923	0.30004	19	-1	-	0.63±0.49
Pterospermum canescens Roxb.	Sterculiaceae	250	208	8.87413	9.35771	250	-42	-	1.10±0.60
Sapindus emarginatus Vahl	Sapindaceae	2	1	0.05545	0.04969	2	-1	-	0.00±0.00
Sapium insigne (Royle) Trimen	Euphorbiaceae	9	9	0.32795	0.27098	9	-	0	0.31±0.67
Streblus asper Lour.	Moraceae	0	1		0.00385	0	-	1	0.00±0.00
Strychnos nux-vomica L.	Loganiaceae	240	178	3.57752	4.03002	240	-62	-	1.16±0.62
Srytchnos potatorum L. f.	Loganiaceae	9	6	0.12529	0.13491	9	-3	-	0.86±0.11
Syzygium cumini (L.) Skeels	Myrtaceae	30	27	5.85676	6.6335	30	-3	-	2.03±1.73
Tarenna asiatica (L.) Kuntez ex Schumann	Rubiaceae	36	54	0.04317	0.09468	36	-	18	0.30±0.28
Terminalia bellirica (Gaertner) Roxb.	Combretaceae	2	1	2.10605	1.51698	2	-1	-	0.00±0.00
Vitex altíssima L. f.	Verbenaceae	50	45	3.05733	3.37573	50	-5	-	0.89±0.59
Walsura trifolia (A.Juss.) Harms	Meliaceae	1	1	0.00258	0.02246	1	-	-	0.00±0.00
Wrightia tinctoria (Roxb.) R.Br.	Apocyanaceae	4	4	0.01009	0.01761	4	-	-	0.65±0.07

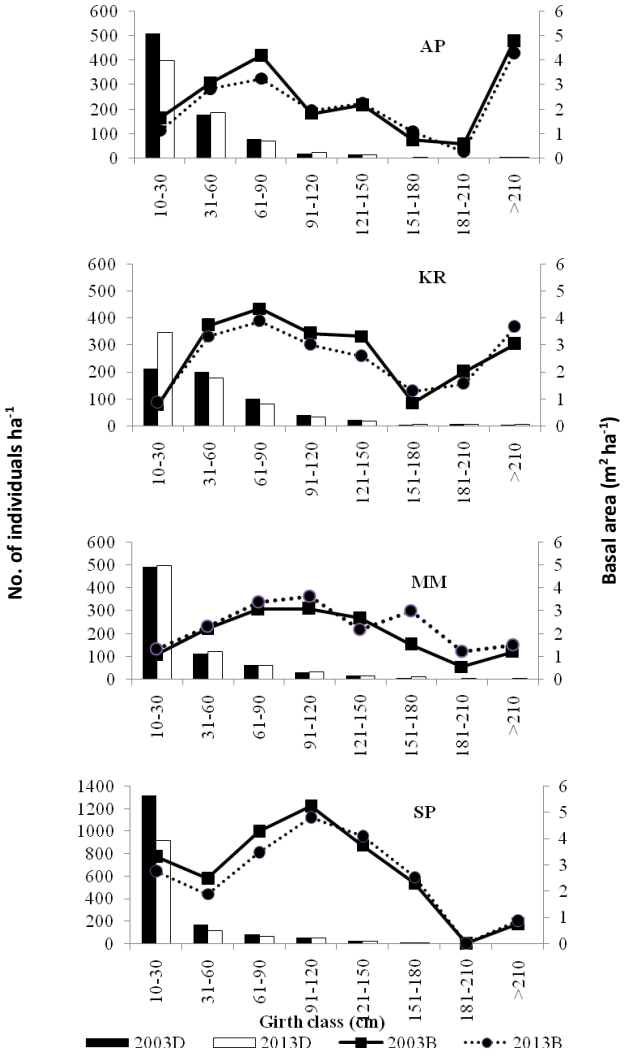


Figure 2. Decadal changes (2003 & 2013) in basal area (B) and tree density (D) by tree girth class in Araiyapatti (AP), Karisakkadu (KR), Maramadakki (MM) and Shanmuganatha-puram (SP) tropical dry evergreen forest sites

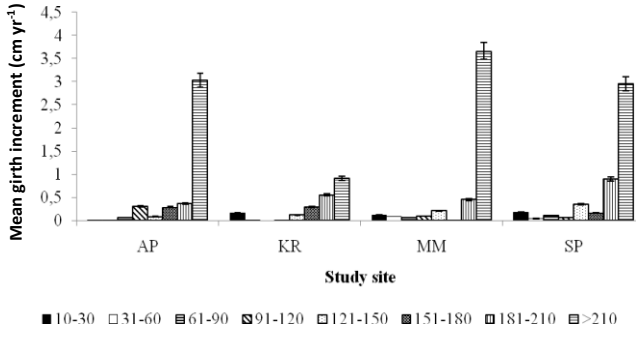


Figure 3. Mean girth increment (cm yr^{-1}) of trees by girth classes in four study sites - (AP, KR, MM and SP)

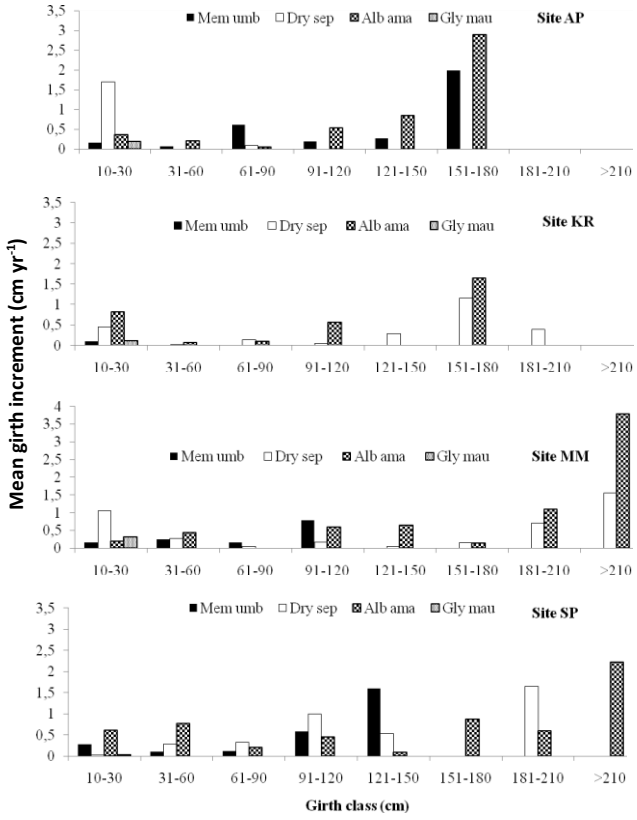


Figure 4. Comparative mean girth increment (cm yr^{-1}) by girth classes for four abundant tree species (≥10 gbh) enumerated in all the four sites (from left to right): (a) *Memecylon umbellatum*, (b) *Drypetes sepiaria*, (c) *Albizia amara*, (d) *Glycosmis mauritiana*

This due to selective felling trees for temple construction and greater resource extraction, such as fuelwood, fodder collection, and grazing pressure. This kind of disturbances leads to thinning of the woody layer and change the forest microclimate, which in turn might impair regeneration process of tree species resulting in low abundance. A marginal reduction in the tree density from 3825 stems in 1996 to 3814 stems in the 1999 Kolli hills, with an annual mortality rate of 0.73% was reported by Sundaram and Parthasarathy (2002) across the four sites. Phillips et al. (1994) reported mortality rate of 1 to 3% yr^{-1} in Asian rainforests. Manokaran and Kochummen (1987) reported that tree density decreased between the years 1947-1951 in Sungei Menyala, Malaysia. The maximum recruitment rate was registered at site KR (21.8 % yr^{-1}) and MM (11.5 % yr^{-1}) which are the less disturbed sites, but the recruitment rate was less in the other disturbed sites AP (9.1 % yr^{-1}) and SP (5.1% yr^{-1}) (Table 3). This could be assigned to human disturbance as well as natural disturbances that facilitate favorable conditions for recruitment of new individuals or the growth of pre-existing ones in the understory species. However, the majority of saplings in the understorey of tropical rainforests grow in the shade (Chazdon and Fetcher 1984; Clark and Clark, 1999) and the trajectories to the canopy can be long (Clark and Clark 2001; Brienen and

Zuidema 2006) putting strong selection pressure on traits that increase survival in the shade. Elevated recruitment rates were reported after periods of acute disturbance (Lugo and Scatena 1996; Boucher and Mallona 1997) or chronic disturbance, such as in especially dry years (Condit et al. 1996), in secondary stands (plot 2 in Milton et al. 1994; selectively logged area (in Elouard et al. 1997) and/or forest edges (Laurence et al. 1998). Tree turnover rate ranged from 9.7 in site MM to 25.4 % yr^{-1} in site SP.

The calculated tree turnover rate (mean of mortality and recruitment rate) for stems ≥ 10 cm gbh ranged from 9.7 % in site MM to 25.4 % in site SP (Table 3).The turnover rates of tropical forests are often said to be high, with mortality rates >1% and sometimes >2% per year (Lieberman and Lieberman 1987; Manokaran and Kochummen 1987; Swaine et al. 1987; Proctor et al. 1989; Phillips et al. 1994) and Mani (2006) also reported turnover rate range of 1.1 to 1.6% yr^{-1} over three years (2003-2006) at five inland tropical dry evergreen forests of peninsular India. However, over a longer interval time the precision of recruitment and mortality estimates declines as more trees would have recruited and died undetected in the time interval (Phillips et al. 2004). In other tropical forests, a turnover rate of 0.68% was reported in wet evergreen forest of Kolli hills, Eastern Ghats (Sundaram and Parthasarathy 2002) and this is low when compared to neotropical (1.84% in Añangu, Ecuador- Korning and Balslev 1994; 0.91% in Reserva Ducke, Brazil- Rankin de Merona et al. 1990; and 2.15% in La Selva, Costa Rica-Lieberman and Lieberman 1987) and Asian tropical (0.89% in Sungei Menyala, Malaysia-Manokaran and Kochummen 1987; 1.89% in Pasoh, Malaysia-Condit et al. 1992 and 1.36% in Lambir, Malaysia-Hall 1991) forests.

In summary, there has been a considerable change in tree diameter increment, mortality and recruitment rate over a decade in four inland Indian tropical dry evergreen forest sites. These long-term studies are important for understanding the tropical forest tree dynamics; it is vital to know the forest status especially those of growth, mortality and recruitment rate and also this data will useful further research. Tree diversity decreased in sites AP and SP, whereas other two sites moderately increased this indicate forest threatened by disturbance multiple reasons (Clearing the trees inside the forest, Grazing by goats, Road construction for the temple (width), and Cultural attachment of local people), those disturbances promoting tree diameter increment. Further, we suggested the need for long-term monitoring studies to get deeper insights in understanding the forest growth rate and to ascertain the role of trees in forest dynamics on a long-term basis, and, in turn, for forest management.

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Table S1. Past and present site disturbance score status (1- ranked as rare: 2-occasional: 3-frequent) of four inland tropical dry evergreen forest sites, Araiyapatti (AP), Karisakkadu (KR), Maramadakki (MM) and Shanmuganathapuram (SP) in peninsular India

Attributes	AP		KR		MM		SP	
	2003	2013	2003	2013	2003	2013	2003	2013
Site encroachment (land use within the forest)								
Temple construction	1	2	2	3	3	3	2	2
Bridle path use	1	3	2	2	3	3	3	3
Temple visitors impact: area used for								
Vehicle parking (area occupied)	1	2	3	3	2	2	3	3
Cooking	1	2	3	3	3	2	2	3
Festive occasion use	1	3	2	2	3	3	2	3
Grazing (cattle/goat)	2	3	2	2	3	3	2	2
Culture attachment of local people	1	2	2	2	3	3	2	3
Resource removal								
Firewood	1	1	1	1	2	1	1	2
Timber	1	2	1	1	1	1	1	2
Other: medicinal plants, edible fruits and soil	1	2	1	1	2	2	2	2
Nearest habitation & people's forest dependence	1	2	2	2	3	3	3	3
Approach road to temple (width)	1	2	2	2	3	3	3	3
Fodder (native forest species) collection	0	3	0	2	0	2	0	2
Habitat fragmentation	0	2	0	1	0	1	0	2
Solid waste dumping (plastic, polythene, glass, rexin)	0	2	0	1	0	1	0	2
Ground clearing for temple related work	0	1	0	1	0	1	0	1
Total score	13	34	23	29	31	33	26	38

Note: Pandian and Parthasarathy (2016)

Table S2. Summary of density (stems ha^{-1}) and mean girth increment (cm yr^{-1}) of all tree species (≥10 gbh) by girth class during 2003 and 2013 inventories in four inland tropical dry evergreen forest sites AP, KR MM and SP. Data were pooled

Species name	Family	Tree density by girth class - 2003								Tree density by girth class -2013								Mean girth increment rate of trees (cm yr^{-1}) by girth class							
		10-30	31-60	61-90	91-120	121-150	151-180	181-210	>210	10-30	31-60	61-90	91-120	121-150	151-180	181-210	>210	10-30cm	31-60	61-90	91-120	121-150	151-180	181-210	>210
Acacia leucophloea (Roxb.) Willd.	Mimosaceae	1	0	2	0	0	0	0	0	2	1	1	0	0	0	0	0	0.07	0	0.65	0	0	0	0	0
Albizia amara (Roxb.) Boivin	Mimosaceae	53	29	26	25	17	6	4	4	44	29	15	22	16	11	3	4	0.02	0.11	0.18	0.43	0.03	0.39	0.42	1.07
Albizia lebbeck (L.) Benth.	Mimosaceae	1	0	0	0	3	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0.75	0	0	1.1
Albizia odoratissima (L.f.) Benth.	Mimosaceae	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0.74	0.98	0	0	0	0
Allophylus serratus (Roxb.) Kurz	Sapindaceae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atalantia monophylla (L.) Correa	Rutaceae	9	0	1	0	0	0	0	0	6	3	1	0	0	0	0	0	0.16	0	0.4	0	0	0	0	0
Azadirachta indica A. Juss.	Meliaceae	7	5	2	3	0	0	0	0	3	4	2	2	0	0	0	0	0.35	0.48	0.22	0.54	0	0	0	0
Benkara malabarica (Lam.) Tirven.	Rubiaceae	1	0	1	1	0	0	0	0	8	1	0	1	0	0	0	0	0.23	0	0	0.35	0	0	0	0
Borassus flabellifer L.	Arecaceae	0	0	1	9	1	0	0	0	0	0	1	7	0	0	0	0	0	0	0.1	0.17	0	0	0	0
Cadaba trifoliata (Roxb.) Wight & Arn.	Capparaceae	11	7	3	0	0	1	0	0	22	11	6	0	0	1	0	0	0.35	0.28	0.14	0	0	1.35	0	0
Canthium coromandelicum (Burm.f.) Alston	Rubiaceae	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0.45	0	0	0	0	0	0	0
Canthium dicoccum (Gaertn.) Teijsm. & Binn.	Rubiaceae	31	47	2	0	0	0	0	0	41	54	5	0	0	0	0	0	0.34	0.29	0.7	0	0	0	0	0
Cassia fistula L.	Ceasalpiniaceae	8	10	1	1	0	0	0	0	8	6	5	1	0	0	0	0	0.58	0.04	0.03	0.15	0	0	0	0
Cassia roxburghii DC.	Caesalpiniaceae	2	1	1	0	0	0	0	0	0	1	2	0	0	0	0	0	0	1.15	1.02	0	0	0	0	0
Cassia siamea Lam.	Caesalpiniaceae	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroxylon swietenia DC.	Flindersiaceae	48	26	12	4	1	1	0	0	42	35	20	5	0	1	0	0	0.02	0.01	0.24	0.64	0	0.4	0	0
Clausena dentata (Willd.) Roemer	Rutaceae	9	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0.20	0	0	0	0	0	0	0
Commiphora caudata (Wight & Arn.) Engle.	Burseraceae	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0.25	0	0	0	0	0	0	0
Cordia obliqua Willd.	Cordiaceae	0	0	4	8	4	0	1	0	0	1	5	2	2	0	1	0	0	0	0.88	1.16	0.85	0	1.33	0
Crateva magna (Lour.) DC.	Capparaceae	3	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0.08	0	0.75	0	0	0	0	0
Dalbergia paniculata Roxb.	Papilionaceae	0	0	0	0	1	0	0	0	5	0	0	0	1	0	0	0	0	0	0	0	0.6	0	0	0
Diospyros ebenum Koen.	Ebenaceae	5	3	1	2	2	1	0	0	8	4	1	0	2	2	0	0	0.59	0.85	1.11	0	0.95	0.97	0	0
Diospyros ferrea (Willd.) Bakh.	Ebenaceae	2	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0.16	0	0	0	0	0	0	0
Diospyros montana Roxb.	Ebenaceae	6	2	0	0	1	0	0	0	5	2	0	0	1	0	0	0	0.33	0.3	0	0	0.59	0	0	0
Drypetes sepiaria (Wight & Arn.) Pax. & Hoffm.	Euphorbiaceae	67	133	78	58	29	6	6	3	55	103	78	37	31	8	8	2	0.08	0.03	0.15	0.08	0.08	0.63	0.25	1.7
Ehretia pubescens Benth.	Cordiaceae	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euphorbia antiquorum L.	Euphorbiaceae	27	46	23	1	0	0	0	0	30	31	19	1	0	0	0	0	0.32	0.05	0.37	0.95	0	0	0	0
Ficus amplissima J.E.Smith	Moraceae	0	0	1	1	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0.7	2.05	0	0	0	2
Ficus benghalensis L.	Moraceae	1	0	0	1	0	1	0	2	2	0	0	1	0	1	0	2	0.89	0	0	0.3	0	0.55	0	2.25
Ficus microcarpa L.f.	Moraceae	0	0	1	0	0	0	0	2	0	0	1	0	0	0	0	2	0	0	2.4	0	0	0	0	1.96
Ficus religiosa L.	Moraceae	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1.4	0	0
Flacourtia indica (Burm.f.) Merr.	Flacourtiaceae	2	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0.35	0	0	0	0	0	0	0
Gardenia resinifera Roth	Rubiaceae	13	3	3	0	0	0	0	0	18	6	2	0	0	0	0	0	0.39	0.41	0.53	0	0	0	0	0
Glycosmis mauritiana (Lam.) Yuich. Tanaka	Rutaceae	164	1	0	0	0	0	0	0	348	4	0	0	0	0	0	0	0.2	0.16	0	0	0	0	0	0
Gmelina asiatica L.	Verbenaceae	1	1	0	2	1	0	1	0	1	3	0	2	0	0	1	0	0.38	0.26	0	0.7	0	0	0.85	0
Jatropha gossypiifolia L.	Euphorbiaceae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lannea coromandelica (Houtt.) Merr.	Anacardiaceae	2	10	6	1	4	0	0	0	7	6	6	2	5	0	0	0	0.32	0.45	0.295	1.2	1.06	0	0	0
Lepisanthes tetraphylla (Vahl.) Radlk.	Sapindaceae	71	37	16	2	1	2	0	0	45	36	16	7	1	1	0	0	0.13	0.12	0.18	0.46	1.01	0.8	0	0
Manilkara hexandra (Roxb.) Dubard	Sapotaceae	5	5	19	14	13	5	5	5	2	5	10	12	13	5	5	5	0.26	0.13	0.52	0.53	0.05	0.09	0.48	0.49
Maytenus emarginata (Willd.) Ding Hou	Celastraceae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Memecylon umbellatum Burm.f.	Melastomatace	1432	149	40	6	5	1	0	0	977	147	33	11	3	0	0	0	0.18	0.11	0.27	0.37	0.76	0	0	0
Morinda pubescens J.E. Smith	Rubiaceae	6	4	1	2	1	0	0	0	9	4	1	2	0	0	0	0	0.14	0.26	0.33	0.4	0	0	0	0
Phyllanthus polyphyllus Willd.	Euphorbiaceae	0	2	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1.28	0	0	0	0	0	0
Pleiospermium alatum (Wall. ex Wight.&Arn.) Sw.	Rutaceae	4	2	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0.61	0.55	0	0	0	0	0	0
Pongamia pinnata (L.) Pierre	Papilionaceae	22	18	17	5	6	1	1	2	6	14	10	11	5	2	1	2	0.05	0.4	0.27	0.41	0.32	0.25	0.65	1.35
Premna serratifolia L.	Verbenaceae	0	0	2	0	0	1	0	1	1	0	2	0	0	0	0	1	0	0	0.925	0	0	0	0	3.65
Prosopis juliflora (Sw.) DC.	Mimosaceae	9	5	3	1	1	0	0	0	7	5	3	1	2	0	0	0	0.27	0.58	1.42	0.05	0.15	0	0	0
Pterospermum canescens Roxb.	Sterculiaceae	88	56	44	28	20	8	3	3	58	52	42	20	16	8	6	6	0.137	0.14	0.18	0.31	0.18	0.22	0.38	1.07
Sapindus emarginatus Vahl	Sapindaceae	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0.7	0	0	0	0	0
Sapium insigne (Royle) Trimen	Euphorbiaceae	5	2	0	0	2	0	0	0	4	3	0	0	2	0	0	0	0.11	0.52	0	0	0.9	0	0	0
Streblus asper Lour.	Moraceae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strychnos nux-vomica L.	Loganiaceae	155	40	19	11	4	5	1	5	78	54	15	14	8	3	2	3	0.04	0.09	0.23	0.12	0.38	0.9	1.65	1.61
Sryrchnos potatorum L. f.	Loganiaceae	3	4	1	1	0	0	0	0	0	4	1	1	0	0	0	0	0.6	0.54	0.45	0.4	0	0	0	0
Syzygium cumini (L.) Skeels	Myrtaceae	0	3	4	3	4	5	3	8	0	2	2	3	4	4	3	9	0	0.1	0.35	0.77	0.65	0.21	0.64	2.68
Tarenna asiatica (L.) Kuntez ex Schumann	Rubiaceae	36	0	0	0	0	0	0	0	51	3	0	0	0	0	0	0	0.18	0	0	0	0	0	0	0
Terminalia bellirica (Gaertner) Roxb.	Combretaceae	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	7.8
Vitex altíssima L. f.	Verbenaceae	4	16	13	4	8	4	0	1	6	6	15	8	6	3	0	1	0.11	0.4	0.84	0.19	0.47	0.15	0	1.46
Walsura trifolia (A.Juss.) Harms	Meliaceae	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Wrightia tinctoria (Roxb.) R.Br.	Apocyanaceae	3	1	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0.32	0.5	0	0	0	0	0	0

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Biodiversity of subterranean termites on the *Acacia crassicarpa* plantation

NOOR FARIKHAH HANEDA[1,♥], ICHMA YELDHA RETMADHONA[2], DODI NANDIKA[3], ARINANA[3]

[1]Department of Silviculture, Faculty Forestry, Institut Pertanian Bogor. Darmaga Campus, Bogor16680, West Java, Indonesia
Tel./fax.: +62 -251-8626806/8626886, ♥email: nhaneda@yahoo.com
[2]Graduate Program in Silviculture, Faculty of Forestry, Institut Pertanian Bogor. Darmaga Campus, 16680, West Java, Indonesia
[3]Departmen of Forest Product, Institut Pertanian Bogor. Darmaga Campus, Bogor 16680, West Java, Indonesia.

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Abstract. *Haneda NF, Retmadhona IY, Nandika D, Arinana. 2017. Biodiversity of subterranean termites on the* Acacia crassicarpa *plantation. Biodiversitas 18: 1657-1662.* The development of industrial plantation forests is one of the efforts to meet the needs of raw material of processing industry of timber forest products. In the last 15 years, the development of industrial plantation forest has grown rapidly. Plant species widely developed is fast-growing plants (fast growing trees) such as *Acacia crassicarpa* Cunn ex Benth. However, *A. crassicarpa* has a low resistance against pests, evidenced by the presence of subterranean termites attack occurred in the industrial plantation forest of *A. crassicarpa*. This aim of the study is to determine the diversity of subterranean termites in plantation forest of *A. crassicarpa*. Two methods used for data collection: baiting system and survey. Five species of subterranean termites found on sample paths were *Coptotermes curvignathus* Holmgren, *C. gestroi* Wasmann, *Schedorhinotermes javanicus* Kemner, *Longipeditermes longipes* Haviland, and *Pericapritermes mohri* Kemner. These species can be distinguished from the morphology of the mandible. *Coptotermes* is most commonly found Genus in study plots.

Keywords: *Acacia crassicarpa*, baiting system, *Coptotermes curvignathus*, fast-growing trees, survey

INTRODUCTION

The development of industrial plantation forests is one of the efforts to meet the raw material needs of processing industry of timber forest products. In the last 15 years, industrial plantation forest has grown rapidly. The plantation area increased from 1.13 million ha in 1995 to 9.97 million ha in 2009 (Sumargo et al. 2011). Plant species widely developed is the fast-growing plants (fast growing species) because they have a relatively short harvest period. *Acacia crassicarpa* is one of the plants that has many advantages. Thus they are widely used in pulp and paper industries. *A. crassicarpa* is one of the major tropical Australian and South-East Asia, especially in Indonesia and Vietnam (Lam et al. 2014). The fiber of the plant is in the category of medium to high and the yield is quite higher compared to *Acacia mangium* (Sugesty et al. 2015). In addition, highly adaptive to the extreme environment, makes it very easy to plant in peat. According to Suhartati et al. (2014) this species is able to grow well in the marginal land condition (in swamps, or in an area flooded periodically) with high organic matter content and low pH. The plant began to be developed and grown on a large scale in various areas of industrial plantation forest. However, Acacia is included in the category of low durability wood, which makes it very vulnerable to pest attacks. As reported by Hutabarat et al. (2015), some trees are potentially attacked by pests, especially termites such as rubber, pine, eucalyptus, acacia, and palm oil.

It is also recently reported that *A. crassicarpa* plantations in Riau has been attacked by termites (Tjahjono 2016 (personal communication)). Termites attack has caused death on some stands, which should be a particular concern in the future. Termites are known as pests that attack the building and construction, but in some cases, subterranean termites also potentially become a pest in plantations, agriculture, and industrial plantation forests. One example of the plantations potentially attacked by subterranean termites is palm oil. Arinana et al. (2012) identified three species of termites attacking the palm oil plantation in Bogor, namely: *Macrotermes gilvus*, *Coptotermes curvignathus*, and *Nasutitermes javanicus*. Another subterranean termites attacks on plantation forests have been reported by Ngatiman (2014) and Herlinda et al. (2010). Subterranean termites have invaded meranti plantation forest in East Kalimantan and community-owned rubber plantations in West Sumatra.

One of the termites species that should be taken into account is *Coptotermes* species. The termites have a very large population and wide range of habitat. *Coptotermes* is also known to be very greedy and harmful (Nandika 2015). Moreover, not all species of subterranean termites are harmful, some species of subterranean termites are needed in nature as decomposers. Therefore, proper identification is needed for effective control. The necessity to conduct a study of the diversity of subterranean termites, especially in plantations *A. crassicarpa* gives an important information in designing the pest control of subterranean termites. The purpose of this study is to determine the diversity of subterranean termites in the industrial plantation forest of *A. crassicarpa*.

MATERIALS AND METHODS

Study area

Specimen collection of subterranean termite was conducted for 5 months from May to September 2016 in Bukit Batu Subdistrict, Bengkalis District, Riau Province, Indonesia. The specimen was identified in Forest Entomology Laboratory, Department of Silviculture, Faculty of Forestry, Institut Pertanian Bogor, Indonesia. The study site is presented in Figure 1. There were nine sample plots used in this study and they were classified by the age of *A. crassicarpa* stands (1, 2, and 3 years old).

Equipments and materials

The equipments used in the study were tape measure, digital camera, bottle for termites collection, a binocular microscope with lens of 0.67 to 4.5 x magnification. The main materials used were pine wood cut according to the standard of ASTM-D-1758-96-2008 (ASTM 2008) as stakes in baiting method, and 70% alcohol.

Procedures

Sample plots. The plots were set using stratified random sampling methods with intensity sampling of 0.6% of overall plots (1,500 plots). There were nine sample plots used as study site. One plot has more or less 25 ha of width, thus to shorten the scope of research, two sample paths were made in the sample plot. The sample paths of 250 m x 25 m were made in transect across contour. Collection of subterranean termite specimens in sample paths was carried out by survey and baiting system.

Baiting system. The baiting system was conducted using pine wood as stakes in accordance with ASTM-D-1758-96-2008 (ASTM 2008), which referred to the study by Arinana et al. (2014). A total of 60 wooden stakes were installed in sample paths or 120 wooden stakes in one sample plot. The wooden stake was installed with the distance of 8 m x 6 m (four trees from the edge of the canal and two trees between wooden stakes in one lane) (Figure 2). Monitoring was done after one month of installation of wooden stakes, then termites attacking the stakes were collected and put into a collecting bottle containing 70% alcohol.

The stakes were planted vertically into the ground. Half of the stakes was below the soil surface, and the other half was above the ground (Figure 3) to facilitate the monitoring. The upper stakes were coded using light-colored to facilitate the monitoring activities.

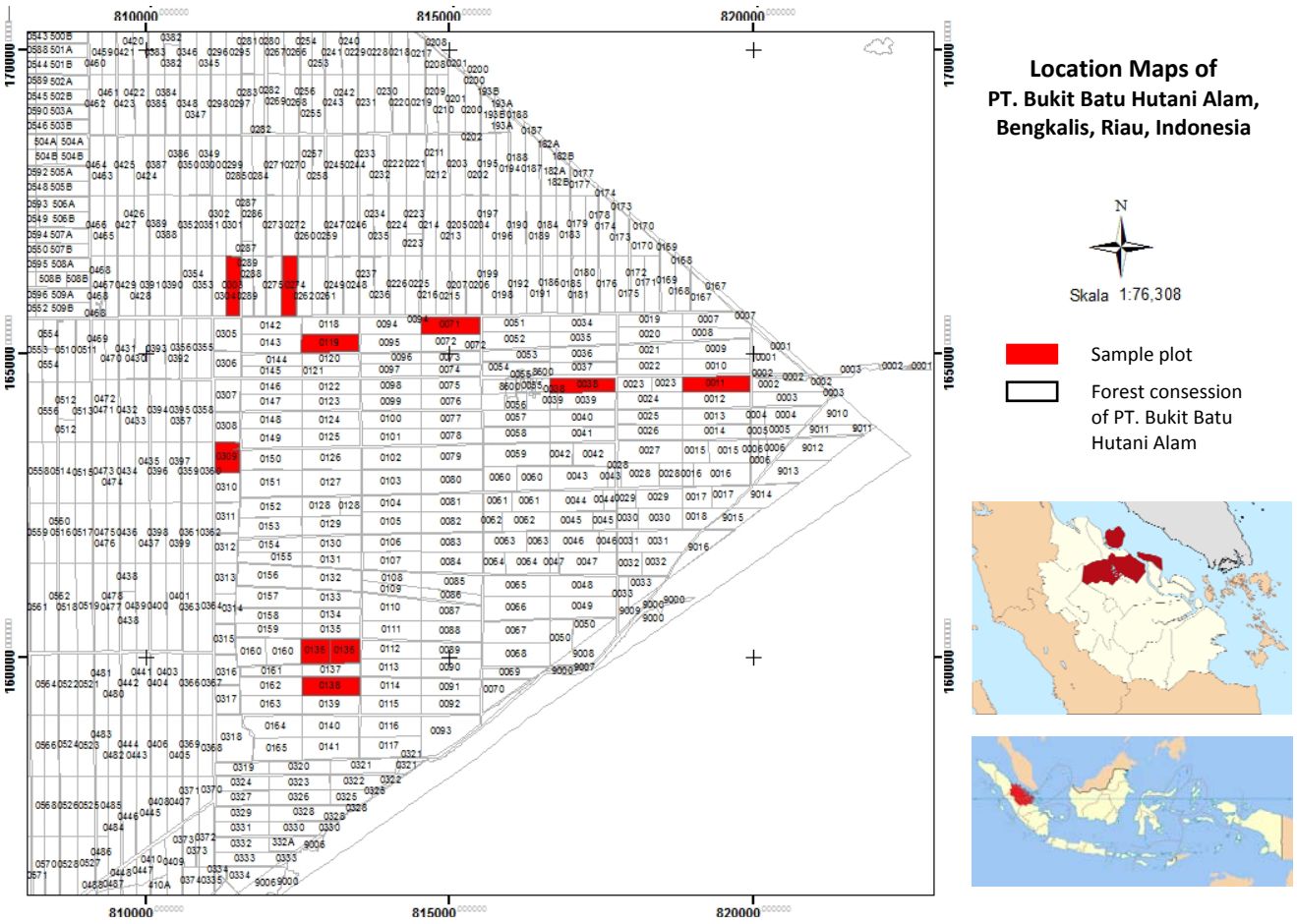


Figure 1. Study site of A. crassicarpa industrial plantation forest, Bukit Batu Subdistrict, Bengkalis District, Riau Province, Indonesia

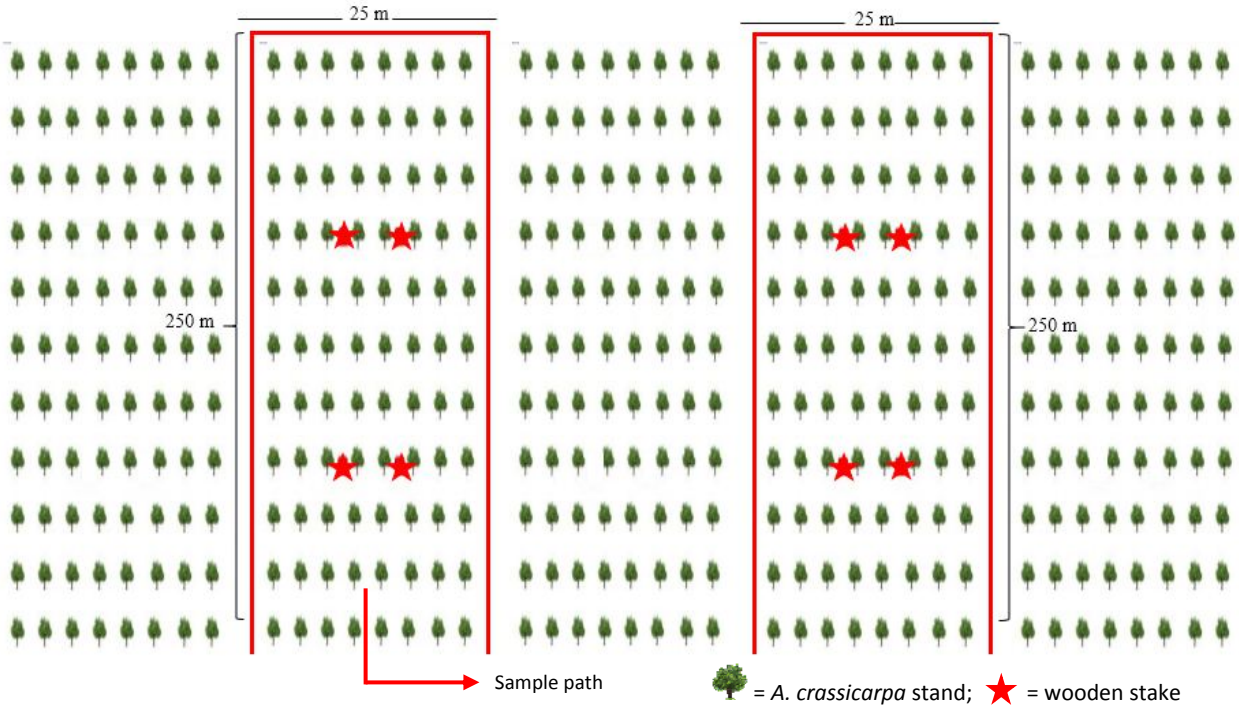


Figure 2. Illustration of bait stake placement in sample paths

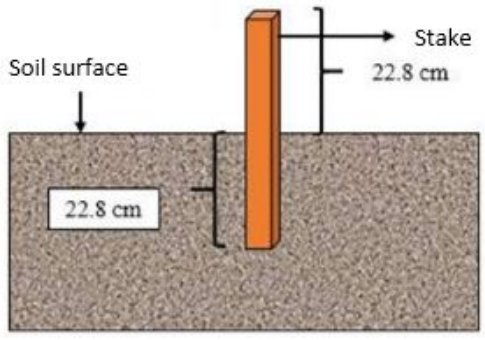


Figure 3. Installation of the stakes

Survey. Survey was conducted by directly observing the physical condition of the tree. The attacked trees were indicated by the presence of tunnel of termites' path on a tree. In addition to the tree condition, the observations were done around the sites such as: logged over stumps and litter. The termites which were from the soldier caste were then collected and put into a collecting bottle. The number of affected trees was recorded. .

Identification. The specimens were identified using a binocular microscope with lens of 0.67 to 4.5 magnification. The termites species determination was performed using the determination key of termites by Tho (1992) and Ahmad (1958). The observation of termites included the entire body, especially on the head (mandible).

RESULTS AND DISCUSSION

The results of the survey show five species of subterranean termites found in the sample paths namely *Coptotermes curvignathus*, *C. gestroi*, *Schedorhinotermes javanicus* (Family: Rhinotermitidae), *Longipeditermes longipes*, and *Pericapritermes mohri* (Family: Termitidae). These five types of subterranean termites can be identified by the shape of the mandible and physical size. In nine sample plots, genus of *Coptotermes* was found in all sample plots. Compared to other species, *Coptotermes* have the most extensive attack, i.e. of 17,280 observed trees, 560 *A. crassicarpa* stands were attacked by *Coptotermes*. It showed that all the trees were attacked by *Coptotermes* termites. The distinctive feature of this genus is that when the soldier caste of the colonies is disturbed, their heads will secrete a white liquid. It is a *Coptotermes'* defense from predators or enemies. *Pericapritermes* was the fewest termites found in the sample paths. This termite was only found on sample paths with 3 years old of *A. crassicarpa* stands.

In the bait method, three species termites of family Rhinotermitidae were found namely *C. curvignathus*, *C. gestroi*, and *Schedorhinotermes javanicus*. A total of 1,080 wooden stakes were installed in nine sample plots, but only 105 wooden stakes were identified to be infested by termites (9.7%), of which 91 (8.4%) wooden stakes were attacked by *Coptotermes* and 14 (1.3%) by *Schedorhinotermes*.

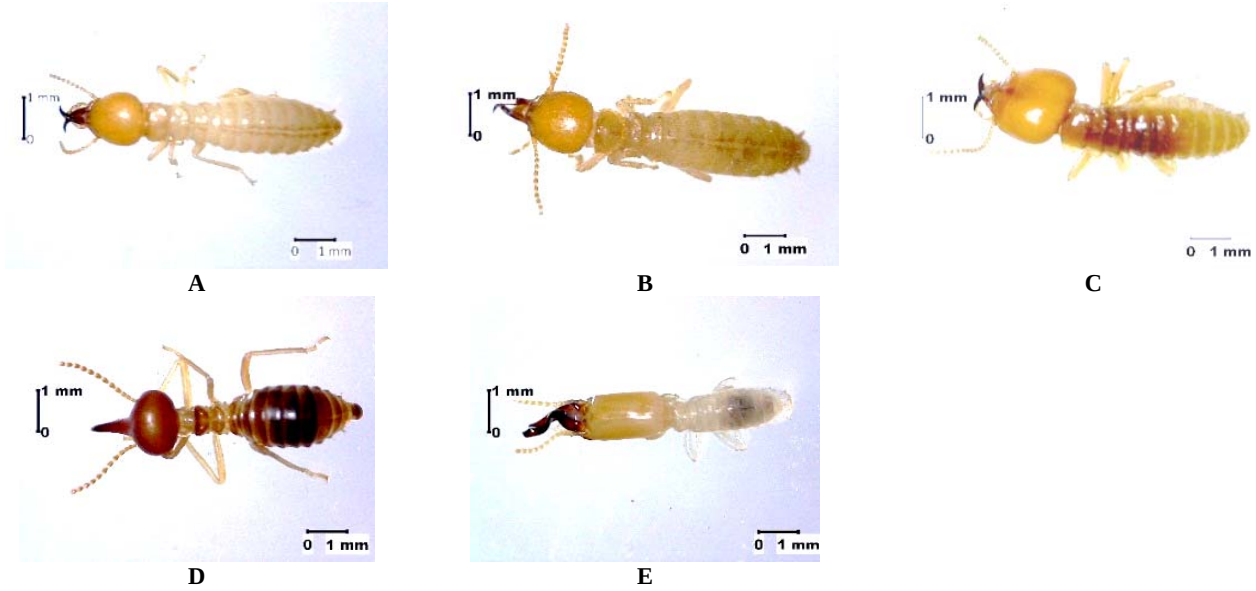


Figure 3. Species of subterranean termites: A. *C. curvignathus* (Holmgren); B. *C. gestroi* (Wasmann); C. *Schedorhinotermes javanicus* (Kemner); D. *Longipeditermes longipes* (Haviland); E. *Pericapritermes mohri* (Kemner)

Table 1. Species of subterranean termites in *Acacia crassicarpa*

Age of stand (years)	Species of termite	Family	Location
1	*Coptotermes curvignathus*	Rhinotermitidae	Stand, stump, litter
	C. gestroi	Rhinotermitidae	Stand, stump, litter
2	*C. curvignathus*	Rhinotermitidae	Stand, stump, litter
	C. gestroi	Rhinotermitidae	Stand, stump, litter
	Schedorhinotermes javanicus	Rhinotermitidae	Stand, stump, litter
	Longipeditermes longipes	Termitidae	Dead tree, stump, litter
3	*C. curvignathus*	Rhinotermitidae	Stand, stump, litter
	C. gestroi	Rhinotermitidae	Stand, stump, litter
	S. javanicus	Rhinotermitidae	Stand, stump, litter
	L. longipes	Termitidae	Dead tree, stump, litter
	Pericapritermes mohri	Termitidae	Stump

Coptotermes was commonly found in *A. crassicarpa* stands, but *Longipeditermes* and *Pericapritermes* were not found in these stands. Sample plot with 3 years old of *A. crassicarpa* stands has the largest number of species when compared to sample plots with other standing age. There were five species termites found in sample plots with 3 years old of *A. crassicarpa* stand. While sample plot with 1-year-old stands have the lowest number of species in which only two species were found. The total number of each termite species were presented in Table 2. Specimens of termites were collected at some points especially on *A. crassicarpa* stands and around the study site. *Coptotermes*, especially *C. curvignathus*, have the longest body size compared to other types. The next species with long body is *S. javanicus*. This termite has two types of soldier caste: mayor and minor. The morphology of these two soldier types of *S. javanicus* can be differentiated from the size and color of the body.

Table 2. Number of subterranean termites in *A. crassicarpa* plantation

Family	Species	Number of termites
Rhinotermitidae	*Coptotermes curvignathus*	198
	C. gestroi	130
	Schedorhinotermes javanicus	128
Termitidae	*Longipeditermes longipes*	78
	Pericapritermes mohri	33

Discussion

Differences in the number of subterranean termite species can be caused by several things, such as differences in soil type and inundation of land conditions (Cheng et al. 2008, Kon et al. 2012). According to Cheng et al. (2008), the land with mineral soil type will be dominated by

members of Termitidae, but the number of its members will be decreased on peatland. In contrast, members of the Rhinotermitidae will dominate on peatlands but their species richness decrease in mineral soil type. However, the history of land use can also affect species richness in an area. Haneda and Firmansyah (2012) also found that the highest diversity occurred in the Termitidae family in the forest area with mineral soil type. In primary forests, the diversity of termites is very high, but decreases in the monoculture forest (Handru et al. 2012). Some studies also show the same phenomenon that the richness of termite species in relatively undisturbed areas is higher than in other areas.

Termites are a very important group of insects in the humid tropics. This colonizing insect has high species diversity and populations. Some species can potentially become pests as they eat the cellulose material. This insect is also known as the destroyer of buildings and constructions made of wood, but in the last century, termites superbly become pests in agriculture, plantation, and forestry. Termites are insect species in the order Isoptera in which about 200 species and 179 species have been identified in Indonesia. Termites are polymorphic which live in a caste system of colonies: breeders, workers, and soldiers. Each caste have the distinctive body with their respective duties in the colony. According to Nandika et al. (2015), this insect can be identified by observing the size of the head and mandible of the soldier caste.

In nature, termites have a very important role as soil macrofauna to make the tunnels on the ground, which makes the soil to have a good porosity for plant growth. Some particular species of termites are able to accelerate the nutrients cycling to improve soil fertility. Termites are one of the critical decomposing organisms for decomposition process in the tropical forests. However, termites also often destroy the wood or attacking trees and crops which affect a big economic loss. Termites are recently problem which is not easy to overcome and have become a big problem due to their increasing attacks intensity. The attacks have a close relationship with the high population number, wide range of habitat and high adaptability to the unfavorable environment.

Coptotermes is a group of subterranean termites nesting below the ground. But unlike other subterranean termites, the group is able to build temporary nests (secondary nest) away from the ground. Therefore, the attack can reach higher parts. *Coptotermes* is potential termites as a destructive pest of *A. crassicarpa* stands. The termites' existence should be wary. Some cases have been reported by Ngatiman (2014) and Herlinda et al. (2010) that termites do not only damage the wood but also living trees. The attack occurs on the stand causing an injury to death. The diversity of *Coptotermes* in Indonesia is higher than in other countries. Favorable habitat in tropic is certainly very pleasant habitat for termites. According to Savitri et al. (2016), species that have long been known as wood and building destroyers in Indonesia are *C. curvignathus*. This insect is able to adapt to various environmental conditions, in addition, it has a large enough population. *C. gestroi* is endemic termites of South East Asia. The soldier caste has similarities with *C. formosanus*, in which the fontanelle is equal between both mandibles. The difference is in the number of hair on the fontanelle, in which *C. formosanus* has a lot of hair while *C. gestroi* has only one hair. In addition, the morphological shape of fontanelle of *C. gestroi* is more prominent than *C. formosanus* (Subekti 2010). Other biological characteristics of *C. formosanus* and *C. gestroi* has also been described by Su et al. (2017).

Schedorhinotermes is a member of Rhinotermitidae family and Rhinotermitinae subfamily. Soldier caste of *Schedorhinotermes* has two types, i.e. the large soldier (major) and small soldier (minor). *S. javanicus* is easy to find in the entire java island, up to an altitude of 1000 meters above sea level. This is consistent with the study conducted by Haneda and Firmansyah (2012). They reported that *S. javanicus* has been found in Gunung Walat University Forest at 460-715 meters above sea level. In addition, *Schedorhinotermes* has a wide range of habitat. Rismayadi (1999) reported that the home range of the termite colony around Institut Pertanian Bogor (IPB) rectorate building reaches 295 m. The termites are commonly found in tree trunks, logged or stumped of wood in the forest. The species can also be found on the islands of Borneo and Sumatra (Gathome-Hardy et al. 2002). In the sample paths, *S. javanicus* is found in litter and stumps but it is very rarely found to attack *A. crassicarpa* stands.

The existence of Rhinotermitidae, especially *Coptotermes* spp. and *Schedorhinotermes* spp., which belongs to the termites group of wood and litter eater and is a potential pest on *A. crassicarpa* stands. According to Hanis et al. (2014) groups of wood-eating termites are potential to be pest. *Coptotermes* and *Schedorhinotermes* are the most common termites found in the sample paths. However, genus of *Schedorhinotermes* is not found in *A. crassicarpa* stands, but it is found only in litter and tree stumps.

Longipeditermes is one of the termites from subfamily Nasutitermitinae which is often found on the floor of tropical forests (Syaukani 2013). Their activities outside the colony are often done in the morning and evening to reduce the predation risk by predators. The dark-colored body and active movement among litter make the individual of termites is not easy to find and collect. The soldier caste of this genus consists of two groups, the large soldiers (major) and small soldiers (minor). Worker caste is composed of three groups (trimorphic), namely: the major, medium, and minor workers (Syaukani 2013). Recently, three species of termites from the genus is found spreading in the Indo-Malayan subregion, *L. longipes* (Haviland), *L. kistneri* (Ahmad and Akhtar), and *L. mandibulatus* (Thapa) (Miura and Matsumoto, 1998). The termites are distributed in Sumatra, Peninsular Malaysia, Java, and Borneo.

The diversity of subfamily Termitinae is distinguished by the food and morphology. Based on the food source, it can be divided into two groups: wood-eating termites and humus-eating termites. *Pericapritermes* itself is included in the group of termites eating humus, the rest of weathered wood, and clay (Pribadi 2015). On the humus-eating termites, the soldier caste is divided into two groups based on mandible morphology: bilaterally symmetrical

biting/slashing group and snapping group known as *Capritermes* group. *P. mohri* was found near logged tree stump and the nest was found below ground. The nest of the termites was charcoal-like (black) and very hard. According to Faszly et al. (2005), genus *Pericapritermes* is included in category III i.e. termites which consume humus and high organic material. The species requires a very specific habitat, so they are very difficult to find. As Haneda and Firmansyah (2012) have observed in Gunung Walat Educational Forest, Sukabumi that the genus was found only at one point. The finding is in line with the recent study.

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Population structure, distribution pattern and microhabitat characteristics of *Aglaonema simplex* in Pasatan Protected Forest, Jembrana, Bali, Indonesia

DEWI LESTARI♥, NI PUTU SRI ASIH
Bali Botanic Garden. Candikuning, Baturiti, Tabanan 82191, Bali, Indonesia. Tel.: +62-368-2033170, 2033170, Fax. +62-368-2033171 ♥email: dewi016@lipi.go.id

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Abstract. *Lestari D, Asih NPS. 2017. Population structure, distribution pattern and microhabitat characteristics of Aglaonema* simplex *in Pasatan Protected Forest, Jembrana, Bali, Indonesia. Biodiversitas 18: 1663-1668. Aglaonema simplex* Blume is one species of *Aglaonema* that is distributed in Bali, Indonesia. This species can be found in the forests of Jembrana, Karangasem, Tabanan, Buleleng and Bangli. There is no recent data for *A. simplex's* population and microhabitat in Bali, while this data is needed to develop future conservation policy. By tracking populations over time, ecologists can see how these populations have changed and may be able to predict how they are likely to change in the future. Monitoring the size and structure of populations can also help ecologists to manage populations. This study was aimed to determine the current condition of *A. simplex* in the Pasatan forest and to find out the population structure, distribution, and its microhabitat characteristics. The study was conducted along two tracks at Pasatan Forest, Bali on June 9-11, 2015. Data were collected using the purposive quadrant plot method and analyzed descriptively, tabulated in tables and graphs. The population pattern distribution was as defined by standardized Morisita's index and microhabitat differences in both tracks were determined by the Mann-Whitney test in SPSS 16. The total number of *A. simplex* was 114 individuals. Thirty-seven percent of plants were juveniles, while 36% were mature plants without fruit and 27% were mature plants with fruit. The population structure along the first track was dominated by a mature population of plants without fruit, while fruiting mature plants dominated the structure of the population along the second track. The distribution of the population along the first track was clustered, while distribution along the second track was uniform. *A. simplex* was found at an altitude of 367- 448 m asl., oil pH of 6.7-6.8, soil humidity of 73%-84%, air temperature 27°-28° C, air humidity at 80%-86%, with low light intensity of 170-225 lux.

Keywords: *Aglaonema simplex*, Jembrana, microhabitat characteristics, Pasatan protected forest, population

INTRODUCTION

Aglaonema is a genus of flowering plants in the arum family, Araceae, a monocot, herbaceous plants with fibrous roots. Leaf shape varied between oval, oblong or deltaloid and smooth leaf surface, hairless with various leaf tips. Position of the leaves are alternate or opposite with the stalk hugging the stem. As with other Araceae, *Aglaonema* inflorescence consists of two main parts, the spathe and spadix. The flowers are arranged on the spadix. At the top of the spadix, there are male flowers, with sterile flowers in the middle and female flowers at the base (Mayo et al. 1997).

All species of *Aglaonema* come from Southeast Asia, India, China, Indonesia and New Guinea (Nicolson 1969; Mayo et al. 1997; Allen 2011). This plant is known as Chinese evergreen (Antosh 2001). *Aglaonema* was first introduced to Europe at the Kew Botanic Gardens in 1885 and cultivated in the USA in 1930 (Chen et al. 2004). It soon became widespread and popular.

It is estimated that there are 22 species of *Aglaonema* in the world (Boyce et al. 2010). It can be found growing in primary or secondary forests, ravines and humid places. Yusof et al. (2013) said that this genus is also found in pyroclastic areas. A few species are restricted to kerangas or monsoonal perhumid semi-deciduous forest (Boyce and Wong 2012). They are predominantly found at an altitude of less than 1500 meters above sea level (asl), although some species could be found at more than 2000 meters asl on Mount Kinabalu, such as *Aglaonema simplex* Blume (Nicolson 1969).

Aglaonema was favored as an indoor ornamental plant in Indonesia (Qodriyah and Sutisna 2007). Their varied and beautiful leaves attract more and more hobbyist. Soon, *Aglaonema* became popular and source of income for many farmers and was cultivated in many regions (Roza et al. 2012), but most of them are hybrids. Therefore, we have to explore more *Aglaonema* germplasm to conserve and develop it for wider purpose.

One of *Aglaonema* species distributed in Bali is *Aglaonema simplex* (Kurniawan and Asih 2012). This species can also be found in Sulawesi (Nugroho and Santika 2008), Southern Myanmar, Java (Central and East Java), Sumatra, Madura, Kalimantan, Sula Islands and part of the Philippines (Yuzammi 2000; Erlinawati 2010), Laos (Nguyen et al. 2014) and Malaysia (Mansor et al. 2012). In Bali, this species can be found in the forests of Jembrana (Hanum and Asih 2016), Karangasem (Asih et al. 2015), Tabanan, Buleleng and Bangli (Kurniawan and Asih 2012). According to Asih et al. (2014), *A. simplex* can be used as medicine to relieve fever and heal ulcers.

Aglaonema simplex can reach 100 cm high, with a white-grey trunk. There are two variations of leaf morphology of *A. simplex*, a plain green leaf with a slightly wavy edge and a white patterned leaf with a flat edge (Winarti 2002). The leaves are elliptical to elongated, with a rounded leaf base, tapered leaf tips, primary leaf veins slightly prominent adaxially and prominent abaxially. Leaf sheaths can be found approximately ½-7/8 length of the petiole. The spathe is yellowish-green or whitish-green when it first opens and during anthesis, it becomes more elongated. The length of the spadix is about 5-7 cm and the ripe fruit is red (Hanum and Asih 2016).

According to the IUCN, only 7 species that have been declared on IUCN Red List, from more than 600 spesies of Araceae that were estimated to be distributed in Indonesia. It means that Indonesia still needs many researches on ecology of Araceae. In IUCN, *A. simplex* is categorized as least concern, not too affected by forest destruction or degradation. However, the data is old, so more monitoring and data surveys are needed to update this data (Allen 2011). Tracking populations over time is needed to see how populations have changed and may be able to predict how they are likely to change in the future. Monitoring the size and structure of populations can also help ecologists to manage populations (Openstax 2017).

Actually, Araceae's ecology research in general have been held in West Kalimantan (Ilhamullah et al 2015) and Kelantan, Malaysia (Yusof et al 2013). However, this research did not state about *A. simplex* population and microhabitat characteristic, while this information is needed to understand its ecology and to establish its future conservation policy (Kusuma & Astuti 2009). Therefore, this study is held in Pasatan protected forest, Bali, as one of distribution areas of *A. simplex*. The aim of this study was to determine the current condition of *A. simplex* in the Pasatan protected forest, to find out the population structure, distribution and its microhabitat condition.

MATERIALS AND METHODS

Study area

The study was conducted in Pasatan protected forest, Poh Santen, District Mendoyo, which is included in Tegalcangkring Resort Forest Management (RFM), on June 9-11, 2015. The area covers of 740 hectares, but some of them has been disturbed and converted into cultivated land, planted with bananas, coffee and cacao. However, there are some areas that have not been converted yet, and were still in virgin state, such as the top of the hill and the riverside. Therefore, we held this research in those virgin areas, i.e. the hill and riverside. There are two tracks that have been explored: the path to sumber air (riverside) and path to puncak (top of the hill). The first track was along the side of Pergung River from 346 m asl to 432 m asl. The second track was along the left side of Pergung River until it reached an elevation of 586 m asl where there were no *A. simplex* found.

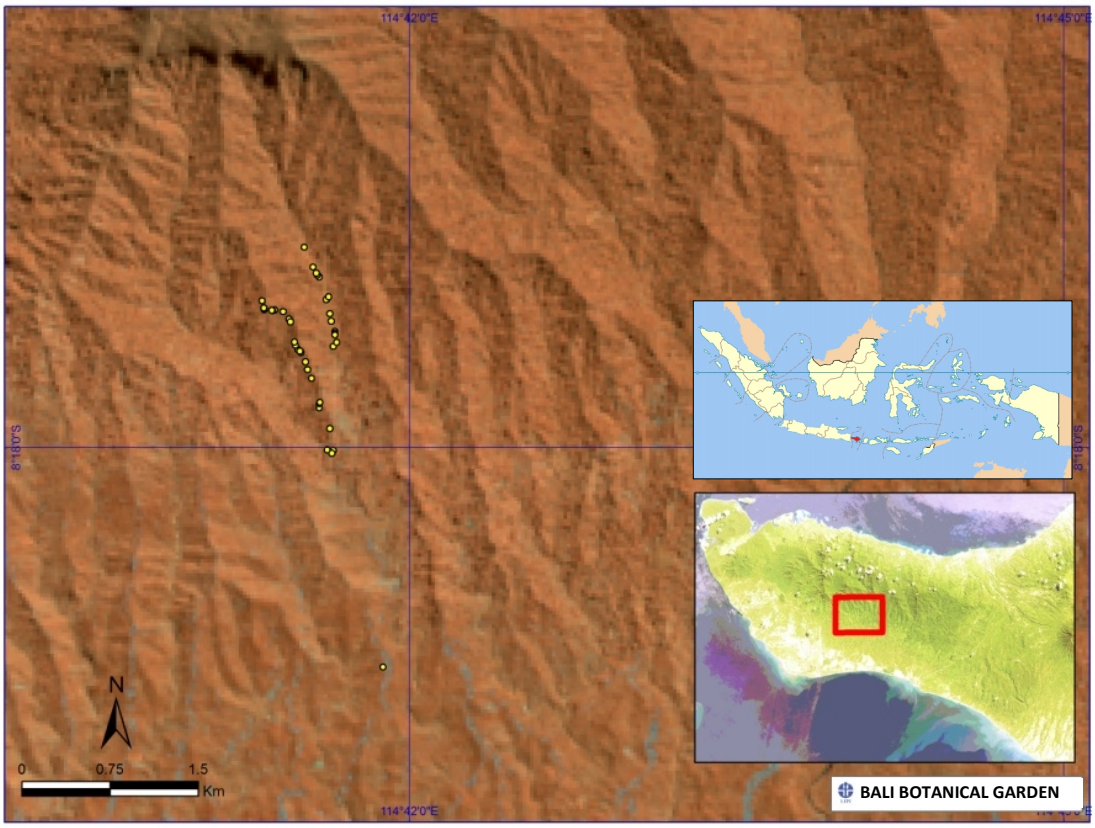


Figure 1. There were two tracks that were explored: the right is first track (a path to sumber air), while the left is second track (a path to puncak). Location of research in Pasatan Protected Forest, Jembrana, Bali, Indonesia

The forest vegetation along the first track was moist with dense undergrowth. Trees were dominated by *Sauraia* sp. while plants that grow on the surface of the rocks (lithophytes) included *Begonia coriacea* and other Araceae genera such as *Homalomena* sp. Forests along the second track were dominated by trees from the Clusiaceae and Macadamia genera. Endemic climbers such as *tiying-lut lut* (*Dinochloa sepang*) were also found along this track.

Procedures

The data was collected purposively (purposive sampling). The quadrant plots of 2 m x 2 m were made at each encounter of *A. simplex*. The parameters were the number of individuals that were found, *A. simplex*'s stage of growth (juvenile, mature, fruiting/flowering mature) soil pH, soil moisture, humidity, altitude, air temperature and light intensity. Juvenile was characterized by the presence of two leaves that were still attached to the stem. While mature was characterized by leaf that its clasps and encircle the stem and fruiting/flowering mature was characterized by the presence of fruit/flower (Nicolson 1969).

Data analysis

The data were analyzed descriptively and tabulated in graphs and tables. The population distribution pattern was defined by standardized Morisita's index to determine how the population was distributed: randomly, uniformly or clumped. The standardize of the Morisita index is used because this index is an improvement of the Morisita index, by placing an absolute scale between -1 and 1. This index is also the best method for measuring the spatial distribution patterns because it does not depend on population density and sample size (Rani 2003). Standardized Morisita index (Ip) was calculated as follows:

When, $I_{d_i} \geq M_{c_i} > 1$, $I_{p_i} = 0.5 + 0.5 \times \left[\frac{I_{di} - M_{c_i}}{n - M_{ci}} \right]$,

When, $M_{c_i} > I_{d_i} \geq 1$, $I_{p_i} = 0.5 \times \left[\frac{I_{di} - 1}{M_{u_i} - 1} \right]$,

When, $1 > I_{d_i} > M_{u_i}$, $I_{p_i} = -0.5 \times \left[\frac{I_{di} - 1}{M_{u_i} - 1} \right]$ and

When, $1 > M_{u_i} > I_{d_i}$, $I_{p_i} = -0.5 + 0.5 \times \left[\frac{I_{di} - M_{ui}}{M_{u_i}} \right]$,

Differences in microhabitat along both tracks were determined by non parametric statistical test, the Mann-Whitney test in SPSS 16, because microhabitat data were found to be uncommonly distributed. A significance value of <0.05 means that the hypothesis that two tracks did not differ significantly is rejected, whereas a significance value of >0.05 means that the hypothesis that two tracks did not differ significantly is accepted.

RESULTS AND DISCUSSION

Results

Aglaonema simplex was found to have green leaves and slightly wavy edges (Figure 2). Eight other Araceae species were also found, namely *Alocasia longiloba, Amorphophallus muelleri, Amorphophallus* sp., *Amorphophallus variabilis, Homalomena* sp., *Rhaphidophora* sp., *Schismatoglottis calyptrata*, and *Scindapsus hederaceus*. Over an elevation of 459 m asl, none of *A. simplex* was found, while *Amorphophallus variabilis* was found at a highest altitude, i.e. at 498 m asl.



Figure 2.A. *Aglaonema simplex* leaf morphology with green leaves and curly edges; B. *A. simplex* inflorescence with cone and white spathe with ripe red fruit

Population structure

Eighteen plots were observed on both tracks with 114 individuals of *A. simplex* in total. The population was predominantly found along the first track (74%), while the remainder was found along the second track. Nevertheless, the average number of individuals per plot on each track did not differ significantly. This is demonstrated by the Mann-Whitney test that showed a significance value of 0.0583> 0.005. Similarly, the population density did not differ significantly (Table 1).

The population was dominated by juveniles (37%) followed by mature plants without fruit (36%) and fruiting mature plants (27%). However, the structure of the population in both tracks was different (Figure 3). Figure 3 shows that the population structure along the first track was dominated by a mature population of plants without fruit, while the structure of the population along the second track was dominated by fruiting mature plants.

Distribution pattern

Standardized Morisita's index values for the first track was 0.50356, so its distribution pattern was clumped, while the standardized Morisita's index along the second track was -0.50296, so *A. simplex* in this track was dispersed uniformly. The distribution along both tracks is shown in Figure 4.

Microhabitat condition

In this study, *A. simplex* was found to live at an altitude of 367-448 m asl., with pH soil 6.7-6.8, soil humidity at 73%-84%, air temperature 27°-28° C, air humidity 80%-86%, with a low light intensity of 170-225 lux (Table 2). The soil texture along the first track was top soil, black color, moist, porous with a little sandy. Soil texture in the second track was dominated by dried compacted soil, slightly clay with red color.

Table 1. Altitude, number of plots, average population and population density of *Aglaonema simplex*

Track	Altitude (m asl)	Plot nos.	Average population (ind. /plot)	Population density
Sumber air	< 400	13	6.46	1.03
Puncak	> 400	5	6	0.96

Table 2. Microhabitat condition of *Aglaonema simplex* in both track

Parameter	Site		Asymp Sig (2-tailed)
	Sumber air (mean + SE)	Puncak (mean + SE)	
Altitude	366.46 ± 2.53	448.2 ± 7.92	0.001
Soil pH	6.73 ± 0.28	6.8 ± 0.07	0.605
Soil humidity	83.46 ± 8.49	73.23 ± 9.67	0.065
Temperature	27.71 ± 0.65	26.9 ± 0.19	0.002
Air humidity	85.95 ± 2.29	79.88 ± 2.39	0.003
Light intensity	170.31 ± 62.89	225 ± 26.96	0.034

Note: This table showed comparison of two tracks. Asymp sig (2 tailed) is a result of Mann Whitney test on both tracks. * significant in α=5%

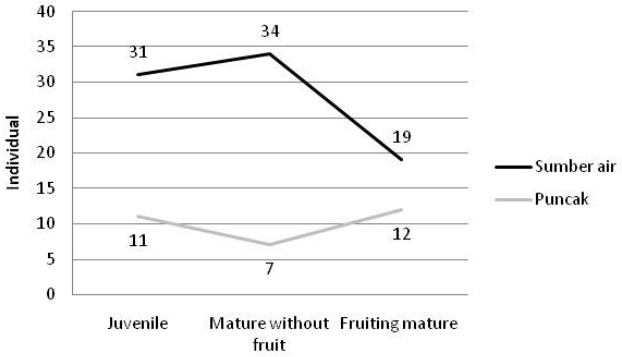


Figure 3. Population structure along both tracks

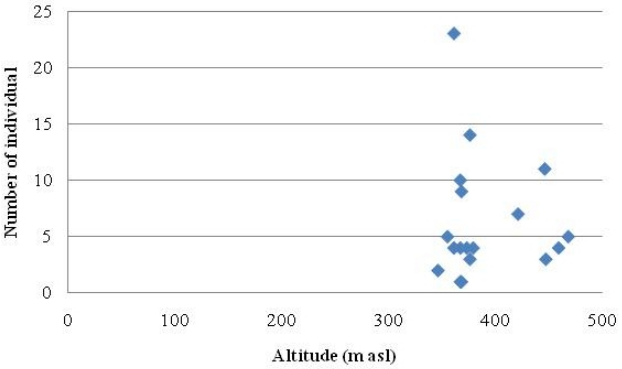


Figure 4. Population distribution of *Aglaonema simplex*

Mann-Whitney test show that there was a significant difference between altitude, humidity and temperature on both tracks, while soil moisture, soil pH and light intensity did not differ significantly.

Discussion

The population was predominantly found along the first track (74%), while the remainder was found along the second track. It is thought to be related to their microhabitat. Budiarto (2006) stated that the important factor for *Aglaonema* growth is light, temperature, humidity and air circulation. *Aglaonema* generally thrives in places with limited lighting, like in the shade of large trees, although it also needs sunlight for photosynthesis to occur. To grow optimally, it needs temperatures of 30° C during the day and 23° C at night with 50-60% humidity (Djojokusuma 2006). This plant also likes porous soil so that air circulates well and water flows smoothly (Antosh 2001). The air temperature along the first track was close to the ideal temperature of *A. simplex* as mentioned above, its light intensity was also lower and its soil was more porous than media at the second track. So, the microhabitat at the first track was better for the growth of *A. simplex* than the second track.

The population was dominated by juveniles. Similar results was obtained by Kusuma and Astuti (2009) that the population was supported by many numbers of juveniles. This condition indicates satisfactory regeneration behavior (Saxena and Singh 1985; Bharati and Prasad 2015).

Figure 3 shows that there were differences in population structure between the two tracks. The number of fruiting mature plants along the second track was more than the number of mature plants without fruit, while along the first track, mature plants without fruit was more than the number of fruiting mature plants. This was due to the different light intensity. The light intensity at the second track was higher (Table 2), enabling a higher amount of photosynthesis to occur, so that *A. simplex* along the second track grow faster and achieve the reproductive stage faster than *A. simplex* along the first track. As Shibao et al (2005) said that photosynthesis and reproduction of *Cyripedium flavum* are affected by light availability in the under-storey, higher photosynthetic rate increases reproductive success and fitness of *C. flavum*. Widiatuti et al. (2004) also said that there was interaction effect of light intensities on relative growth rate of Chrysanthemum.

Plants of the same species may have a different distribution pattern because it depends on other factors in the habitat such as seed predators, seed spreading agent and seedling pathogens (Russo and Augspurger 2004). It also happens to *A. simplex* in Pasatan protected forest. *A. simplex* along the first track was clustered and along the second track was distributed uniformly. Plant distribution is usually affected by the seed spreading agent. Plants distributed in groups usually do not have an agent so seedlings only grow around the parent, while plants randomly distributed usually have agents that enable seeds to grow away from the parent (Howe 1989). Seeds of *A. simplex* are not spread by water (Mansor et al. 2012). Its large size does not allow it to move away so juveniles only grow around the parent. A lot of water and nutrients along the first track encouraged individuals to grow into large groups. Along the second track, some pieces of *A. simplex* seed - broken by a bird were found, so it is suspected that seeds are also spread by birds but not widely dispersed. They did not reproduce in large groups because the supply of water and nutrients along the second track was not as much as the first track. The second track was away from the river and consisted of compacted soil, not as porous as growing media along the first track. This was a limitation for individuals to grow in large groups. This condition supports Kleiner (2016) statement that uniform distribution usually happens because of something that prevents individuals to live in close proximity to each other, such as competition for light, nutrients, and water or because their roots are allelopathic.

Nonparametric statistical test - Mann-Whitney - determined that there was a significant difference between altitude, humidity, and temperature on both tracks, but the average population and the population density did not differ significantly. So, it is assumed that *A. simplex* can tolerate the differences of altitude, humidity and temperature.

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Conformation traits of dairy cattle populations in selected Districts of Northwestern Amhara, Ethiopia

MISGANAW MAMMO[1], KEFYALEW ALEMAYEHU[2,♥], ASAMINEW TASSEW[2]

[1]Department of Animal Science, Debre Markos University, Bure Campus, Ethiopia
[2]Department of Animal Production and Technology, College of Agriculture and Environmental Sciences, Bahir Dar University, Bahir Dar, Ethiopia.
♥email: kefyale@gmail.com

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Abstract. *Mammo M, Alemayehu K, Tassew A. 2017. Conformation traits of dairy cattle populations in selected Districts of Northwestern Amhara, Ethiopia. Biodiversitas 18: 1669-1679.* The study was conducted in three selected districts of northwestern Amhara. The objective was to quantify the characteristics of conformation traits of dairy cattle populations at smallholder farmers' level. 279 cattle keepers were selected for measurement of conformation traits. About 105 indigenous cattle from each district and 42 crossbred female cattle were considered for measurement. SPSS (2007) was used to analyze qualitative data and SAS (2014) for quantitative data. The results revealed that the conformation trait measurements (cm) of indigenous cattle had significant variation ($P< 0.001$) on neck length for dairy type (54.07 ± 4.80) and meat type (51.96 ± 4.05) traits. The width at the point of the shoulder was 7.72 ±1.96, 6.60 ± 1.68 and 5.35 ± 1.61 for meat type, dual type and for dairy type cattle, respectively. A male cattle had significant variation over female except neck length. All qualitative traits were laid intermediate levels (4-6 point) except crossbred which was 63.64 %. About 30% of dairy type cattle had high rear udder depth and 43.33% dairy type cattle had strong central ligament. Crossbred cattle had 42.1% sickled rear leg. In general, about 57.5% dairy type cattle and 95.2% crossbred cattle have very angular shape as 75% of meat type cattle lack angularity. From this research, it could be concluded that there were significant differences of conformation traits between dairy type and beef type cattle from the cattle population considered in the study for developing specialized dairy or beef breed types. As well as indigenous dairy type cattle are comparable to other known dairy cattle breeds like Kanan, Fogera and Barka. However, farmers did not exercise in developing product specific and specialized breeds with their corresponding production system than multipurpose. Thus, selection and appropriate planed breeding program is crucial for future dairy cattle genetic improvement to match genotype and environment and meet producers' production objectives. Hence, conformation traits can be a vital tool to make decisions on cattle selection and breed improvement.

Keywords: Cattle types, conformation traits, breed types, breed performance

INTRODUCTION

In order to ensure proper conservation and utilization of indigenous breeds, it is necessary to evaluate genetic variations that exist within and among breeds. A large proportion of indigenous livestock populations in the developing world have yet to be characterized or evaluated at phenotypic and genetic levels (Hanotte and Jianlin 2005). Most existing classification of livestock breeds in Africa is based on historical, anthropological and morphological evidence, which are most often not enough for the purpose of conservation (Mwacharo et al. 2006).

Ethiopia has one of the largest livestock inventories in Africa with a national herd estimated at 57.83 million cattle's consisted of local breeds (98.59%, crossbred (1.22%), and exotic breed (0.19). The country has also 28.89 million sheep, 29.70 million goats and 10.37 million pack animals (CSA 2015/2016). Ethiopia is endowed with the highest cattle populations in Africa which serve as one of the most important sources of food and cash income. Cattle production system in Ethiopia is mainly smallholder substance farming, with animals having multipurpose use and as such, no specialized and systematic breeding is used (Tadesse et al. 2014).

Indigenous cattle populations were divided into three groups: dairy type, dual type and meat type due to great variability of conformation traits. Especially cattle have developed traits like long neck and sharp width at point of shoulder has characterized under dairy type and cattle develop wide shoulder, thick and short neck characterized under meet type.Variation of conformation traits among indigenous cattle population is great opportunity for selection and genetic improvement for the future. similarly Bahashwan (2014) reported that, morphometric traits measurements is conducted for characterize breeds of animals and can be an essential tool for the program selection and breed improvement. Because of low productivity, adapted indigenous breeds are subjected to crossbreeding with imported dairy cattle like Holstein and Jersey.Mistakes in breeding program have missed the opportunity to improve and preserve the indigenous breed. Similarly, Tesfa et al. (2016) reported that, Fogera cattle breed is well known for its milk production but, currently its productivity is declined because of different problems like genetic admixture with uncharacterized local breeds and inbreeding, shift of production system, absence of well-defined breeding programs, poor selection strategy and genetic gain.

The role of conformation traits an indigenous dairy cattle improvement program is not well described Northwestern Amhara, West Gojjam administrative zone is one of administration zone which has high cattle population (471,816 cows of 1648 cross and 595,075 oxen) potential and suitable weather conditions for cattle production from Amhara regional state. Lack of proper characterization of conformation traits, identification, valuation, and maintenance of different traits of cattle and trait preference and identification of farmer were the main problems in the area. The quantification of conformation traits like udder related conformation traits had paramount importance in identifying dairy type traits. Since none of the conformation traits data for indigenous cattle types has previously been studied, in the present we analyzed conformation traits measurements to characterize this cattle types.Therefore, the objective of this paper was to undertake characterization of conformation traits for identifying and developing dairy type cattle from the indigenous cattle population.

MATERIALS AND METHODS

Description of the study areas

The research was done in three selected districts (Debecha, Quarit and Finote Selam) of West Gojjam administrative zones of western Amhara located at 300 km (Debecha), 427 km (Quarit), and 385 km (Finote Selam) from Addis Ababa (Figure 1, Table 1).



Figure 1. Map of the study areas in three selected districts of West Gojjam, Amhara, Ethiopia. Note: 1. Quarit, 2. Finote Selam, and 3. Debecha

Table 1. Geographic coordinates of the sampling location and number of livestock populations in 2016

District	Altitude (a.s.l)	Temp. (°C)	Annual rainfall (mm)	Cattle populations (head)		Other livestock's (head)	
				Indigenous	Cross	Sheep	Goat
Quarit	1920-3000	16-25	1250	293, 118	-	84,245	119,926
Finote Selam	1862	14-32	1250	7821	-	2580	1403
Debecha	2080	18-24	1006	133052	175	45763	12714

Note: District Agricultural Offices, 2015

Sampling technique and data collection procedures

Before sampling, series of discussions were made with the Zonal and District livestock health and marketing agency experts in order to have information on the infrastructure, cattle and its distribution in the areas. The first stage involved selected three districts purposively based on cattle population. The district agricultural development offices and livestock development and health agencies were contacted for brief discussion on traditionally recognized indigenous cattle types. The distribution of households in the districts and selection of sample were done together with experts and development workers. A total of 279 cattle keepers were interviewed for the study (93 from each district). A multistage sampling technique was employed to gather the required information on conformation traits for dairy and meat type characterization.

Morphological measurements were taken using graduated measuring tape in full. Body measurements were made on adult cattle of both sexes, which were selected based on dentition technique. Because farmers often did not have birth records, age was estimated by examining each animal's teeth as suggested by Pace and Wakeman (2003), besides of asking cattle owners. Only animals with an eruption of the fourth pair of teeth, indicating maturity, were included in the study. All measurements were recorded after humanely restraining the randomly selected animals. Veerkamp et al. (2002), subjective visual assessment of animals by classifiers is undertaken for several different purposes in livestock.

The linear conformation traits were measured in centimeter (cm) using graduated measuring stick and flexible tape. A separate form was prepared for each animal measured. Visual observations were made and morphological features were recorded based on cattle morphological characteristics descriptor list of FAO (2012), and International Committee for Animal Recording (ICAR 2015), breed descriptor list. Traits for both sexes had been calculated with Angularity (ANG (number of finger between last ribs and point given. Traits for both sexes had been calculated with Angularity (ANG (number of finger between last ribs and point given for angularity)), Rump angle (RA), Rear legs set (RLS) and 6 udder measurement traits Udder Depth (UD), Rear Udder Height (RUH), Central Ligament (CL), Rear Teat Placement (RTP), Front teat placement (view from behind) (FTP), Fore Udder Attachment) (FUA)) were female at milking stage. All above measurements were classified on a scale of 1 to 9. Traits on the shape and appearance of the animals were recorded in pre-coded format. Some photos were taken on matured animals for ease of comparison and description with other standard cattle types.

Before taken measurement, indigenous cattle were visually assessed by types (done by researcher). All measurements were recorded after humanely restraining the randomly selected animals. All measurements were carried out by the same person in order to avoid between individual variations; a separate form was prepared for each animal.

Body length (BL), Weather height (WH), Stature (ST), Width at point of shoulder (WAS), Navel flap length (NvF), Body depth (BD), scrotum length (SL), Perpetual sheath width (PSL), scrotum circumference (SC) and neck length were measured using a flexible centimeter while a heart girth meter was used. The definitions of conformation traits are indicated in Table 2.

According to emphasize of autochthonous traits indigenous cattle were classified in three groups: dairy type, dual type, meat type.

Data management and analysis

The data was collected from 357 cattle (315 indigenous,53 male and 262 female and 42 cross female) cattle. Indigenous cattle were grouped into three (123 dairy type, 111 dual types, and 81 meat types) cattle on the basis of variables and visual judgments of conformations traits. Categorizations were also done in cattle type, district, and sex. Before analysis, the data was encoded and filled in Microsoft Excel as per the categories. Data was analyzed according to the statistical package with the General Linear Model (GLM) procedure of SAS (SAS 9.1.3), in Duncan multiple range test and the qualitative phenotypic data and information from the questionnaire were analyzed using Statistical Package for Social Sciences SPSS (2007).

The quantitative and qualitative data were analyzed for both sexes separately to avoid confounding effect due to sex and each cattle type to see their variation. General Linear Model (GLM) procedure of SAS (9.1.3) was used for metric data to differentiate between location and cattle type (with cross, dairy, dual and meat type cattle) and correlation analysis.

Significant differences in means were separated using Duncan's Multiple Range Test. The model used for GLM analysis was:

$$Yijkl = \mu + Nj + Sk + Bl + SKxBL + Eijkl$$

Yijkl = Observed value of the trait of interest
μ = over all mean
Nj= the effect of j^{th} sex (male and female)

Table 2. Definitions of conformation traits

Traits		Specifications
Body length	BL	The distance between point of shoulder and the pin bone
Neck length	NL	Proximal end of cervical region to the distal end of the cranial region
Heart girth	HG	Circumference of the body behind the base of the hump and just behind the front legs
Height at withers	-	The vertical distance between ground and the point of wither
Rump width	RW	Width between the two pins
Perpetual sheath width	PSL	Widest part vertically from the base of the abdomen
Teat length	-	From the base of the udder to the point of the teat
Rump Angle	RA	pins high/low) is the degree of slope from hooks to pins when the cow is standing.
Rear Legs Sets	RLS	posty/sickle) is a measurement of the set of hock. A score of 1 indicates extremely posty legs and a score of 9 indicates sickle hocked legs.
Fore Udder Attachment	FUA	is an evaluation of the strength of attachment of the fore udder to the body wall.
Rear Udder Height	RUH	is measured as viewed from the rear, at the crease where the udder meets the leg, in relation to the midpoint between the points of hock and pins.
Central ligament	CL	is a measure of the depth of cleft of the udder between the rear teats from the base of the rear teats to the point where the halves of the udder come together.
Udder depth (deep/shallow):	UD	is a measure of the depth of udder floor relative to the hock.
Front Teat Placement (wide/close):	FTP	is a measurement of the placement of the front teat on the quarter



Figure 2. Morphological body measurements in the field. Note: A=HG, B=RH, C=RL, D=WH, E= NL, F=CW, G= SL, H= PSL, I= RW, J= BD, K= BL

SK = the effect of k^{th} agroecology /districts (Quarit, Finote Selam and Denbcha)

BL = then effect of l^{th} breed (dairy type, dual type, meat type and cross)

SKxBL= interaction effects of breed with agroecology

Ejkl = the random residual error

RESULTS AND DISCUSSION

Conformation traits characterization

In this research, the neck length (NL) of dairy type had significant variation (P<0.01) than meat type and dual purpose type cattle (Table 3 and Figure 3). Neck length of dairy type was higher than Kanan cattle 43.04 ± 0.43 (Aamir et al. 2010) since Kanana cattle is dairy cattle in Northern Sudan as reported by Rege (1999) and Bagit/ Barka cattle 47.14 ± 0.09 (Ftiwi 2015). The length and thin neck is an indicator of angularity a common conformation of ideal dairy type. The short and thick neck is an indicator of rectangularity a common conformation of meat type/beef cattle. Long, lean and blending smoothly into the shoulder, clean cut throat is an excellent dairy character (ICAR 2015). Dairy form is an important indicator of cow's ability to mobilize energy (fat reserves) to milk (Tsuruta 2005).

Dairy type cattle had significant variation (P<0.01) on BD than dual and meat types in female populations (Table 3). It was an indicator of the wide hind quarter and more angularity in dairy-type than others. Crossbred cattle had significant variation (94.38±7.91, P<0.001) than all the rest of indigenous cattle. The measurement of BL in Fogera cattle was similar to dairy types and dual type cattle in the study area but lower than meat type cattle. Fogera breed is considered as a high milk producer compared to other indigenous breeds of Ethiopia (Anteneh et al. 2010; Tesfa et al. 2016). The measurement of BL in this result was accordance with (Khalid 2011) who reported that BL of female Kanana cattle was 87.29 ± 1.92 cm as Kanana cattle is dairy cattle in Northern Sudan (Rege 1999). The measurement of WH of dairy cattle had higher value than Sheko cattle (101.5 cm) and Kanan cattle (112.67 ± 1.07 cm) as reported by Khalid (2011).

There was higher significant variation (P<0.0001) of wider at the point of shoulder (WAS) in meat type than the rest of cattle types (Figure 4). But there was no significant variation of WAS between dairy type and crossbred cattle. Narrowness WAS (excellent sharpness over the withers) is a common conformation of ideal dairy cattle. Wideness (thick over the withers) of WAS is a common conformation of ideal meat/ beef cattle. As indicated by ICAR (2015), sharpness over the withers leads more (dairyness,



Figure 3. Indigenous dairy type cow at Debecha (A), dual type cow at Finote Selam (B) and meat type cow at Debecha (C)



Figure 4. Crossbred (A) and indigenous dairy cattle (B) in Debecha, dual type cattle (C) and meat type cattle (D) in Finote Selam(The arrow shown the sharpness of WAS)

Table 3. Conformation traits measurement of indigenous cattle populations

Effect and Level		ST (cm)	CW (cm)	NL (cm)	WAS (cm)	BD (cm)	BL (cm)	WH(cm)	HG(cm)	RW(cm)	RL(cm)
	N	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Over all		119.17±5.36	18.68±2.52	53.24±4.77	6.40±1.97	86.65±7.13	116.56±8.53	115.94±8.12	149.61±13.88	9.76±1.49	36.62±2.85
Cattle type		***	NS	**	***	NS	**	**	**	NS	**
Dairy	123	$119.04±5.44^{B}$	$18.35±2.40^{A}$	$54.07±4.80^{A}$	$5.35±1.61^{C}$	$87.46±6.79^{A}$	$115.14±5.94^{B}$	116.20±5.28B	$146.80±14.44^{B}$	$9.98±1.41^{A}$	$37.00 ±2.99^{A}$
Dual	111	$117.33±5.42^{C}$	$18.53±2.53^{A}$	$53.25±5.06^{AB}$	$6.60±1.68^{B}$	$85.95±7.34^{A}$	$115.52±8.01^{B}$	$113.421± 11.16^{C}$	$147.57±14.03^{B}$	$9.86±1.64^{A}$	$35.84±2.60^{B}$
Meat	81	$121.88±3.92^{A}$	$19.37±2.57^{A}$	$51.96±4.05^{B}$	$7.72±1.96^{A}$	$86.37±7.29^{A}$	$120.12±11.23^{A}$	$118.99±5.15^{A}$	$156.68±9.92^{A}$	$9.30±1.29^{A}$	$37.12±2.76^{A}$
District		**	**	NS	*	**	NS	NS	**	***	***
Finote Selam	105	$119.61±5.76^{A}$	$18.67±2.73^{A}$	$52.49±5.74^{A}$	$6.14±2.17^{B}$	$85.83±7.65^{B}$	$116.81±9.93^{A}$	$116.44±4.69^{A}$	$147.51±19.24^{B}$	$10.62±1.5^{A}$	$35.24±3.35^{B}$
Debecha	105	$120.21±4.54^{A}$	$19.29±2.45^{A}$	$53.37±4.07^{A}$	$6.94±2.09^{A}$	$88.85±6.93^{A}$	$116.58±8.25^{A}$	$116.14±11.15^{A}$	$153.67±10.68^{A}$	$9.19±1.29^{C}$	$37.06±2.39^{A}$
Quarit	105	$117.60±5.54^{B}$	$17.99±2.22^{B}$	$53.77±4.50^{A}$	$6.03±1.46^{B}$	$84.92±6.22^{B}$	$116.30±7.48^{A}$	$115.27±6.33^{A}$	$146.94±9.71^{B}$	$9.63±1.25^{B}$	$37.37±2.39^{A}$
Sex		***	**	NS	***	***	***	***	***	***	***
M	53	124.40±4.92A	19.87±2.27A	53.96±4.45A	7.87±2.23A	93.32±6.29A	$123.64±6.58^{A}$	$121.94±5.74^{A}$	$160.43±14.18^{A}$	$8.89±1.72^{B}$	$39.13±2.56^{A}$
F	262	118.11±4.81B	18.44±2.50B	53.09±4.83A	6.10±1.77B	85.30±6.51B	$115.12±8.16^{B}$	$114.73±7.99^{B}$	$147.42±12.77^{B}$	$9.94±1.37^{A}$	$36.11±2.63^{B}$

Note: ST= stature, CW chest width, NL= neck length, WAS= width at point of shoulder, BD= body depth, BL= body length, WH weather height, HG= heart girth, ANG =angularity, pt= point given, No.F= number of finger, RW= rump width, RL= rump length; M=male. F=female. Means with the same letter within the same column are not significant. * Significant at 0.05level= p 0.05, ** significant at 0.01 level = p 0.01 and *** significant at 0.001 level = P 0.001; NS=not significant and SD= Standard deviation.

angularity, milkier appearance), longer and linear neck, less excessive conditions. Refer to Tsuruta (2005), physical characteristics openness of the ribs, sharpness of bones, the thinness of the tail and thigh are all indicators of the cow's ability to make milk. Data from ICAR (2015) noted that cow wideness on WAS lacks in dairy character; utilizes feed to accumulate meat or fat rather than produce milk. The round of beef cattle should be deep and wide, with the widest portion about midway between the tail head and hock. Wide shoulders are the major indicator of muscularity; the wider the shoulders must be a balance, maximum meat on the rump. Comparative measurements of conformation traits can provide evidence of breed relationships and size (Abdulmojeed et al. 2010).

Conformation traits which were significant variation between each cattle type indicated that each cattle type had a distinct feature. A few of conformation traits had no significant variation also indicated that genetic admixture between cattle type; since our breeding system is traditional and natural selection. Alderson (1999), conformation trait assessment is only one factor in selection procedures. Alphonsus et al. (2012) also indicated that the significant effect of sire on the body conformation traits indicates that appropriate breeding plan that will ensure the use of proven sire in the dairy herd combined with good mating system will yield good results.

The overall results of conformation traits measurement of crossbred cattle were a higher significant value ($P<0.001$) over indigenous cattle but the lower value ($P<0.001$) on WAS. The small body size of indigenous cattle might be the result of natural selection to reduce the sensitivity to fodder shortage in harsher environments, thus its high adaptive capacity to harsher environmental conditions and inbreeding effect. Tesfa et al. (2016) reported that, Fogera cattle breed is declined because of different problems like genetic admixture with uncharacterized local breeds and inbreeding, shift of production system, absence of well-defined breeding programs, poor selection strategy and genetic gain. In the former time there were high cattle population in the area due to large grazing area and availability of feeds as well as enough breeding bulls, but now a day cattle population and breeding bulls are reduced (personal communication).

Conformation traits measurements are essential for selection and identifications of cattle in their types since body conformation traits have high heritability traits. Conformation traits classification is an important tool in decision making as it focuses on the selection of animals that should have a longer herd life, expressing their productive and reproductive potential based on their morphologic traits (Elisandra et al. 2014).

The district had a significant effect ($P<0.05$) on ST, CW, BD, HG, RW, RL and NvL. But there was no significant effect on NL, BL and WH in female cattle populations (Table 3). The variation was lower in male cattle populations than female in each trait. It might be due to farmers gave higher attention due to management and feeding for his male cattle started from a younger age to replacement oxen and could express its biological development than female.

Sex had a highly significant effect on most of the linear body measurement. As expected conformation traits measurements in males were higher than those in the females except NL (Table 3). Different reports revealed that the mean values of indigenous males are usually greater than their counter female groups (Taye 2005; Garoma 2006).

There was no significant difference of PSW in both effects. The district had a significant effect ($P<0.001$) on SL and ($P<0.01$) on SC. Cattle type had no significant effect on SL but was significant effect on SC ($P<0.01$). Dairy type cattle had a higher value than dual, and meat type cattle on SC and can be used as an indicator of a cow is developing good udder circumference born from such a bull.

Dairy type conformation traits

The overall result of most frequent udder conformations was observed at intermediate levels (4-6 points). The average strong fixed udder was found 3.46% of the populations. A comparable result was indicated by Aamir et al. (2010), a well strong fixed udder was found in 8.0% of the samples studied in Sudanese Kenana Cattle.

Over all udder conformations found at intermediate level except low RUH in crossbred cattle and broken CL in meat type cattle. Almost all the udder related traits were laid an intermediate level in all districts.

The overall results of udder conformations were listed in Table (5 and 6) and Figure 5. A high percentage of loose FUA was found in crossbred cattle (50%) than dairy type cattle (1.67%). Loose and weak FUA was an indicator of poor udder conformation. But indigenous cattle had better (90% intermediate and 8.33% extremely strong in dairy-type; 100% intermediate both dual and meat type FUA. Deep UD was found in dairy-type cattle breed (8.33 than crossbred cattle (4.55%) meat (3.33%) and dual type creed (2.38%). A research found by Berry et al. (2004) indicated that there is a strong genetic correlation between UD and FUA (0.92). Cows with shallow udders possessed tighter fore-udder attachments. Mavrogenis et al. (1988) also explained that the heritability of udder circumference and udder depth was moderate to high suggesting that selection should be effective.

A high percent of RUH was laid an intermediate level >68% for indigenous cattle and 31.82 % in crossbred cattle. About 63.64% of crossbred cattle were laid low RUH but not more than 5% of indigenous cows. A high percent of CL was possessed by dairy cattle which is an indicator of cows with good teat placement. Strong CL was found in dairy-type cow 43.33% than the rest (dual type 21.43%, meat type 4.17% and cross cow 0%). Selection of cow which had good central ligaments will be possible to improve rear teat placement and teat length indirectly. Milerski et al. (2006) reported that a low correlation was found between udder attachment and central ligament ($r= 0.29$) on the contrary low negative correlation ($r=-0.33$) between central ligament and udder attachment in Tsigai dairy sheep.

Table 5. Number of cattle (head) according to udder traits description of indigenous female cattle based on judging method (point except TL(cm))

Phenotypic variance	District			Overall
	Debecha N(%)	Finote Selam N(%)	Quarit N(%)	
Fore udder attachment (FUA)				
Weak and loose (1-3)	0(0)	0	1 (2.13)	1(0.71)
Intermediate (4-6)	29(100)	48 (96)	43 (91.49)	120 (95.83)
Extremely strong and tight (7-9)	0	2 (4)	3 (6.38)	5(3.46)
Teat length (TL)				
Short (2-4)	13(44.83)	11(22)	17 (36.17)	41(34.33)
Medium (5-7)	14(48.28)	32(64)	28 (59.57)	74 (57.28)
Long (8-10)	2(6.89)	7(14)	2 (4.26)	11(8.38)
Udder Depth (UD)				
Deep (1-3)	2(6.89)	1 (2)	2 (4.26)	5(4.38)
Intermediate(4-6)	25(86.21)	39 (78)	44 (93.61)	108 (85.94)
Shallow(7-9)	2(6.89)	10 (20)	1 (2.13)	13(9.67)
Rear Udder Height (RUH)				
Low (1-3)	2(6.90)	2(4)	0	4(3.63)
Intermediate (4-6)	23(79.31)	41(82)	39(82.98)	103(81.43)
High(7-9)	4(13.79)	8(14)	8(17.02)	20(14.77)
Central Ligament (CL)				
broken ligament (1-3)	9(31.03)	8(16)	14(29.79)	31(25.60)
Intermediate (4-5)	15(51.72)	22(44)	22(46.81)	59(47.51)
Deep cleft/strong ligaments (7-9)	5(17.24)	20(40)	11(23.40)	35(26.88)
Rear Teat Placement (RTP)				
Outside of quarter (1-3)	5(17.24)	5(10)	11(23.40)	20(16.88)
Intermediate (4-6)	21(72.41)	33(66)	33(70.21)	87(69.55)
Inside of quarter (7-9)	3(10.35)	12(24)	3(6.38)	18(13.57)
Front teat placement (view from behind) (FTP)				
Outside of quarter (1-3)	0	4(8)	0	4(2.66)
Intermediate (4-6)	27(93.10)	37(74)	35(74.47)	99(80.52)
Inside of quarter (7-9)	2(6.90)	9(18)	12 (25.53)	23(16.81)

Note: N: number of observation

Table 6. Number of cattle (head) according to udder traits description based on judging method of FAO (2012) and International Committee for Animal Recording (ICAR, 2015) conducted by researcher

Phenotypic variance	Cattle Type				Overall
	Dairy (%)	Dual (%)	Meat (%)	Cross (%)	
For udder attachment (FUA)					
Weak and loose (1-3)	1(1.67)	0	0	11(50)	12(20.23)
Intermediate (4-6)	54 (90)	42(100)	24(100)	11(50)	131 (85)
Extremely strong and tight (7-9)	5(8.33)	0	0	0(0)	5(2.08)
Udder Depth (UD)					
Deep (1-3)	2(8.33)	1(2.38)	2(3.33)	1(4.55)	6(4.65)
Intermediate(4-6)	21(87.50)	40(95.24)	47(78.33)	13(59.09)	121(77.79)
Shallow(7-9)	1(4.17)	1(2.38)	11(18.67)	8(36.36)	21(15.40)
Rear Udder Height (RUH)					
Low (1-3)	1(1.67)	2(4.76)	1(4.17)	14(63.64)	18(18.49)
Intermediate (4-6)	41(68.33)	35(83.33)	16(66.67)	7(31.82)	110(67.12)
High (7-9)	18(30.00)	5(11.90)	7(29.17)	1(4.54)	22(14.45)
Central Ligament (CL)					
Convex to flat floor (flat), broken ligament (1-3)	2(3.33)	14(33.33)	15(62.50)	6(27.27)	35(31.61)
Intermediate (4-5)	32(53.33)	19(45.24)	8(33.33)	16(72.73)	75(51.16)
Deep cleft/strong ligaments (7-9)	26(43.33)	9(21.43)	1(4.17)	0(0)	36(17.23)
Rear Teat Placement (RTP)					
Outside of quarter (1-3)	5(8.33)	16(38.10)	0	2(9.09)	23(13.88)
Intermediate (4-6)	44(73.33)	19(45.24)	24(100)	17(77.27)	104(73.96)
Inside of quarter (7-9)	11(18.33)	7(16.66)	0	3(13.64)	21(12.16)
Front teat placement (view from behind) (FTP)					
Outside of quarter (1-3)	2(3.33)	1(2.38)	1(4.16)	3(13.64)	7(5.88)
Intermediate (4-6)	48(80.00)	32(76.19)	19(79.17)	17(77.27)	116(78.29)
Inside of quarter (7-9)	10(16.67)	9(21.43)	4(16.67)	2(9.09)	25(15.90)



Figure 5. Indigenous and cross cows udder conformation. Note: (i) Dairy types cows (A, B, C) at Debecha, (ii) Cross cows (D, E, F) at Finote Selam, (iii) Dual type cows (G, H) at Finote Selam and Quarit, (iv) Meat typecows (I, J) at Quarit and Debecha



Figure 6. Sickle leg and high pin cross cow (A, B), and normal leg and rump position indigenous cow (C)

Table 7. Number of cattle (head) according to leg, angularity and rump structure traits based on judging method of FAO (2012) and International Committee for Animal Recording (ICAR 2015) conducted by researcher

Parameter	Cattle type				District		
	Dairy	Dual	Meat	Cross	Debecha	Quarit	Finote Selam
Angularity							
Point given	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Lacks angularity: close ribs, coarse bone (1-3)	0	20 (20.2)	43 (75.4)	1 (2.4)	22 (20.8)	19 (19.2)	26 (31.7)
Intermediate: with open rib (4-6)	45(42.5)	75(75.8)	14(24.6)	1 (2.4)	49(46.2)	66(66.7)	29 (35.4)
Very angular: open ribbed flat bone (7-9)	61(57.5)	4 (4.0)	0	40(95.2)	35(33.0)	14(14.1)	27 (32.9)
Number of fingers							
narrow 1-3=1-1.5	7 (6.6)	9 (9.1)	13(22.8)	6 (14.3)	19(17.9)	3 (3.0)	8 (9.8)
intermediate 4-6=2-2.5	58(54.7)	66(66.7)	31(54.4)	22(52.4)	69(65.1)	52(52.5)	50 (61.0)
wide 7-8= 3-3.5	41(38.7)	24(24.2)	13(22.8)	13.2(33.3	18(17.0)	44(44.4)	24 (29.3)
Rump angle							
High Pins (1-3)	1 (.9)	0	0	3 (7.1)	0	1 (1.0)	0
Intermediate(4-6)	95(89.6)	95(96.0)	55(96.5)	34 (81.0)	101(95.3	91(91.9)	75 (91.5)
Extreme slope(7-9)	10 (9.4)	4 (4.0)	2 (3.5)	5 (11.9)	5 (4.7)	7 (7.1)	7 (8.5)
Rear leg seats							
Straight (1-3)	1 (.9)	0	0	0	0	1 (1.0)	0
Intermediate(4-6)	97(91.5)	93(93.9)	57(100.0	18 (42.9)	103(97.2	88(88.9)	80 (97.6)
Sickled (7-9)	8 (7.5)	6 (6.1)	0	24 (57.1)	3 (2.8)	10(10.1)	2 (2.4)
Naval flap length							
Absent (0-0.5)	6 (5.7)	4 (4.0)	1 (1.8)	28 (66.7)	7 (6.6)	1 (1.0)	6 (7.3)
Small (1-4.5)	75(70.8)	69(69.7)	43(75.4)	10 (23.8)	78(73.6)	74(74.7)	45 (54.9)
Medium (5-8)	21(19.8)	19(19.2)	13(22.8)	3 (7.1)	17(16.0)	22(22.2)	22 (26.8)
Large (9-13)	4 (3.8)	7 (7.1)	()	1 (2.4)	4(3.8)	2 (2.0)	9 (11.0)

Note: Angularity (ANG), Rump angle (RA), Rear leg sets (RLS)

The overall results of angularity about 57.5% very angular in dairy type, about 75% intermediate in dual type, about 75.4% lack angularity in meat type and about 95.2% very angular in crossbred cattle (Table 6,7 and Figure 3). In respect to RLS about 91.5%, 93.9%, and 100% were laid at the intermediate level dairy, dual and meat type cattle, respectively (Table 7). But 57.1% of crossbred was sickle legs (Figure 4.A and B).Farmers stated that, sickle legs were difficult to walk in long distance in fragile land for forage and to perform draught work, especially in rural community. Similarly to the current findings, Zavadilová et al. (2009) reported that, feet and legs are potential indicators of a cow's longevity. Crossbred cattle had short longevity and not persist suitable for rural community work for trashing, plowing and discover long distance for forage and water in the field. Wiggans et al. (2010) reported that, RLS were importance for locomotion and longevity. Bad leg conformation strongly affects animal welfare, and feet defects lead to diseases and lameness. Similarly Gordon et al. (2008), locomotion is a qualitative observation of a cow's ability to walk normally to identify functionality Canadian Holstein.

All conformation traits were found at the intermediate point in all districts even if the percentage varied. So district had no such big effect on the above qualitative traits.

In conclusion, this research investigated the existence of three main cattle types: dairy, dual and meat types based on the conformational characterization of indigenous cattle. Most of the conformation traits were significantly different ($p<0.01$) especially NL, WH, WAS and BL among cattle types. Most of the udder conformation traits were found an intermediate level of in dairy-type cattle but low RUH and broken CL in a crossbred cow. Over 50% of RLS was sickle in crossbred cattle but intermediate in dairy-type cattle. Dairy type cattle had distinct traits from the rest of indigenous cattle. The conformational traits data obtained in this study are vital and valuable in management and useful tool in future strategies and should be well utilized to improve the performance of indigenous dairy and meet type cattle. Hence, establish adequate selection, and breeding plan can help to exploit their traits for the future dairy cattle genetic improvements. However, farmers did not select cattle for a specific purpose rather keep for multipurpose.

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Impact of climate change on potential distribution of xero-epiphytic selaginellas (*Selaginella involvens* and *S. repanda*) in Southeast Asia

AHMAD DWI SETYAWAN[1,2,♥], JATNA SUPRIATNA[1], DEDY DARNAEDI[3], ROKHMATULOH[4], SUTARNO[2], SUGIYARTO[2], ILYAS NURSAMSI[5,6], WULAN RATNA KOMALA[5], PRAKASH PRADAN[7]

[1]Program of Conservation Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Depok 16424, West Java, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: volatileoils@gmail.com
[3]Division of Botany, Research Center for Biology, Indonesian Institute of Sciences, Cibinong-Bogor 16911, Indonesia
[4]Department of Geography, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Depok 16424, West Java, Indonesia
[5]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jatinangor, Sumedang 45363, West Java, Indonesia
[6]Bird Conservation Society (BICONS). Bandung 40184, West Java, Indonesia
[7]West Bengal Biodiversity Board, Department of Environment, Government of West Bengal, Salt Lake, Sector-III, FD415A, Poura Bhawan, 4th Floor, Kolkata, West Bengal, India

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Abstract. *Setyawan AD, Supriatna J, Darnaedi D, Rokhmatuloh, Sutarno, Sugiyarto, Nursamsi I, Komala WR, Pradan P. 2017. Impact of climate change on potential distribution of xero-epiphytic selaginellas (*Selaginella involvens *and* S. repanda*) in Southeast Asia. Biodiversitas 18: 1680-1695.* Climate change is one of the greatest challenges for all life on earth, as it may become the dominant driver of changes in ecosystem services and biodiversity loss at the global level. *Selaginella* is a group of spike-mosses that seem easily affected by global warming (climate change) due to requiring water medium for fertilization. However, some species have been adapted to dry condition and may grow as epiphytes, such as *S. involvens* and *S. repanda*. Both species are commonly found in opposing a range of elevation. *S. involvens* is often found in high-altitude regions, whereas *S. repanda* is often found at lower-altitude regions. The difference in this altitudinal distributions is expected to limit redistribution mechanism of each species to adapt the climate change projections. This study model examines the potential geographic distribution of *S. involvens* and *S. repanda* under current climatic conditions and models the impact of projected climate change on their potential distribution. Future climate predictions are made with four detailed bioclimatic scenarios (i.e. RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5) and three-time intervals (2030, 2050, 2080), which combine various climatic factors. In this modeling, it can be concluded that *S. involvens* and *S. repanda* can adapt to future climate change, and continue to be sustainable, although it is strongly influenced and shifting habitat distribution in some areas.

Keywords: Climate change, *Selaginella involvens*, *Selaginella repanda*, Southeast Asia, potential distribution, xerophytic

INTRODUCTION

Current trend on climate change is recognized to be one of the greatest challenges for all life on earth (Fitzpatrick et al. 2008). Intergovernmental Panel on Climate Change (IPCC) developed scenarios which project a further increase in global mean surface temperature of 2.6-4.8°C above pre-industrial levels by the year 2100, spatial and temporal changes in precipitation patterns, and increased incidence of floods and droughts (IPCC 2014). The negative impact of climate change, if combined with continuous environmentally destructive human activities, will become more intense and frequent. The debate of potential ecological impact of ongoing climate change has intensified over the last decade (e.g. Gilman et al. 2010; Pereira et al. 2010; Salamin et al. 2010; Beaumont et al. 2011; Dawson et al. 2011; McMahon et al. 2011; Alice et al. 2012; Bellard et al. 2012; Belgacem and Louhaichi 2013). It has been forecasted that climate change and its impacts may become the dominant driver of changes in ecosystem services and biodiversity loss at the global level (Deb et al. 2017). In the context of understanding the ecological responses to climate change, it may be more relevant to understand the changes in region which are spatially heterogeneous rather than the approximated global averages (Walther et al. 2003; Bonebrake and Mastrandrea 2010). There are five global climate domains in the world: i.e., tropical, subtropical, temperate, boreal, and polar (FAO 2000, 2012). Among all of these global climate domains, the tropical biome has the highest rate of forest degradation and destruction (Morris 2010; Hansen et al. 2013; Deb et al. 2017). Therefore, it becomes very important to study the biodiversity-climate interaction in highly modified tropical landscapes (Wilson et al. 2016).

One of the most vulnerable areas to the impact of climate change is Southeast Asia (SEA), due to its large and growing population, the economic importance of its agricultural sector, its long coastlines and low-lying areas, and its high dependence on natural resources for development (NIC 2009). The climate of Southeast Asia is monsoonal with summer-dominant rainfall and a large-scale seasonal reversal of the wind regimes (Loo et al. 2015). A significant increase of mean temperature, acceleration of annual rainfall, and extreme climate events

such as drought, floods, and cyclones are several projected negative impacts of climate change in SEA (IPCC 2014; Loo et al. 2015). Frequent changes and shifts in monsoon precipitation up to 70% below normal level, and the delayed of monsoon by up to 15 days are also projected as the effects of the predicted increase in mean temperature (Schewe and Levermann 2012). Along with unsustainable utilization of natural resources, climate change is believed to negatively affects the current plant diversity patterns. These threats are expected to lead to reduced plants biodiversity, low emergence of annual species, the life cycle of plants, and changes in phenology and the timing of reproduction (Thuiller et al. 2008; Belgacem et al. 2008; Hilbish et al. 2010; Hill and Preston 2015).

Among the biotic components of vegetation, spike-mosses are one of the earliest groups to be affected by climate change, through the change in their response to climate, distribution, and phenology. The gametophyte generation of spore-bearing vascular plants such as spike-mosses is highly dependent on wet environment (Bellard et al. 2012). Therefore, the disruption of spike-mosses life cycle is also being one of negative effect of climate change. Although plants generally respond slowly to climate change, the short lifespans of spike-mosses will likely make them adapt fast enough to avoid several negative impacts of climate change, such as temperature and moisture stress. Spike-mosses, like any other plants groups, may develop various mechanisms to adapt to those changes in temperature and moisture level by reducing growth rates, photosynthetic rates, mineral absorption and tissue regeneration, and by increasing concentrations of secondary metabolites (Jochum et al. 2007). However, these mechanisms may not be able to cope with all the anticipated severe climate change. Several scientists (Philips et al. 2006; Minteer and Collins 2010; Chen et al. 2011; Morueta-Holme et al. 2015) expect that to cope with severe climate change; plants will migrate to other areas with more suitable climatic-ecological requirements.

Selaginella Pal. Beauv. is the single remaining genus of vascular plants from the order Selaginellales, or mostly well-known as spike-mosses or lesser club-mosses. It has a wide range of distribution specialized on tropical regions of the world, rare in the temperate regions, and almost absent in the alpine zone. *Selaginella* species can be found in both very dry and very humid environments and in both open and shaded habitats. The tropical-hot and high humidity characteristics of climate in SEA is highly suitable for the wide distribution of Selaginella in this region. *Selaginella involvens* (Sw.) Spring. and *Selaginella repanda* (Desv. & Poir.) Spring. are two examples of dry-adapted selaginellas grow on the rock crevices or surface of plants and physiologically dependent on atmospheric sources of water and nutrients. Which are released as water vapor or rain (Nadkarni 2010). Thus, we may expect that both water and temperature directly control relevant eco-physiological process influencing growth rates and species distribution (Nascimbene and Marini 2015).

Selaginella involvens is a typical species of highland that adapt to dry conditions; grows on cliffs, rocks crevices, and epiphytes on trees. In Java, It is usually found in the highlands (> 1000 m asl.), such as Mts. Sindoro-Sumbing (Setyawan 2012), Mt. Merapi (Setyawan et al. 2012, 2015a) and Mt. Lawu (Setyawan et al. 2013), and Dieng Plateau (Setyawan et al. 2015c), but it is sometimes found in lower altitude (~ 500 m asl.), such as Mts. Sewu (Setyawan et al. 2015b), and Bromo Tengger Semeru National Park (Setyawan and Sugiyarto 2015). On the other hand, *Selaginella repanda* is a typical species of hilly lowland that adapt to dry conditions; it very easy to find in karstic mountain of Mts. Sewu (Setyawan et al. 2015b) and southern Java lowlands, although it can also rarely found in the higher altitude (500-1000 m asl.) (Setyawan et al. 2016). It usually attaches to the rocks and uses leaf litter as source of nutrients, with strong, pointed or tapered leaves, to adapt the dry condition.

In the last few decades, there was a marked increase of interest in the use of Species Distribution Models (SDM) (Merow et al. 2013; Fourcade et al. 2014). Empirically, these models were built by relating species occurrence data (either presence-only or presence-absence) to environmental predictor variables. These predictors can exert either direct or indirect effect on species, and must be selected optimally to reflect the three main types of influences on the species, i.e. (i) limiting factors related to species eco-physiology (e.g. temperature, water, soil composition); (ii) natural or human-induced disturbances, and (iii) resources (e.g. energy and water) assimilated by organisms (Guisan and Thuiller 2005). Since these environmental variables contain spatial data, it is best to manipulate it within a geographical information system (GIS) method. One of the most growing approaches of SDM is through the use of Maximum Entropy algorithms (Belgacem and Louhaichi 2013).

Maxent (Maximum Entropy Modeling) is a general-purpose machine learning method with a precise and simple mathematical formulation, and it has some aspects that make it well-suited for species distribution modeling (Phillips et al. 2006). These approaches are based on presence-only data which are necessary since the true absence of species is difficult, and because false absence can decrease the reliability of the predictive models (Phillips and Elith 2013). Maxent can utilize different climatic scenarios to estimate the extent of occurrence of species (Beaumont et al. 2007). Therefore, allowing the evaluation of species' vulnerability to climate change (e.g., Rondinini et al. 2006; Botkin et al. 2007; Randin et al. 2008; Engler and Guisan 2009; Garavito et al. 2015). Scientists (e.g., Carter et al. 2007; IPCC 2014) tried to define the vulnerability to climate change as "the degree to which a system is susceptible to, or unable to cope with, adverse effects of climate change, including climate variability and extremes".

Assessments of species vulnerability consider the vulnerability as the extent to which a species (within a defined geographical region) is susceptible to harm from climate impacts (Schneider et al. 2007). These assessments are useful to identify which species are likely to be impacted significantly by climate changes, and to enhance the understanding of why they may be vulnerable (Belgacem and Louhaichi 2013). Climate correlates to

species in the range of both horizontal and vertical spatial scales. Horizontally, species distribution range at a large extent is shaped by macroclimate. Nevertheless, at the smaller extent, topography shaped the macroclimate to produce an altitudinal climatic gradient along the local elevation (Mandar et al. 2008). Whether considering species ranges in horizontal or vertical terms, climate imposes range limits either indirectly, by mediating biotic interactions such as competition, or directly, through physiological constrains on survival or reproduction (Gavin and Hu 2006). *S. involvens* and *S. repanda* are two species which generally found at opposing range of elevation. *S. involvens* is often found in high-altitude areas, while *S. repanda* is usually found at lower-altitude areas (Setyawan et al. 2016). Therefore, we assumed that there will be altitudinal barrier between these species, which in this case will limit the redistribution mechanism of each species to adapt the climate change projections.

This study model the potential geographic distribution of *S. involvens* and *S. repanda* under present climate condition, and models the impacts of projected climate change on their potential distribution. We generate future predictions under four detailed bioclimatic scenarios (i.e., RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5) over three-time intervals (2030, 2050, 2080), which combine a range of climatic factors. Quantifying the potential impacts of a range of climatic scenarios offers the opportunities to develop understanding the response of plants to climate change, and develop mitigation strategies under all projected scenarios of climate change to effectively conserve biodiversity.

MATERIALS AND METHODS

Study area

The study was conducted to model the effect of climate change in Southeast Asia (SEA) region. SEA region consists of eleven political countries which can be divided into mainland SEA (i.e., Cambodia, Laos, Myanmar, Peninsular Malaysia, Thailand, and Vietnam) and maritime SEA (i.e., Indonesia, Phillippines, East Malaysia, Brunei, Singapore, and East Timor) (United Nations 2015). The geographic scope of this study includes the region of approximately 23.5 °N to 10 °S latitude and 97 °E to 141 °E longitude (Figure 1.). Southeast Asia covers an area of roughly 4,687,481 square kilometers with Mount Hkakabo in northern Myanmar on the border with China, at 5,881 meters, as the highest peak of mainland Southeast Asia (Leinbach and Frederick 2015). This vast altitudinal range and wide areas create a wide variation in climatic, physiographic, topographic, and edaphic conditions resulting in rich biodiversity.

SEA region has been experiencing climate change and its impact; Average annual surface temperature increased by 0.5-1.1ºC during the period 1901-2005 (NIC 2009). Similarly, climate model projection shows temperature increase by approximately 1ºC through 2030, and will keep increasing through the remainder of the 21st century (IPCC 2014). Although, there is no clear projection in precipitation patterns in the region, climate model suggests that net precipitation rates will increase across the region, but there will likely be local decrease that will vary geographically and temporally (NIC 2009).

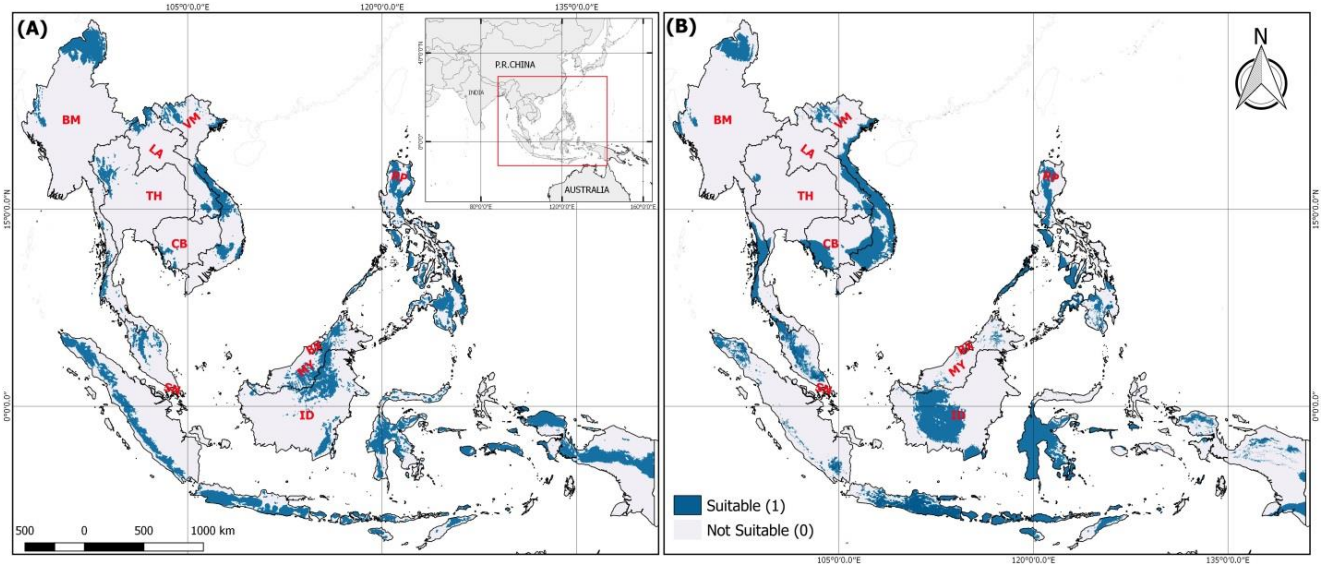


Figure 1. Predicted potential distribution of (A) *Selaginella involvens*, (B) *Selaginella repanda* under current climate condition

Procedures

Species locality data

Occurrence data for *S. involvens* and *S. repanda* were compiled from two main sources. The first source of locality points for both species was from field survey conducted in all Indonesian provinces in Java island between July 2007 and January 2014. The specimens were identified using several references on *Selaginella* of the Malay Archipelago and adjacent regions, both early (Alston 1934, 1935a,b, 1937, 1940) and latest literature (Wong 1982, 2010; Tsai and Shieh 1994; Li and Tan 2005; Chang et al. 2012; Zhang et al. 2013), as well as our previously publications (e.g., Setyawan 2012; Setyawan et al. 2012, 2013, 2015a,b,c; Setyawan and Sugiyarto 2015).

Altogether, 126 occurrence points of *S. involvens* and 68 occurrence points of *S. repanda* distributed in central, western and eastern region of Java island were collected using Garmin eTrax GPS series. None of error-correction step was conducted to adjust these data as we ensuring the accuracy of these points were less than 0.01 km at the time of data collection (www.garmin.com). Our judgment was based on allegation stated by Montgomery et al. (2011), that said the level of telemetry error on modern GPS (normally between 0.01 km and 0.05 km), which is much smaller than the resolution of the predictor variables used in this study, has a little effect on the accuracy of predicted models. The second source of occurrence data of both species was from Global Biodiversity Information Facility (GBIF 2017) database which provides freely accessible occurrence points on its website (http://www.gbif.org). Despite the use of "geo-referenced points only" filter on the data acquiring process from this website, several adjustments must be conducted. All coordinates were verified carefully and error that may occur were corrected using Google Earth software (Google Earth Pro 2017). Data records which lack coordinate were geo-referenced using Biogeomancer Workbench guided by locality description on each datum (Guralnick et al. 2006). Ambiguous data (i.e., those that does not have specific locality and cannot be geo-referenced) were removed, then the remaining 809 occurrence points of *S. involvens* and 124 points of *S. repanda* were combined with the occurrence points collected from the field survey.

The recent availability of biodiversity and environmental datasets globally (e.g. gbif and bioclim) was the main factor in the increasing number of regional to continent-wide SDM study (Hijmans et al. 2005; Kozak et al. 2008). Nevertheless, such datasets derived from collection of records and/or opportunistic observation often exhibit a strong geographic bias (Stolar and Nielsen 2015). Sampling bias correction was performed to minimize the strong influence of such bias to the modeling prediction ability (Fourcade et al. 2013, 2014). For this study, we conducted two out of five option methods of sampling bias correction proposed by Fourcade et al. (2014): (i) We conducted systematic sampling (often called spatial filtering) by creating a grid of a 2 km × 2 km cell size and randomly removed the occurrence points existed, until one point of occurrence left per grid cell. This size of this grid cell was not meant to represent the approximate of species' dispersal capabilities, but rather to reduce the geographic biases by modified the 10-km radius rule proposed by Pearson et al. (2007); Anderson and Raza (2010). The grid cells creation and points removal were conducted using QuantumGIS ver 2.18.10. (ii) We created bias file grids which later can be fed to Maxent software ver. 3.4.1by its setting options (Dudik et al. 2005; Elith et al. 2010; Phillips et al. 2017). Bias file is a probability surface represented by cell value which reflect the intensity of sampling effort across the area of study and gives a gradual weight to random background data used for modeling. Creating bias file can also be estimated using the aggregation of occurrences from closely related species (Phillips et al. 2009). However, in most real modeling situation, this information is limited. Thus, following Elith et al. (2010), we produced a Gaussian kernel density map of the occurrence locations, then rescaled it from 1 to 20 to be derived from bias file instead of using our knowledge to create artificially bias file (Fourcade et al. 2014). As the distribution of both species occurs in different countries (of different areas), we used the political state boundary extracted from Global Administrative Areas website (www.gadm.org/), to limit the background areas for the species.

Environmental and bioclimatic data on current climate condition

Selecting the predictors to build the model in this study was based on the model-driven selection process. Model-driven selection will use all possible predictors and select those with greatest importance in the model to be considered as the main factor affecting the distribution of species, rather than expert-driven selection where the expert priory will choose the predictors expected to directly affect the distribution of the species (Fischer 2011). We first collected 19 bioclimatic layers ver. 2.0 and one altitude layer. The 19 grid-based bioclimatic layers were downloaded form WorldClim Bioclimatic datasets website (www.worldclim.org). Bioclimatic layers were generated by interpolating the average monthly climate data from between 9,000 and 60,000 weather stations at approximately 1 km^2 spatial resolution (Fick and Hijmands 2017). Altitude layer was collected from the same website with the same spatial resolution. To use in Maxent software, these layers must be processed through several steps including image cutting to the extent of area study, resampling data, and file converting from .tif format to .asc format. Land use/land cover changes, human disturbances, species dispersal or biotic interaction changes were not taken into account as the availability of these data were limited.

It is widely known that many climate variables are highly correlated with each other. Even though including all these variables will not affect the predictive quality of Maxent model greatly (Elith et al. 2011), it does, however, significantly limit any inference of the contribution of any correlated variables since Maxent often excludes all other highly correlated variables from being incorporated (Van Gils et al. 2012, 2014). Since this study is interested in interpreting how each variable contributes to the model, we

decided to reduce autocorrelation of the climatic variables by removing the highly correlated variables. We used the SDM toolbox ver 2.0 (Brown 2014) in ArcGIS ver. 10.1 to perform the calculation and automatically removed each one of two correlated variables. The remaining six bioclimatic variables (i.e., bio_1, bio_2, bio_3, bio_4, bio_12, and bio_19) plus altitude layer were then used as predictors variables to model the potential distribution of *S. involvens* and *S. repanda* under current and future climate condition.

Environmental and bioclimatic data on projected future climate condition

Future predictive maps for potential redistribution of *S. involvens* and *S. repanda* were generated using the future climate projection datasets provided freely by CGIAR Research Program on Climate Change, Agriculture, and Food Security (www.ccafs-climate.org). The general circulation model which often used in climate modeling was originally has a spatial resolution at 100 to 200 km^2. To achieve the 30 arc seconds resolution (1 km^2), CCAFS downscaled it using the delta method by interpolating it from its original resolution (Ramirez and Jarvis 2008). We selected four RCP (Representative Carbon pathways), which represents the future greenhouse gas (GHG) trajectories, namely RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5 for three different time periods (2030, 2050, and 2080). In this study, we selected the HadGEM2-CC (Hadley Global Environment Model-2 Carbon Cycle) global circulation model, which was developed by the Hadley Center, United Kingdom (Collins et al. 2011). HadGEM2-CC model has been used to perform all the CMIP5 (Coupled Model Inter-comparison Project Phase 5) centennial experiments including ensembles of simulations of the RCPs (Shrestha and Bawa 2014). RCP 2.6 assumes that global GHG will increase slowly to reach its peak at 3.1 W/m^2 in between 2010-2020, with emissions declining substantially thereafter to 2.6 W/m^2 by the year 2100 (Van Vuuren et al. 2007; Moss et al. 2010). Emissions in RCP 4.5 is assumed to be stabilized at 4.5 W/m^2 by the year 2100 due to the employment of a range of technology and strategies to reduce GHG emissions (Clarke et al. 2007). Likewise, the emissions in RCP 6.0 is projected to reach its peak around 2080 and stabilizes by the year 2100 at 6.0 W/m^2. In RCP 8.5, emissions continue to rise throughout the 21st century, reaching around 8.5 W/m^2 as the highest value by the end of the century (Riahi et al. 2011). The same altitude layer was used since this variable was static variable that does not change with time.

Despite the widely usage of global climate model (GCM) to provide the basis for climate change impacts assessments of climate change at local to global scales and to be used as primary source of information for constructing climate scenarios, these models exhibit systematical error (biases) due to the limited spatial resolution, numerical schemes, simplified physics and thermodynamic processes, or incomplete knowledge of climate system processes (Ramirez-Villegas et al. 2013). Therefore, in this study, we implemented the bias correction data provided by CGIAR-CCAFS under two different calibration approach: (i). The Change Factor (CF) approach, where the raw GCM outputs current values are subtracted from the future simulated values, resulting in "climate anomalies" which are then added to the present day observational dataset (Tabor and Williams 2010). 2). (ii) The Quantile Mapping (QM) approach, it was selected to complement the CF approach, since the CF works well for only more non-stochastic variables (i.e., temperature). Thus, a more sophisticated approach for bias-correcting stochastic variables such as this approach (e.g., precipitation and solar radiation) was needed.

Maxent modeling

We used freely available Maxent software ver. 3.4.1 (Philips et al. 2017b) to model the potential distribution of *S. involvens* and *S. repanda* habitat under current climate condition and assess its changes under the effect of projected future climate condition. Maxent has been proved to gives the best results among other modeling algorithms available on the basis of presence-only (PO) data and environmental variables (Philips and Dudik 2008; Summers et al. 2012). The popular utilization of Maxent is due to the higher predictive accuracy than any other methods (Elith et al. 2006; Summers et al. 2012). Maxent also performs well to estimate the effect of climate change on the potential shifting range of species (Kou et al. 2011; Johnston et al. 2012; Duan et al. 2016), with more than 1000 published distribution modeling has been conducted since 2005 using Maxent software (Merow et al. 2013; Fourcade et al. 2014).

Despite the fact that Maxent has been shown to give robust and reliable results by just using default settings (Phillips et al. 2008), we modify several parameter values to adjust the calculation with our presence data and predictor variables. The adjusted parameter values were: maximum iterations were set 5,000 to allow the model to have adequate time for convergence, convergence threshold was set to 1×10^{-6}, and ten replicated runs (the averaged value is the one used as the result) with "cross-validate" as the replicated run type. Using "cross-validate" means to split the data ten times (10% per partition), train the model ten times on 90% of the data, and test it each time on the 10% partition alternately. Assuming that both selaginellas are responded directly to the predictors (vs to correlated factors) and to avoid overfitting (Elith et al. 2010), we decided to smooth the Maxent by choosing only hinge features to model both *S. involvens* and *S. repanda*. We doubled the default "regularization multiplier" value as we assumed that it was necessary because we use a large collection of species from diverse regions to be projected to different climate condition (Elith et al. 2006; Merrow et al. 2013; Radosavljevic and Anderson 2013). Furthermore, increasing the "regularization multiplier" value as high as 2.00 or 40.00 are necessary to reduce over-fitting to lower levels (Radosavljevic and Anderson 2013). We used the "projection" feature to predict the impact of future climate change to the redistribution of climatically suitable habitat for both species (Van der Wall et al. 2009).

Data analysis

Maxent will produce predictive maps which represent potential maps of species distribution, these maps show where the ecological niche is potentially suitable for the species across the study area. The degrees of potential suitability are linearly scaled between 0 (lowest) to 1 (highest) probability 1 (highest) (Philips and Dudik 2008). In addition, Maxent calculates the bioclimatic' relative contribution to the model and how much of these variables affect the Maxent prediction. Alternate estimation of variable importance was also collected by running the jackknife test in Maxent. The results of jackknife test show which variable have the most useful information by itself and which variable appears to have the most information that is not present in the other variables.

The predictive maps, which by default are in ASCII format, containing the probability were further analyzed using QuantumGIS software ver. 2.18.11 (QGIS 2017). To allow us to quantify and compare the redistribution of suitable habitat, we conducted binary calculation (suitable vs unsuitable) by using selected threshold rule. Selecting threshold rule is one of the many possible biases in SDM (Phillips and Dudik 2008; Nenzen and Araujo 2011; Bean et al. 2012; Syfert et al. 2013). Therefore, in determining the threshold rule, one should avoid arbitrariness and should consider the relative importance of commission and omission error (Hernandez et al. 2008; Nenzen and Araujo 2011; Liu et al. 2013). Considering to reduce omission error as more important determinant than commission error, Norris (2014) in his study proposed "minimum training presence" or "fixed cumulative value 1" to be the most appropriate rule. However, Liu et al. (2016) stated that for rare species, omission errors might be weighted more than commission errors but when considering a more common species, commission errors are more important than omission errors. Therefore, following Liu et al. (2016) we selected "maximum training sensitivity plus specificity" as threshold rule since this rule produces lower commission errors.

Maxent will calculate an area under the receiver operating characteristic (ROC) curve (AUC) as a way to evaluate model performance. Several studies (e.g., Pearson and Dawson 2003; Pearson et al. 2007; Rodder et al. 2010; Jiménez-Valverde A. 2012) have used AUC value to evaluate their model and to convince that their model can not be categorized as a merely random prediction. AUC value range between 0.5 and 1.0, with 0.5 indicating that the model is no better than random prediction; values below 0.7 are low, values between 0.7 and 0.8 are good, and values >0.9 indicate high discrimination, or it means that the model is far better than random prediction. Even though the AUC was written as the established method for assessing the fitness of the model in some papers, unfortunately, it is also proved wrong by Lobo et al. (2008); Bahn and McGill (2013); and Aguirre-Gutiérrez et al. (2013). These studies demonstrated that AUC does not provide useful information for assessing SDM performance. Therefore, in this study, the additional evaluation of the model was conducted using True Skill Statistic (TSS) as it has been proved theoretically and empirically better than AUC and also better than Kappa Statistic in measuring the performance of species distribution models (Allouche et al. 2006).

RESULTS AND DISCUSSION

Results

Analysis of variable contributions

Our models indicated that, for each species, there are two different variables which provide the highest relative contribution to explain the predicted geographic distribution of *S. involvens* and *S. repanda*'s suitable habitat in SEA region (Figure 1). Altitude was the highest contributor variable in the distribution pattern of *S. involvens*' suitable habitat (27.3%), while for *S. repanda*, average annual precipitation (bio_12) was the highest contributor variable contributed 36.2% to the model. Two precipitation variables (bio_19 and bio_12) explained in total of 38.7% of the variation in the distribution pattern of *S. involvens*' suitable habitat. Another 66% contribution to this model was apparently from temperature variables (bio_1, bio_2, bio_3, bio_4), with the highest contribution was only 10.1% (Table 1). For *S. repanda* model, another two significant contributor variables were Annual temperature (bio_1) and mean diurnal range (bio_2) which contributed in total of 47.7%. Other variables (alt, bio_3, bio_4, and bio_19) appeared to have no significant contribution to this model with only less than 20% of contribution in total (Table 1).

Alternate estimation of variable importance was also calculated through the jackknife test. The results of jackknife test show which variables have the most useful information by itself and which variables appear to have the most information that is not present in the other variables (Phillips et al. 2008). The Maxent models's jackknife test in this study shows a different conclusion on the importance of each variable for both species. The jackknife for *S. involvens* model shows that altitude has the highest gain when used in isolation, which therefore appears to have the most useful information by itself (Figure 2). Furthermore, the environmental variable that decreases the gain the most when it is omitted is annual precipitation (bio_12), which therefore appears to has the most information that is not present in the other variables. For *S. repanda*'s model, the jackknife test shows that temperature seasonality (bio_4) variable was both the highest gain when used in isolation and decreases the gain highest when it is omitted. This result indicates that for the *S. repanda* model, bio_4 variable has the most useful information which is not present in the other variables.

Models of present-day distribution

The potential present-day distribution of *S. involvens* and *S. repanda*'s suitable habitat, as derived from Maxent (Phillips and Dudik 2008; Elith et al. 2011; Phillips et al. 2017), are shown in Figure 1. These models were built using 327 unique locality points of *S. involvens* and 190 locality points of *S. repanda* which were the remaining points after spatial filtering applied (see method). Based on

our models, approximately 12% (561,424.7 km^2) of the SEA area is suitable for *S. involvens*, within predicted altitudinal range of between 1050 m asl. to 3500 m asl. (Figure 3). The suitable area for S involvens in mainland SEA spread patchily in Vietnam, Burma, Thailand, Laos and Peninsular Malaysia, with small predicted suitable area in Cambodia. Meanwhile, in maritime SEA the predicted suitable habitat for *S. involvens* spread in all of big islands of Indonesia (Sumatra, Java, Sulawesi, Borneo and Papua), also appears in Lesser Sunda islands and most of the south Maluku islands. Additionally, the predicted suitable habitat also appears in almost all of the Philippines archipelago. Furthermore, our model predicted there are approximately 15% (687,412.1 km^2) of *S. repanda*'s suitable habitat in SEA region, spread mostly in lowland areas between 50 m asl. to 1100 m asl. (Figure 3). The predicted suitable habitat of *S. repanda* in mainland SEA spread across north to south Vietnam, adjoining with Laos national border, and also spread patchily in Burma, Cambodia, and Peninsular Malaysia (Figure 1). The model predicted a very small suitable habitat for *S. repanda* in Thailand. In the maritime SEA, the predicted suitable areas spread across big islands of Indonesia (Sumatra, Java, Borneo, Sulawesi, and very small suitable area in Papua). Additionally, the predicted suitable habitat of *S. repanda* appears in most of the Philippines archipelago.

Table 1. Percentage of variable contribution to the final model

Variables	Description	Contribution (%)	
		S. involvens	***S. repanda***
Altitude		20.3	5.9
Bio_1	Annual mean temperature (°C*10)	15.6	32.9
Bio_2	Mean diurnal range (mean of monthly (max temp - min temp))	22.5	14.8
Bio_3	Isothermality	0.7	4.8
Bio_4	Temperature seasonality (standard deviation*100)	9.2	3.7
Bio_12	Annual precipitation	14.4	36.2
Bio_19	Precipitation or coldest quarter	17.2	3.1

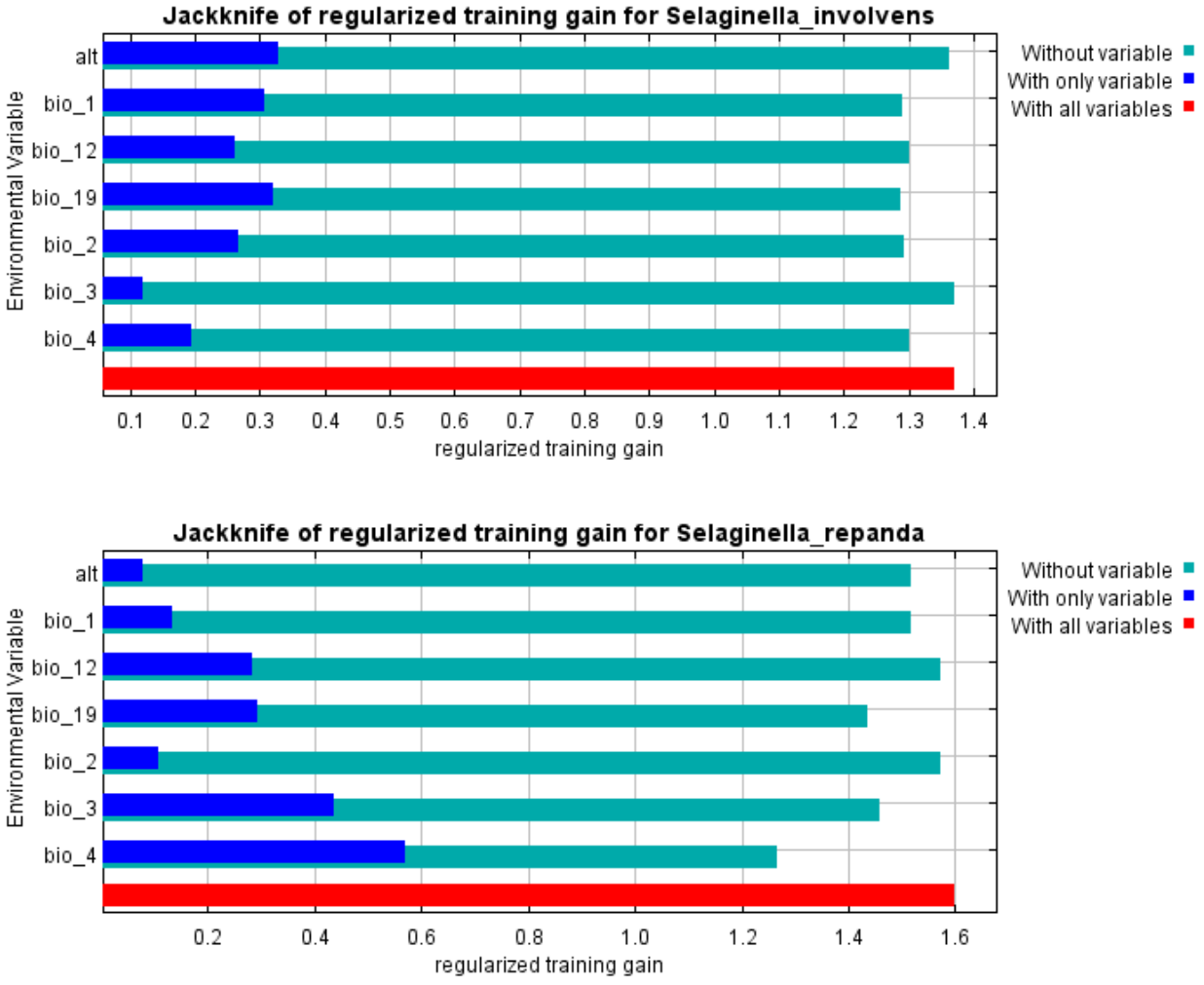


Figure 2. Results of jackknife test of relative importance of predictor variables for *Selaginella involvens* and *Selaginella repanda*

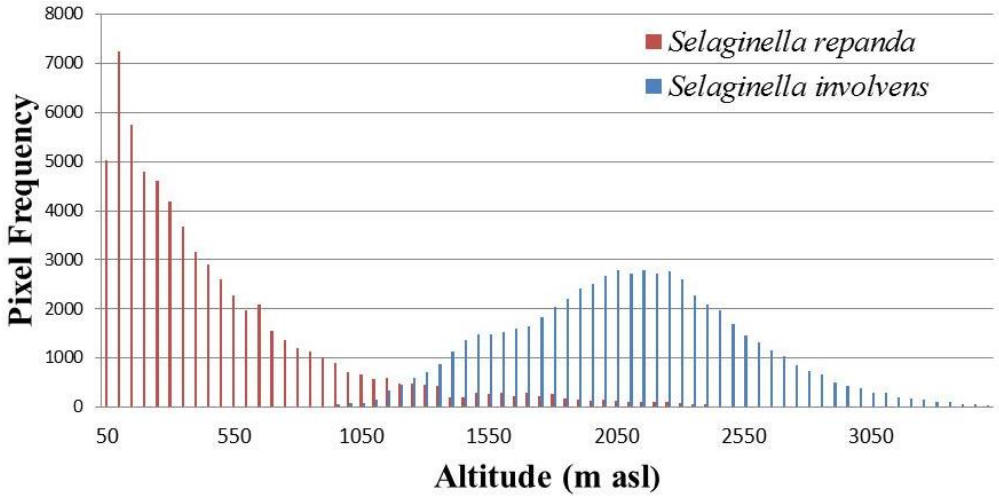


Figure 3. Predicted altitudinal distribution of *Selaginella involvens* and *Selaginella repanda* suitable habitat under current climate condition

The predicted suitable habitat of *S. involvens* and *S. repanda* under current climate condition were mainly concentrated in Java island, covering both the highland and lowland areas, respectively. The vast altitudinal gradient in this island creates a broad diversity in climatic, physiographic, and edaphic condition which become the major factors in influencing the development of the models. Our model predicted about 28,266.5 km^2 (21.1%) areas are suitable for *S. involvens* which spread mainly on highland areas across this island, ranged from 1100 m asl. to about 3000 m asl. On the other hand, the suitable habitat of *S. repanda* predicted mainly on the lowland, spread across the island in a total area of 38,037.8 km^2 (28%). Nevertheless, several small patches of predicted suitable areas for both species also appear overlapped in between the lowland and highland in a total area of 5423.6 km^2.

Despite the fundamental problem when using AUC (Area Under the Curve) for validation (Franklin 2010; Hijmans 2012), we retrieved the AUC value of 0.848 for *S. involvens*' model and the AUC value of 0.910 for *S. repanda*'s model to illustrate that the predictions in this study were far better than random prediction and to indicate that the selected variables have described the distribution of both selaginella acceptably. Furthermore, additional evaluation of the models was conducted using True Skill Statistic (TSS) to give further confidence in the output of these models. The TSS value of 0.81 and 0.85 for *S. involvens* and *S. repanda*'s models, respectively, give the impression that the models built in this study have a very good degree of agreement (Li and Guo 2013). Several studies also demonstrated the use of Kappa statistic for Maxent validation (e.g. Aguirre-Guiteres et al. 2013; Duan et al. 2014), but, regarding of the Kappa value, it is highly correlated to prevalence of the locality points and the size of the study area (Lobo et al. 2008). Consequently, this would generate some sort of bias or misunderstanding, for example, if one uses a small study region or if the locality points are localized in a small area and the prevalence is small, one would get a high Kappa value. Moreover, Kappa, just like AUC, is more reliable if we use PA (Presence-Absence) model, due to the fact that both AUC and Kappa are weighting omission and commission errors equally. Thus, in case of this study where presence only data were used, we thought that the use of Kappa statistic is not suitable.

Potential impact of the future climate change scenarios

The potential impact of several climates changes scenarios on the predicted suitable habitat is illustrated in Figure 4. Across all scenarios in three different periods of time, the predicted climatically suitable areas were predicted to decrease significantly (Figure 5). Under the RCP 2.6 (lowest GHG emission prediction) future climate scenario, the climatically suitable area for *S. involvens* will likely to continuously decrease by approximately 10% in the year 2080, while about 17% of *S. repanda*'s suitable habitat will be lost in the same period of time. Likewise, by the end of 2080, there will be a gradual decrease of predicted suitable area under RCP 4.5 and RCP 6.0 (medium GHG emission prediction) by maximum 20% and 22% for *S. involvens* and *S. repanda* respectively. Furthermore, RCP 8.5 future climate scenario is predicted to have the most impact on the decrease of climatically suitable habitat for both species. Approximately, 27% of current suitable area for *S. involvens* will be lost, while 29% of current *S. repanda*'s suitable habitat will also be vanished by the end of 2080. The continuously declining area of climatically suitable area for both species occurs gradually at the edge of every patch of modeled area. Thus, visual assessment can not clearly reveal which nation will likely to undergo the most suitable habitat loss. However, we can easily identify the progressive process of declining in Borneo and Sulawesi islands.

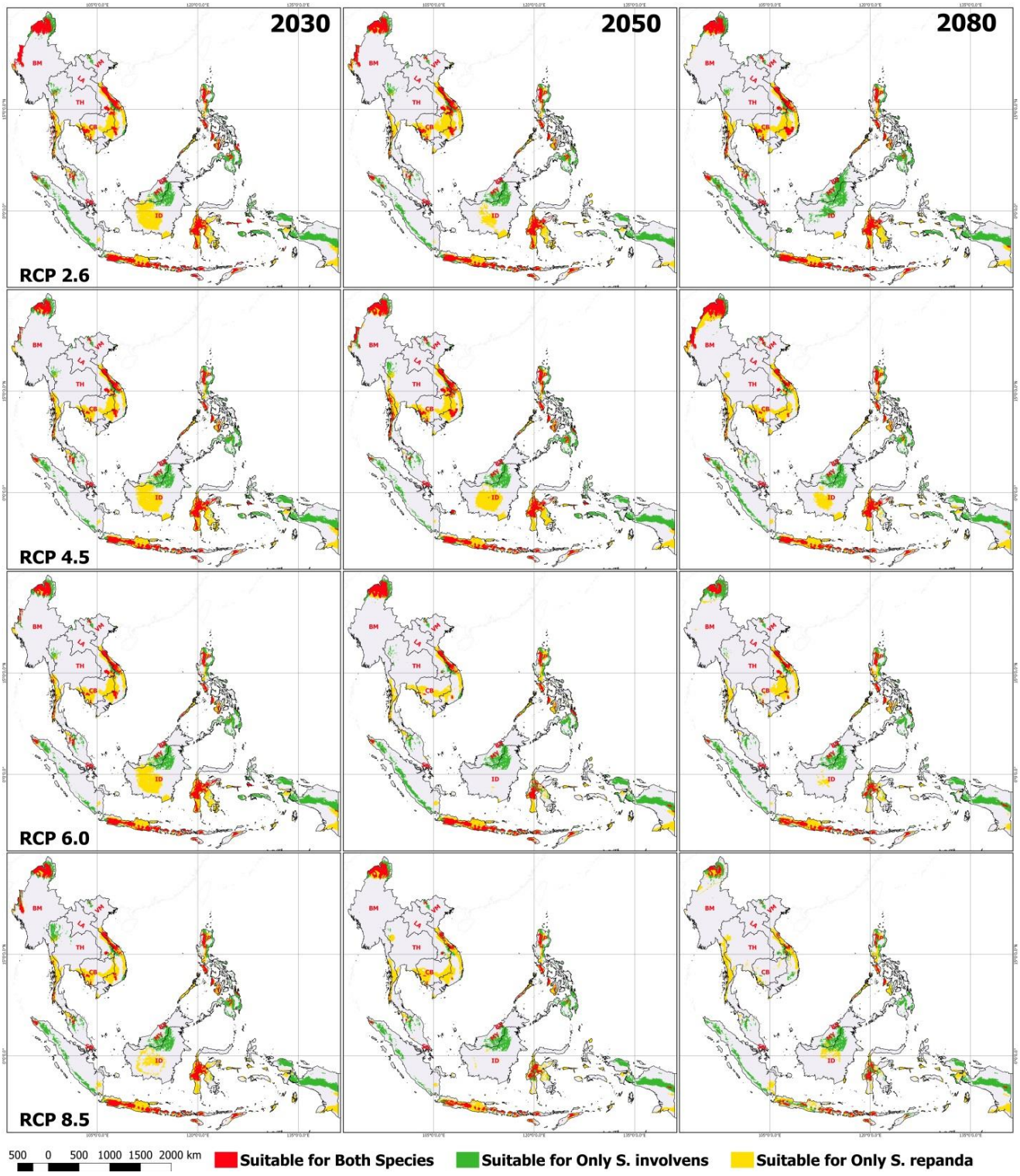


Figure 4. Prediction of future distribution under future climate projections

Discussion

This study represents the primary attempt to model the distribution of *S. involvens* and *S. repanda*'s suitable habitat under current climate condition and to model its redistribution under the effect of future climate change scenarios in SEA region. Our models predict the potential distribution of climatically suitable habitat for both species, under current climate condition, are distributed in all of SEA region countries. The distribution of suitable habitat for *S. involvens* mostly appears in highland areas, while for *S. repanda* appears mainly in lowland areas. Nevertheless, there are also areas which predicted suitable for both species.

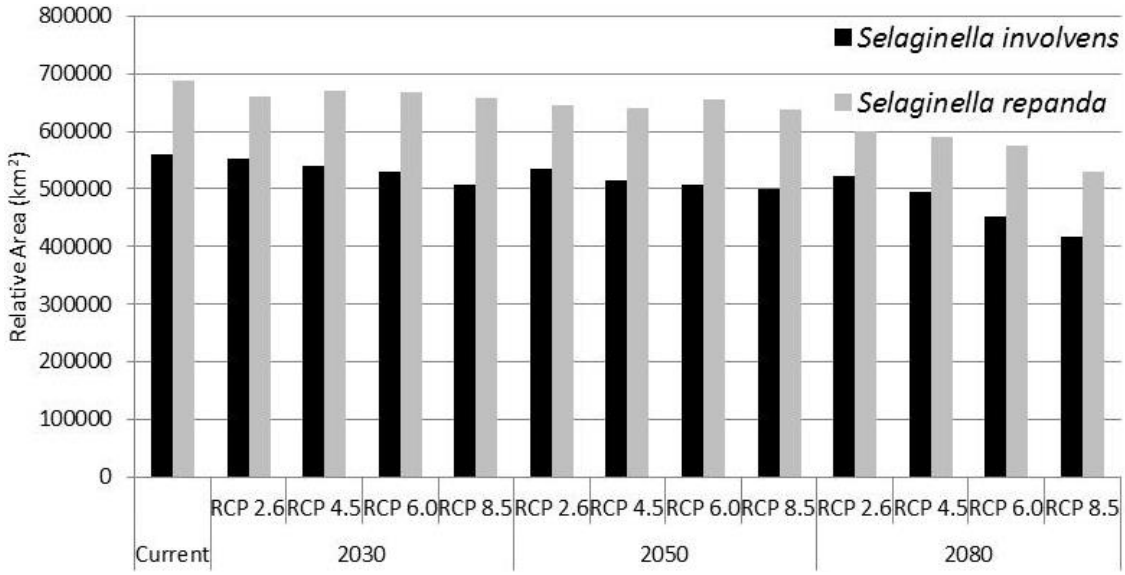


Figure 5. Estimated areas of predicted distribution of *Selaginella involvens* and *Selaginella repanda* suitable habitat

The predictive maps represent the potential distribution of climatically suitable habitat for both species, not the actual distribution of the species. The maps illustrate where the ecological niche is potentially suitable for both species. In the predicted suitable area, the species may not actually exist, for the following reasons (i) The vegetation has been drastically altered causing the predicted areas are no longer suitable for the species (e.g. deforestation, agriculture). (ii) The commission error occurs as the result of inaccuracies and weak resolution in the recorded environmental variables. It should be noted that the climate data were produced by modeling the original climate data from a network of weather stations. (iii) The micro-climate factors are not included in the models. (iv) The current environmental variables dataset have not yet represented the unique environmental condition which necessarily drive the occurrence of the species. (v) Inaccuracy of locality data. However, in the case of this study, the last two of these points may not influence our model strongly. Inaccuracy of locality may not greatly influence our model, because these data have been verified carefully by several correction bias methods (see method). To verified the robustness of the models we may look insight into whether the climatic variables used to build the models are in conjunction with the climatic variable proven to influence the establishment and the existence of species used in this study, and we may look into the comparison of the predictive maps under current climatic condition with the historical data of geographical distribution of both species.

Both *S. involvens* and *S. repanda* are epiphyte plants which derive its moisture and nutrients from the air, rain, or from accumulated debris around it (Everhart et al. 2009). The performance, survival, and distribution of epiphytes are dependent on stand density, microclimate, distance from seed source, tree size and species, type and history of disturbance, population dynamics of epiphytes and trees, and epiphyte physiology (Hietz 1999; Sayago et al. 2013). At the local scale, many study have shown that the host's characteristics and morphology which related to the ability to provide larger area of establishment and colonization, longer period of exposure to light, moisture retention, and better epiphyte-host network structure (e.g. Flores-Palacios and García-Franco 2006; Burns 2007; Zhang et al. 2010; Koster et al. 2014; Rosell et al. 2014) are another important factors influencing the existence of epiphyte plants. Nevertheless, at the given scale of analysis in this study, climate has been assumed as the principal factor controlling species distribution and in dynamic equilibrium with the climate regime (Guisan and Thuiller 2005; Ellis et al. 2007; Zots 2007). In terms of climate condition, the survival and distribution of epiphytes are highly influenced by annual temperature and total precipitation (Eaton and Ellis 2012). Thus, the bioclimatic variables included in this study to build the models were in line with the knowledge about the climatic factors influencing the occurrence of both species.

Similarly, comparing the predicted distribution of climatically suitable habitat of *S. involvens* and *S. repanda* in the countries within the SEA region, with the historical occurrence of both species reported in several studies and documents will represent the level of robustness of this study's results. Out of 11 countries in SEA region, our model predicts the existence of climatically suitable area for *S. involvens* in all of these countries. The studies and documents confirmed the occurrence of *S. involvens* in this region were Spring (1843), (Tagawa 1973), and USDA (2002). Winter and Amoroso (2003), Chang et al. (2012) for Republic in the Union of Myanmar (Burma). Cosico

(1972), Alston (1935b), Lasalita-Zapico (2011), Chang et al. (2012), Tan (2013), and Marjorie and Inocencio (2015) in the Philippines. Spring (1843), Newman et al. (2007) and Chang et al. (2012), in Laos. Spring (1843), Chang et al. (2012), Zhang et al. (2013) and Zhou et al. (2015) in Cambodia. Tsai and Shieh (1994), Chang et al. (2012), Nguyen et al. (2012), Zhou and Zhang (2015) and Zhou et al. (2015) in Vietnam. Boonkerd and Ratchata (2002) Jin et al. (2007) and Chang et al. (2012) in Thailand. Parris et al. (2010) and Youguang and Tan (2013) in Peninsular Malaysia and Singapore. In big islands of Indonesian archipelago, *S. involvens* occurred in Borneo (Spring 1843; Chang et al. 2012), Sulawesi (Spring 1843; Tagawa 1963, 1973; Tsai and Shieh 1994), Java (Spring 1843), and the Lesser Sunda islands (Spring 1843; Tagawa 1963, 1973; Chang et al. (2012). Nevertheless, recent literature study reported that only in Java island, this species occurrence records had been updated.

On the other hand, the documents confirmed the occurrence of *S. repanda* in Southeast Asia were Tagawa (1973), This species can be found in Myanmar (Chang et al. 2012), Thailand (Chang et al. 2012), Laos (Chang et al. 2012), Cambodia (Chang et al. 2012), Vietnam (Chang et al. 2012), the Philippines (Alston 1935a,b; Tagawa 1973; Tsai and Shieh 1994; Chang et al. 2012), Peninsular Malaysia (Wong 1982; Chang et al. 2012). In Indonesian archipelago, *S. repanda* occurred in Java (Alston 1935a; Tagawa 1973; Tsai and Shieh 1994; Chang et al. 2012), Sumatra (Chang et al. 2012), and the Lesser Sunda islands (Alston 1935a; Tagawa 1973; Tsai and Shieh 1994; Chang et al. (2012).

Furthermore, the model predicts the occurrence of climatically suitable habitat for *S. involvens* in highland areas in Sumatra and Papua islands, with no historical or current records to confirm or disconfirm this result. Likewise, several historical and recent studies confirmed the occurrence of this species in mainland SEA (e.g., Spring 1843; Tagawa and Iwatsuki 1967; Mark 2007; Boonkerd et al. 2008). In the maritime SEA the confirmed occurrence was in the Philippines (Tan 2013), while in Indonesia were Java island (Setyawan 2009; Setyawan et al. 2012), Lesser Sunda islands (Spring 1843), and Sumatra (Spring 1843). Meanwhile, there are large areas predicted as climatically suitable in lowland areas of Sulawesi, Borneo and Papua islands which are un-backed by occurrence data from both studies and documents resources. While, it will be impossible to calculate the precise commission error given that we do not have an absence data of these islands, we assume that those areas may just represent suitable but unoccupied habitat.

The predicted distribution of suitable habitat apparently will be significantly disturbed by future climate condition. Our models show a significantly negative trend of *S. involvens* and *S. repanda* distribution under the influence of accelerated global climate change. Under the lowest GHG emission projection (RCP 2.6), when radiative forcing reaches 3.1 W/m^2 before it returns to 2.6 W/m^2 by 2100, there will be a rise in annual mean temperature up to 1.7ºC in all areas of SEA region. Unlike the case of temperature, the changes in precipitation would not be the same in all SEA region areas. There will be both areas where the amount of precipitation shows a decreasing tendency (e.g., southern Indonesia, Thailand, Laos, and Myanmar) and where that shows an increasing tendency (Northern Philippines, Myanmar, and Laos), by the end of 21st century (Figure 6) (IPCC 2014). This condition leads to 10% decrease of *S. involvens* suitable habitat, which occurs at a greater rate in areas where the precipitation predicted to decrease, than in areas which the precipitation predicted to increase (Figure 4, Figure 6). Likewise, under the worst scenario projection (RCP 8.5; radiative forcing 8.5 W/m^2 by 2100), the magnitude of temperature increase to about 3-5ºC and annual precipitation will mostly increase at a great level of changes, with some exception areas where the precipitation is predicted to increase (Figure 6). Under this scenario, 27% and 29% climatically suitable habitat for *S. involvens* and *S. repanda* will be lost, respectively. Several studies have tried to explain the impact of these temperature and precipitation changes on the establishment epiphytic plants. Elis et al. (2005) and Ellis et al. (2009) reported a decreasing trend of the climatically suitable habitat in response to shifting rainfall patterns. Meanwhile, Hsu et al. (2012) and Ellis et al. (2014) reported that under suboptimal climate condition, there will be altitudinal shifting of epiphyte distribution by c. 300-400 m higher than current distribution which shows a high correlation with temperature-related factors in its distribution.

In this study, we extracted the altitudinal value of the predicted suitable habitat under RCP 2.6 and RCP 8.5 climate scenarios in the attempt to ensure whether there will also be altitudinal shifting or not (Figure 7). Across the periods of time, our models showed that both species are projected to marginally shifted to higher altitudes. Although the shifting is relatively small, the figure still represents the attempt of species to cope with the changes in temperature and precipitation variability. Increased temperature and occurrence of severe drought, as indicated by precipitation variability, should increase plant stress in some years (Kelly and Goulden 2008). Thus, expected to decrease the species' ability to survive in the drier, warmer, lower parts of its range (Allen and Breshears 1998; Lenoir et al. 2008a,b) and increase its competitive ability and tolerance in the wetter, cooler, upper parts of its range (Parmesan and Yohe 2003; Parmesan 2006). Despite there are several areas where climatically suitable habitat for both species are overlapped, there is still a gap in the majority of altitudinal distribution between both species' suitable habitats (Figure 7). Therefore, we may assume that there could be an altitudinal buffer related to local microclimate limiting the redistribution of species in response to the climate change. The habitat microclimate and micro-environment itself are not included in the climate data and therefore not modeled.

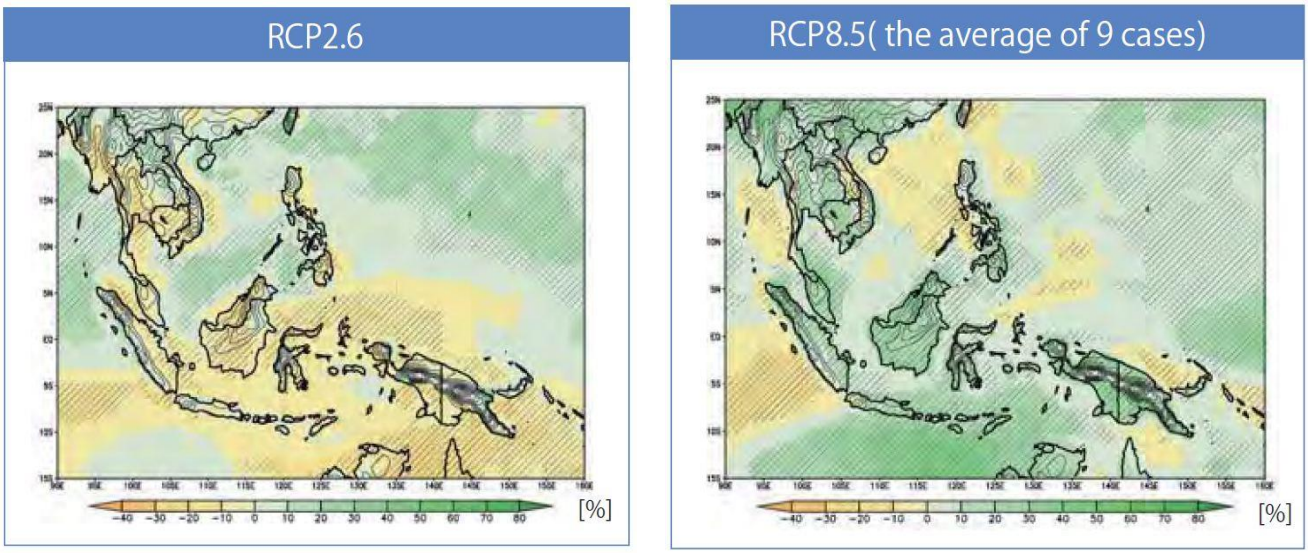


Figure 6. Changes (%) in annual mean precipitation under 2.6 and 8.5 scenarios in Southeast Asia. Colors represent the difference in precipitation between present climate and future climate (2080-2100). (images are acquired from Data Integration and Analysis System (DIAS), Ministry of the Environment, Japan, 2014. Under open license agreement stated in the document and https://dias-dss.tkl.iis.u-tokyo.ac.jp)

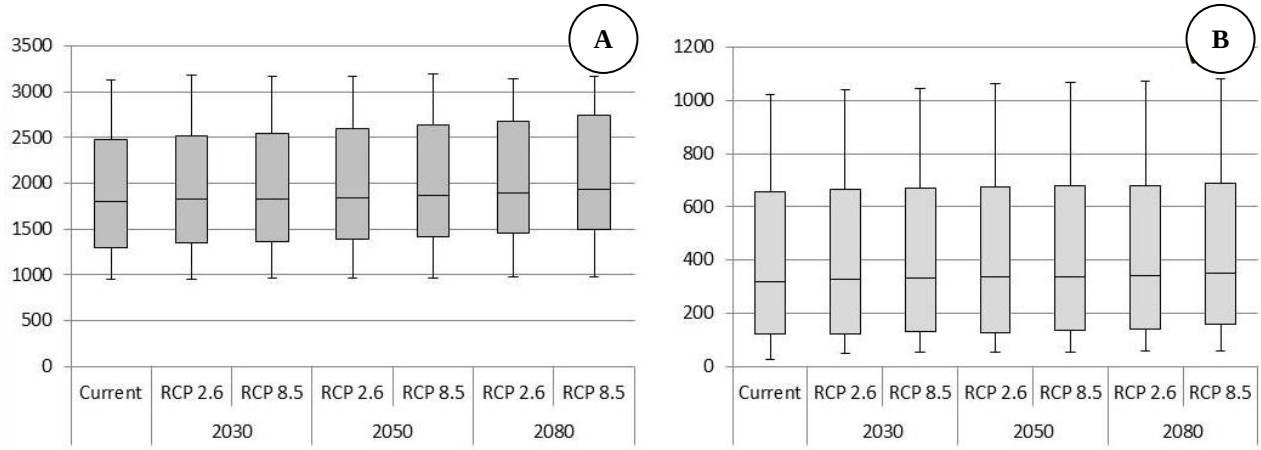


Figure 7. Box plot of median altitudes of (A) *Selaginella involvens*, (B) *Selaginella repanda* suitable habitat distribution under current and future climate scenarios. The plots present median, lower quartile, upper quartile, maximum and minimum observations. Altitude is measured in m asl. (meter above sea level)

The impact of multiple compounding factors acting simultaneously on species and its associated biota under anthropogenic actions and accelerated climate change would be very difficult to model, but the combined consequence is likely to be negative. There were several limitations in the availability of more detailed ecological and physiological data which prevent the build of more ideal models (Morin and Thriller 2009; Sinclair et al. 2010; Ellis 2011). Recent development of the new climate models and the refining of current climate models provide opportunity to build more precise and ideal models of future distribution trends of *S. involvens* and *S. repanda*. Future mapping and modeling process should incorporate potential land use land/land cover changes, more detailed ecological data, and accurately recorded presence and absence data to build the better assessment models. Despite these limitations, our study has established the baseline for assessing the impact of future climate change on *S. involvens* and *S. repanda*. While the quantitative results would be slightly different if using another niche and climate models, we believe that the presents resources of data will provide similar overall trend and projection

results. Thus, it is concluded that the survival of *S. involvens* and *S. repanda* are, and will continue to be, strongly influenced by climate change, and that in most cases the outcome will be negative.

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Phylogenetic relationships within the *Scylla* (Portunidae) assessed by the mitochondrial DNA sequence

HURUL ADILA-AIDA MOHAMAD ROSLY, SITI AZIZAH MOHD NOR, DARLINA MD. NAIM♥
School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia. Tel./Fax: +60-04 653 4056, ♥email: darlinamdn@usm.my.

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Abstract. *Rosly HAAM, Mohd Nor SA, Md. Naim D. 2017. Phylogenetic relationships within the* Scylla *(Portunidae) assessed by the mitochondrial DNA sequence. Biodiversitas 18: 1696-1704.* This study was centered around the phylogenetic of mud crab genus *Scylla* collected across Malaysia based on a 542 base pairs (bp) section of the mitochondrial cytochrome c oxidase I (COI) from 201 individuals. Sampling locations were nine areas including one location from Borneo (Sabah and Sarawak). The Maximum Parsimony (MP) and Neighbor-Joining (NJ) methods was conducted for phylogenetic analysis and performed in MEGA ver. 5.05. We found that *S. olivacea* is the plenteous species collected with 111 individuals, followed by *S. tranquebarica* with 61 individuals. *Scylla paramamosain* is barely found throughout our sampling locations with only 29 individuals caught in this study. Regrettably, no wild samples of *S. serrata* was found during our sampling occasions, accordingly life specimens were purchased from restaurants to complete the analysis. Both MP and NJ phylogenetic trees shows a monophyletic relationships among all four species within genus *Scylla* included in this study. This was also supported by the genetic distance analysis based on Tamura-Nei which indicates that there is high interspecific and low intraspecific genetic distances among and within species of *Scylla* included in the analysis. This investigation divulged a solid proof that supports the occurrence of three species of *Scylla* with the nonappearance of *S. serrata* in Malaysian waters. This current investigations could serve as a guidance for promoting further assessment on aquaculture and conservation management for the species.

Keywords: *Scylla*, phylogenetics, COI, mud crab, conservation

INTRODUCTION

Malaysia is identified as one of the world's mega-diversity center and a biodiversity hotspot, in which it has the total coastline length of 4675 km while the mangroves area occupies 5053.45 km^2 (Wong 2004). This large expanse of water space and various aquatic habitats (e.g. rivers, peat swamps, reservoirs, former mining pools and paddy fields) provide good opportunity for colonization of a large numbers of endemic and unique marine organisms (Abdullah et al. 2017; Imtiaz et al. 2017). Mud crabs of the genus *Scylla* (Portunidae) is also known as swimming crabs due to the broad paddles of the flattened fifth pair of legs that was used to dig into the mud (Ng et al. 2008). They are native to the Indo-West Pacific Ocean such as South Africa, Red Sea, Australia, Philippines, Pacific Islands, Taiwan and Japan (Fratini et al. 2010). In Malaysia, mud crab or locally known as *ketam nipah* or *ketam bakau* inhabits mangrove forests and river mouths in estuarine environments, where they can be found along the west and east coast of Peninsular Malaysia, Sabah and Sarawak.

Mud crabs has an excessive market demand where the price may fetch around Malaysia Ringgit (MYR) 40 - 60 per kilogram in a market, especially females with mature ovaries. As a consequent, the species belong to this genus is the most important crab for commercial culture and export markets (Keenan 2004) particularly in the tropics (Bell and Gervis 1999; Williams and Primavera 2001). To illustrate, the story of mud crab aquaculture has been started in China since centuries ago (Yalin and Qingsheng 1994) and Ikhwanuddin and Oakley (1999) reported that it has been started in other Asian region since more than three decades ago. Due to its high commercial value, mud crab is extensively cultured and captured (Keenan 2004). Further exacerbating the situation is that the broodstocks are almost entirely caught from the ocean (Xu et al. 2009). Additionally, juveniles are caught for seeding in ponds or enclosures while adults and subadults were used for fattening and soft-shell crab production (Le Vay 2001; Sara et al. 2002; Sara 2010). As a result of increased fishing effort over time and unregulated nature of fishery, there are indications that crab populations have decreased significantly (see e.g. Francis and Bryceson 2001; Mahika et al. 2005; David 2009; Xu et al. 2009). In spite of continuous harvest pressure, this species is managed in only a few parts of their range (e.g. northern Australia, Pillans et al. 2005), and, as a consequence, they become smaller and harder to catch in many places especially in developing countries (Gopurenko et al. 2002; Ewel 2008).

Initially, only one species from genus *Scylla* has been revealed namely *Scylla serrata* (Forskål 1775), until the research conducted by Estampador (1949) has made it clear that there are more than just one species occurred within the genus. Consequently, to reveal the puzzles about the taxonomic nomenclature of *Scylla*, several research and reviews were made (see Joel and Raj 1980; Fuseya and Watanabe 1996; Overton et al. 1997), until genetic and molecular research (allozyme electrophoresis, mitochondrial genes and nuclear genes) have come up to provide insight into the taxonomy of the genus (Keenan et

al. 1995; Keenan et al. 1996; Fuseya and Watanabe 1996; Keenan et al. 1998; Keenan 1999; Imai et al. 2004). Accordingly, four distinct species have been successfully revealed (Keenan et al. 1998; Keenan 1999; Imai et al. 2004).

In spite of its commercial interest and the declining status of mud crab genus *Scylla*, globally, knowledge of the genetic and demographic structures of wild populations is limited to a few studies (Gopurenko et al. 2002; see e.g. Keenan et al. 1998; Gopurenko et al. 1999; Gopurenko and Hughes 2002; Xu et al. 2009; for details study). Furthermore, despite the fact that it is an important food resources, only a few documented research on the phylogenetic and population of this species has been reported in Malaysia. In this context, identifying the genetic characteristics of a population and the rate of migration and/or gene flow can give insights into some of the key processes and factors influencing the substantiality of populations (Gauffre et al. 2008). It is also an important prerequisite, which may be crucial for effective conservation (i.e. conservation genetics) of the species concerned. The genetic information obtained would help in delineating proper sustainable management strategies and predicting the effects of proposed management alternatives on the viability of a species.

Additionally, genetic information is crucial in characterizing a species and for monitoring a potential changes in the genetic makeup and adaptive values as a result of interaction between wild and cultured populations, translocation or environmental changes (Jørstad et al. 2004; Utter 2004; Perrier et al. 2013) of this economically important species. The blending of cultured mud crabs with unadulterated wild stock found in open water bodies may accrue in unfit mud crabs contrasted with the local wild stock. Genetic data would, likewise, be helpful in the determination of ideal stocks for an efficient rearing projects and restocking. In the former, populations with high genetic diversity are chosen as broodstocks, while in the later, populations with high intraspecific genetic distance are required as contributor and beneficiary populations to guarantee the compatibility of gene pools of both populations (Lawlor and Hutchings 2004; Utter 2004). Furthermore, as genetic materials is acquired, it manifests heredity and is dependable for studying phylogenetic relationships.

In this study, phylogenetic analysis of mud crab genus *Scylla* collected across Malaysia was conducted by utilizing the mitochondrial DNA cytochrome oxidase subunit I (COI) sequencing. Due to quick changes of animal mtDNA base sequences, mtDNA COI is an effective method for evaluating hereditary connections of individuals or populations within and between species and furthermore to identify and measuring the phylogeny among various species.

MATERIALS AND METHODS

Sample collection

The sampling activity was performed during the years 2010 to 2012 from nine mangrove areas in Malaysia. A total of nine locations were chosen for sampling. Several field trips were conducted across Peninsular Malaysia before the selection of suitable sampling locations. Consequently, nine suitable locations were selected and these include two locations from the east coast of Peninsular Malaysia namely Kelantan and Terengganu, four locations from the west coast of Peninsular Malaysia namely Langkawi, Kedah, Penang, Perlis and Perak, and only one location from Johor (south of Peninsular Malaysia). We also managed to sample individuals from a single population from Sarawak and Sandakan, Sabah, both located within the Borneo region. Justification of choosing these sampling locations is that they encompass the distributional range of mud crab genus *Scylla* within Malaysian waters. Crab pots with fish as bait were used to trap and catch the crab. Approximately seven crab pots were placed at identified mangrove areas in all sampling locations. The trap were spaced at precisely 100 meter apart from each other and were monitored for three successive days, 24 hours per day. Fortunately, our sampling activities were also facilitated by personnel from the Fisheries Research Institute (FRI) and the Department of Fisheries Malaysia (DoF). All samples caught were morphologically identified and classified on the basis of morphology and body coloration.

Approximately 2 cm segment of muscle tissues from a right single claw of each crab was clipped and fully dipped in a labelled 2 ml eppendorf tubes containing 95% alcohol for DNA extraction. These reference specimens were then transported to Lab 308, Universiti Sains Malaysia for molecular analysis. The voucher specimens were deposited at the Centre of Marine and Coastal studies (CEMACS) Collection Centre, Muka Head, Penang following standard preservation protocol.

Preservation of voucher specimen is a central part to record biodiversity samples through time and the most crucial step for this purpose is fixation. In this study, all voucher specimens were thoroughly cleaned with water to remove any contamination. The specimens were initially fixed in 10% formalin for a week. This solution is ideal for fixation of a marine organism in a tropical environment such as Malaysia. After a week, all samples were removed from formalin and rinsed with water to wash out excessed formalin.

Polymerase chain reaction (PCR) and sequencing

Total genomic DNA from all the specimens were extracted based on the protocols from AquaGenomic Solution Kit (BioSyntech, USA) in the presence of Proteinase *K*. A slight modification of the original protocol was made in order to get maximum yield of DNA. The quality and quantity of extracted DNA was checked by electrophoresis, which was conducted on a 1% agarose gel in Tris-Borate-EDTA (TBE) buffer solution at 100V, 500mA for approximately one hour. The agarose gel was stained with ethidium bromide (EtBr) prior to visualization for the presence of the extracted DNA band in a gel documentation system (GENE Flash). The intensity of band indicates DNA quality. The quality and quantity of extracted DNA were also confirmed by use of

spectrophotometer. The DNA quality having values 1.8-2.0 based on OD260/OD280 and quantity above 10 uL suspended in 50 uL of ddH_2O were chosen for Polymerase Chain Reaction (PCR) amplifications.

The isolated DNA template was amplified using primer pair of cytochrome oxidase c subunit I (COI). Primers used for amplification were: Mtd-10 5'- T TGA TTT TTT GGT CAT CCA GAA GT - 3' (Roehrdanz 1993) and C/N 2769 5'- TT AAG TCC TAG AAA ATG TTG RGG GA - 3' (Gopurenko et al. 1999). PCR was conducted in an eppendorf Master Cycler in a total volume of 20 uL based on the optimized conditions. The mixture consists of 10x PCR buffer, 2.5 mM dNTP mixture, 5U of *i-Taq* DNA polymerase, 25mM $MgCl_2$, 0.5 pmol of each forward and reverse primer and 1.6 µl (20 ng) of DNA from each sample. Aliquots were then transferred into each of 0.2 mL labelled tube with DNA individually added for each sample. The thermal regime were 35 x [94°C for 30 s, 50°C for 30 s, 72°C for 1 min, 94°C for 3 min] and a final incubation at 72°C for 5 min. All amplified PCR products were then purified by using MEGA Spin Total Fragment DNA Purification Kit (Intron Biotechnology INC. Korea), an important step to avoid excess nucleotides, salts or primers. The purified PCR product was run on a 1.5% agarose gel with 100 bp ladder to compare the length of amplified PCR products and visualized on a GENEFLASH Syngene Bio Imaging prior to send for sequencing. Purified PCR products were stored at 4°C in a freezer.

The PCR products were then purified according to the protocols from PCR Purification kit (PROMEGA) and sent to First Base Laboratories Sdn Bhd (1st BASE) for sequencing. Sequencing of products was done on the Applied Biosystems machine based on principles of Sanger sequencing method (dideoxy sequencing). The dideoxy nucleotides were fluorescently labelled and each nucleotide position was read based on different wavelengths.

Sequence analysis

Multiple DNA sequences were compiled, edited and aligned to generate unambiguous operational taxa units using Clustal W ver. 1.6 (Thompson et al. 1994) which is integrated in MEGA ver. 5.05 (Tamura et al. 2011). This was also includes four sequences of adult *S. serrata* based on morphological inspection (samples were obtained from a restaurant and it is believed to have originated from Sulawesi -3 sequences and Pulau Jawa -1 sequence, respectively). Another three sequences of *S. serrata* from GenBank that originated from three different countries were also included in this analysis. Sequences were then blast in BOLD (Barcode of Life Database system) and BLAST (Basic Local Alignment Search Tool) database (http://www.ncbi.nlm.nih.gov/blast) to assign each individual into its respective taxon.

Phylogenetic analysis

Intraspecific and interspecific pairwise genetic distances (D_s; Nei 1972; Nei 1978; Kalinowski 2002) were calculated under the Tamura-Nei genetic distance (Tamura and Nei 1993) performed in MEGA ver. 5.05 software (Tamura et al. 2011) for COI gene with gaps treated as missing data. The same software was used to cluster COI haplotypes into a Maximum Parsimony (MP; Farris 1983) and Neighbour-joining (NJ) phylogeny, employing 10, 000 bootstrap replicates. To clearly present the phylogenetic tree, only 51 sequences including the four sequences of *S. serrata* obtained from restaurant were used to construct both MP and NJ trees as representatives of all sequences. Both tree reconstructions were rooted using two indigenous portunids *Stoliczia chaseni* (GeneBank accession number: AB290645.1) and *Johora singaporensis* (GeneBank accession number: AB290641.1) as an outgroup.

RESULTS AND DISCUSSION

A total of 201 samples of genus *Scylla* were successfully collected across the nine sampling locations across Malaysia (Figure 1).



Figure 1. Location of the nine sampling sites across Malaysia

Of these, less than 100 adult crabs were positively assigned to three species based on their morphological characters namely *S. olivacea*, *S. tranquebarica* and *S. paramamosain*. The rest of the samples failed to assign to their respective species based on their morphological characteristics because they are immature samples. Among the total samples, *S. olivacea* was the most commonly occurred species with 111 individuals collected from all sampling localities (except Johor and Sabah), followed by *S. tranquebarica* and *S. paramamosain* with 61 and 29 individuals caught respectively. In this study, *S. tranquebarica* was absent in Langkawi and Penang, but they are the only species inhibit mangrove areas in Johor and Sabah. Interestingly, no *S. serrata* was collected from the sampling locations. Table 1 provides a summary of species occurrences at each sampling site as illustrated in Figure 1.

All samples were successfully sequenced for the COI region, and no evidence was observed in the final 542 bp alignment to indicate the presence of pseudogenes in the data set. The alignment had 77 variable sites with 50 parsimoniously informative sites (Table 2). No insertions, deletions or stop codons were observed among the aligned sequences. Lack of stop codons in the sequences confirmed that the sequences were functional protein coding gene for mitochondrial COI.

Within the four species, intraspecific divergence ranged between 0.3 to 1.0% while interspecific divergence ranged from 10% to 21% with comparatively low divergence between *S. paramamosain* and *S. tranquebarica* and high divergence between *S. paramamosain* and *S. olivacea* (Table 3). The topology generated by MP (Figure 2) and NJ (Figure 3) analysis of COI barcodes showed monophyly of all four species which was strongly supported by bootstrap analysis at all nodes. Above all, the absence of Malaysian *S. serrata* is highlighted. The phylogenetic tree shows that *S. tranquebarica* and *S. olivacea* were more related to each other while *S. paramamosain* formed a sister group to this cluster. *Scylla serrata* was the most basal among the four species in both trees.

Discussion

Results of the mtDNA COI gene analysis in the present study have shed light on the genetic makeup of *Scylla* species, particularly from Malaysia. The high number of parsimonious-informative sites indicates that COI mtDNA is an informative and effective locus candidate for phylogenetic and molecular taxonomy studies (Kamarudin et al. 2011) (Table 2). The phylogenetic analysis of the COI gene confirmed the reciprocally monophyletic status between all *Scylla* sequences (Figures 2 and 3), thus shows less and/or no geographical association (see Rosly et al. 2013). The monophyly of all species was also well established with a very low intraspecific and high interspecific genetic distances respectively (Table 3). Additionally, the genetic relationships revealed by the maximum parsimony (MP) and neighbor-joining (NJ) trees are in high agreement (four monophyletic clusters) with the same phylogenetic sister-species relatedness with the previous molecular phylogeny study of *Scylla*, which was also based on COI sequence data (Keenan et al. 1998).

In this study, *Scylla olivacea* formed the terminal taxon, followed by *S. tranquebarica*, *S. paramamosain* and *S. serrata* which formed the most basal, although the support was not very high (Figures 2 and 3). Despite the fairly wide geographical coverage within the Malaysian waters of *Scylla* sampling in this study (Figure 1), no genetically identified *S. serrata* was detected. Many previous researchers have reported the abundance of *S. serrata* (see Overton et al. 1997; Keenan et al. 1998; Gopurenko et al. 1999; He et al. 2010; Fratini et al. 2010) in the neighboring waters and it seems unlikely and puzzling that the species does not occur in Malaysia.

Table 1. Number of individuals in each sampling site based on mtDNA COI sequence analysis for three species within genus *Scylla* from Malaysia.

Sampling sites	Number of individuals		
	S. olivacea	***S. tranquebarica***	***S. paramamosain***
Perlis	9	1	0
Langkawi, Kedah	15	0	1
Penang	31	0	7
Perak	24	10	10
Kelantan	5	2	7
Terengganu	8	4	2
Johor	0	10	0
Sarawak	19	9	2
Sabah	0	25	0
Total	111	61	29

Table 2. Number of haplotype (*N*hap) and number of site (variable, conserved and parsimonious informative sites) for each member within genus *Scylla*. *N* = sample size

Taxa	*N*	*N*hap	Number of sites		
			Variable	Conserved	Parsimonious informative sites
S. olivacea	111	66	50	492	35
S. tranquebarica	61	12	12	530	8
S. paramamosain	29	16	15	527	7
S. serrata (imported, not from sampling site)	4	3	2	540	1

Table 3. Pairwise Tamura-Nei genetic distances (D_s) among and within species of *Scylla*

Species	***S. olivacea***	***S. tranquebarica***	***S. paramamosain***
S. olivacea	0.011		
S. tranquebarica	0.172	0.003	
S. paramamosain	0.209	0.100	0.006

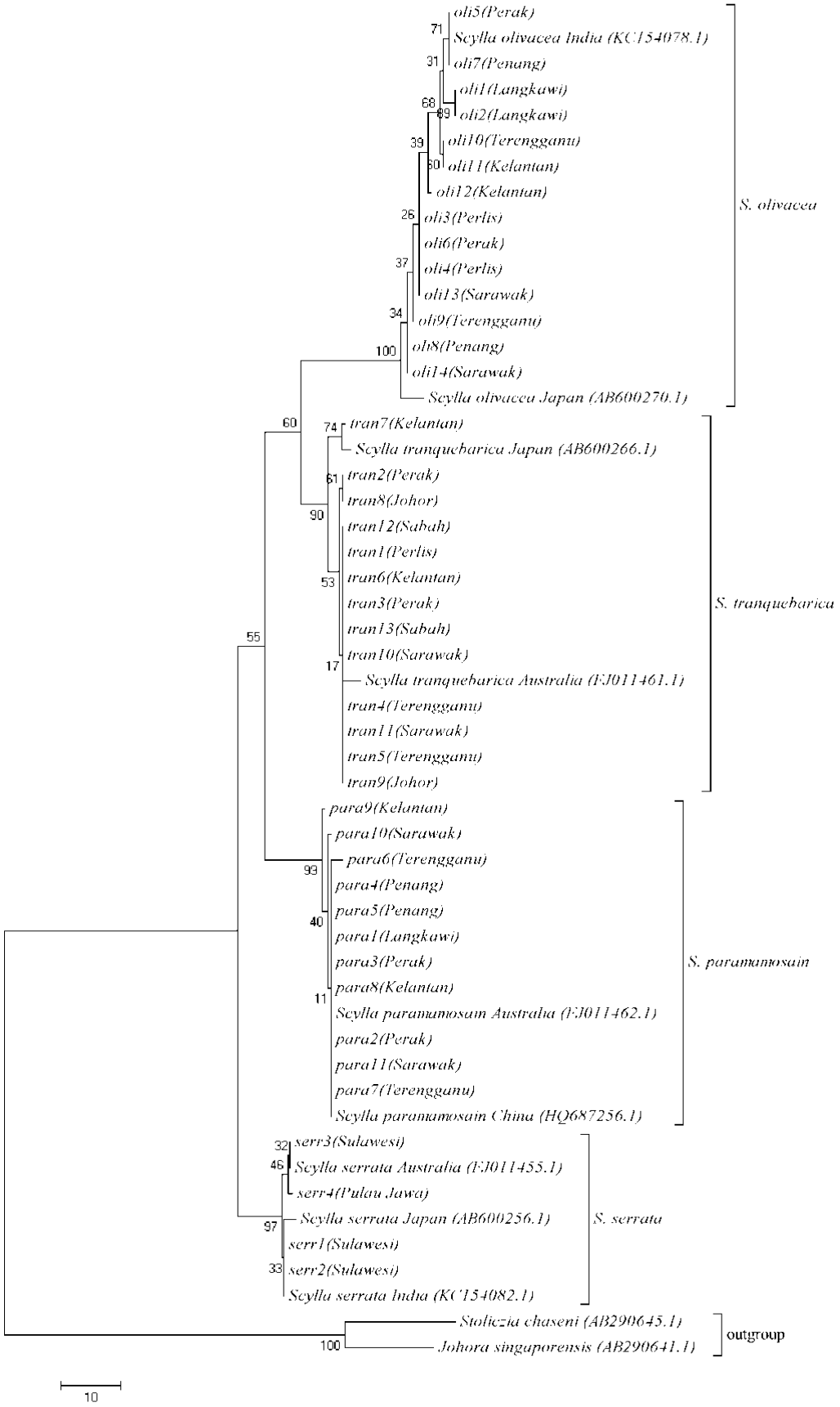


Figure 2. Maximum Parsimony (MP) phylogenetic tree showing the relationships among cytochrome c oxidase I (COI) sequences of *Scylla* with additional sequences from GenBank and sequences of imported *S. serrata* from Indonesia



Figure 3. Neighbour-joining (NJ) phylogenetic tree showing the relationships among cytochrome *c* oxidase I (COI) sequences of *Scylla* species (with additional sequences from GenBank and sequences of imported *S. serrata* from Indonesia)

An obvious explanation is overharvesting may have caused such a decline that the limited number of samples and efforts failed to trap any *S. serrata*. But this species is not considered as an endangered group. However, there may be other underlying reasons, which cannot be pinpointed based on present knowledge. A study by Kosuge (2001) reported that out of 81 individuals, more than 60% of mud crab species sampled from the Matang Mangrove Forest Research off the Straits of Malacca were *S. serrata* while *S. olivacea* made up the remaining samples (no *S. paramamosain* and *S. tranquebaria* were reported). This earlier finding is in wide contrast to this study. However, 80% of the mud crabs sampled by Kosuge (2001) were immature. This could have led to misidentification.

The area which is close to the original sampling by Kosuge (2001) was resampled to further confirm the existence of *S. serrata* in the location recorded in Kosuge (2001). Accordingly, both types (two groups of morphological variants) of *S. serrata* were obtained in this study (unpublished data). However, COI gene did not identify any of the two groups into *S. serrata* species. Furthermore, several researchers (Ikhwanudddin 2001; Ikhwanuddin et al. 2010, 2011; Mohammad Zaidi et al. 2011) reported that this species is not found on the continental coast of the South China Sea based on morphological characters, although they are common in the Indo-Pacific Ocean (Keenan et al. 1998). However, these previous Malaysian studies were only focused on the Setiu Wetlands and Sarawak. The Straits of Malacca are an extension of this wide ocean, which is the native habitat of this species.

While several authors have recorded panmixia for this species over a wide geographical range, Gopurenko and Hughes (2002) recorded a strong genetic structure on a meso-geographic scale in Australia. These differences were attributed to the differential influence of abiotic factors acting on the movement and/or on the survival within the different parts of the species distributional range during its pelagic phases. If it is proven by further extensive and intensive sampling, that *S. serrata* is not present in the surrounding of Malaysian waters and possibly slightly beyond, it could be hypothesized that they may be hydrological factors that prevent recruitment into the Malaysian waters. Fratini et al. (2010) did an extensive phylogeographic study of this species in the Indo-West Pacific Ocean (from Africa right to Australia including Southeast Asia) but it is noteworthy to add that no samples within the present area of study were included in their analysis. Similarly, He et al. (2011) analyzed a total of 439 sequences from 24 locations throughout the Indo-West Pacific but no Malaysian samples were included (presumably because no previous molecular study has been conducted in Malaysia). Thus, more sampling efforts and utilization of molecular markers are needed to confirm their presence as reported by Kosuge (2001), or otherwise, in the Straits of Malacca.

On the whole, in this study we were able to provide useful insights into phylogenetic relationships and genetic identity of *Scylla* species; particularly from Malaysia and surrounding waters. However, further studies using larger samples from other areas of its geographical distribution, sequence data from other mtDNA regions, and information based on nuclear DNA markers are required before any appropriate conservation management strategies for *Scylla* species are implemented.

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Reproductive ecology, spawning potential, and breeding season of blue swimming crab (Portunidae: *Portunus pelagicus*) in Java Sea, Indonesia

TRI ERNAWATI[1], BAMBANG SUMIONO[2], HAWIS MADDUPPA[3,4,5,♥]

[1]Research Institute for Marine Fisheries, Ministry of Marine Affairs and Fisheries, Jakarta Utara 14440, Indonesia
[2]Center for Fisheries Research and Development, Ministry of Marine Affairs and Fisheries, Jakarta, 14440 Indonesia
[3]Department of Marine Science and Technology, Faculty of Fisheries and Marine Science, Institut Pertanian Bogor. Jl. Raya Darmaga, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. ♥email: hawis@apps.ipb.ac.id
[4]Indonesian Blue Swimming Crab Association. Jl. KIG Raya Selatan Kav C-5, Gresik 61121, East Java, Indonesia
[5]Center for Coastal and Marine Resource Studies, Institut Pertanian Bogor. Jl. Raya Pajajaran No. 1, Kampus IPB Baranangsiang, Bogor 16217, West Java, Indonesia

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Abstract. *Ernawati T, Sumiono B, Madduppa H. 2017. Reproductive ecology, spawning potential, and breeding season of blue swimming crab (Portunidae:* Portunus pelagicus*) in Java Sea, Indonesia. Biodiversitas 18: 1705-1713.* The blue swimming crab (Portunidae: *Portunus pelagicus*) is one of the most economically important fisheries species in Indonesia. Little is known about their reproductive ecology and key biological characteristics. This study aimed to investigate population biology and reproductive ecology (Carapace's width to weight relationship, growth, natural mortality, size at first maturity and at first captured, Spawning Potential Ratio (SPR), and breeding season) of *P. pelagicus* at six important landing sites around the Java Sea (Jakarta, Cirebon, Demak, Rembang, Sumenep, and Sampit) from January 2014 to January 2015. Monthly data were collected by fishers at each landing sites, with a total of 14,408 individuals being measured. The study found that males were heavier than females and the smallest mean of individuals' height belonged to Jakarta compared to other locations. The growth parameters varied at the different locations. Crabs could grow up to 100 mm in 10 months with the maximum age being less than three years. The growth rate (K) of more than 1 indicated a fast-growing crustacean with a short lifespan. The lowest carapace width at first capture (L_c) for the BSC was obtained from crabs caught using a bottom gillnet landed in Jakarta, followed by dredged crabs in Cirebon. The mean carapace width at first maturity (L_m) in the Java Sea was 10.6 cm, with the lowest L_m of female crabs being landed in Cirebon and the highest in Sampit. The Spawning Potential Ratio (SPR) ranged from 11% to 24% and the selectivity curve for the BSC fishery at each landing sites is positioned well to the right of the generic maturity curve, except in Demak, suggests that the operation enables almost all crabs to mature and spawn before entering the fishery. The collapsible trap was the most selective gear, with an average catch of 112 mm across at all landing sites, and the least selective was in trawls. Therefore consideration needs to be given to the different gear types used to catch crabs to leave smaller crabs in the water longer to grow to maturity. Considering its fast growth and peak season (February-April and August-October), a short-period is recommended at some sites that have been over-exploited to rebuild a broodstock to support the long-term sustainability of this species.

Keywords: fishing gear, minimum landing size, seafood, sustainable fisheries, small-scale fisheries

INTRODUCTION

The blue swimming crab (BSC; *Portunus pelagicus*, locally called as Rajungan) is one of the most economically important fishery species in Indonesia, after tuna and shrimp (Fauzi, 2013). BSC is found across the Indo-Pacific, the Indian Ocean to the west and eastern Pacific Ocean (Ng, 1998), and in Indonesia, it is widely distributed including the east coast of Sumatra, northern coast of Java, south and east Kalimantan, Southeastern of Sulawesi and south-west of Papua (Sumiono, 1997). The annual BSC catch in Indonesia has fluctuated between 1970-2014. Catch levels increased between 1970 and 2004, but then, they declined in 2004-2005, before they increased again in the following years. In 2008, the estimated crab production reached 34,000 tons, which was approximately 20 % of global production, second only to production in China (FAO 2011 in Chu et al. 2012). Crabs contributed 44% of the total national production (42,000 tons), and 18,500 tons of them came from the Java Sea (MMAF 2012).

Determining life histories of harvested species are important to ensure the sustainability of the species in the wild. Sustainable management requires information on the size and age at maturity, size distribution patterns, age-specific reproductive investment and spawning time (Stearns 1989, Sukumaran and Neelakantan 1996, 1997, Hamid et al. 2016, La Sara et al. 2016)). It is also important to understand the characteristics of the fishery including information on harvest strategy (Campbell and Eagles 1983, Addison and Bennett 1992), and harvesting rules (Gerhart and Bert 2008). Earlier studies have shown intraspecific, and interspecific variation in the reproductive biology characteristics of *P. pelagicus* explained by, area-specific and regional differences; e.g. in Malaysia (Ikhwanuddin et al. 2009, Ikhwanuddin et al. 2012), in India waters (Sahoo et al. 2011), and in Philippines (Ingles and Bkaum 1989). Brachyuran crabs are categorized as

iteroparous species, which have considerable variation in reproduction patterns (Hines 1982, Hines 1989). The reproductive periodicity of *P. pelagicus* follows a continuous pattern in tropical and subtropical regions (Batoy et al. 1987, Sumpton et al. 1994). The reproduction periodicity may be subdivided into three categories (Pinheiro and Fransozo 2002): (i) seasonal reproduction, either the presence of the females with mature gonad or the presence of ovigerous females begin during certain months or seasons; (ii) continuous, when ovigerous females occur across all months of the year; and (iii) seasonal-continuous, the presence of ovigerous females in all months of the year, but with distinguishable peak in abundance of gravid females in some months or seasons.

Since 2015, the National Commission for Stock Assesment under Ministry of Marine Affairs and Fisheries has included The Spawning Potential Ratio (SPR) as one of a method to measure the stock and health of the fishery. The SPR is the ratio of an average crab's lifetime reproductive potential under fishing, and the reproductive potential it would have in the absence of fishing. It measures the proportional reduction in reproductive potential caused by fishing. It is a function of fishing pressure (F), and the productivity of a particular species (M) and so is effectively proportional to F/M (Hordyk et al. 2015b). The SPR is an index of the relative rate of reproduction in an exploited stock. The basic concept of SPR is a proportion of the un-fished reproductive potential left by fishing pressure. Un-fished stock and individuals in an un-fished stock have an SPR of 100% ($SPR_{100\%}$), and fishing mortality decreases $SPR_{100\%}$ from the unfished level to $SPR_{X\%}$ (Prince et al. 2015).

Fishing activities in BSC fisheries carried out in a relatively short time on each trip by small-scale fishers, and they have one-day fishing as seen in Southeast Sulawesi (Madduppa et al. 2016). The common fishing gears used to catch BSC in Indonesia are bottom trawl (locally: "arad"), bottom gill net (locally: "kejer") and collapsible traps (locally: "Bubu"), which have listed as national fishing gears (DJCF, 2012). According to Nomura (1974) and Sumiono and Widodo (2006), the effectiveness of different fishing gears are different based on the gear design, materials and the method in which the gear is used.

Despite the importance of BSC in Indonesia, there is a poor understanding of its population dynamics, especially in the Java Sea, which is one of the most important fishing grounds in Indonesia and has the most fishermen. The aim of this study was to investigate the population biology (including the carapace width to weight relationship, size at first maturity and when first captured, and breeding season) and the spawning potential ratio of *P. pelagicus* in Java Sea at six selected important landing sites (Jakarta, Cirebon, Demak, Rembang, Sumenep and Sampit). We discuss the results of our study in the context of BSC fisheries management and conservation.

MATERIALS AND METHODS

Data collection

The study was conducted at six selected sites around the Java Sea (i.e., Jakarta, Cirebon, Demak, Rembang, Sumenep, and Sampit) from January 2014 to January 2015. Regular monthly sampling was undertaken at each site (Figure 1). A total of 14,408 individual crabs were collected across the landing sites during the study period (Table 1). Biological variables measured included: carapace width (mm), individual weight (g) and sex. The gonadal maturity stages were identified based on Sumpton et al. (1994). Catch, and effort-data were collected from fishermen or middlemen, including catch composition, catch per trip (kg), a number of trips and fishing gear used.



Figure 1. The location of BSC sampling sites in the Java Sea

Table 1. Number of *Portunus pelagicus* samples based on sex (male and female) at each landing site during the sampling period.

Site	Period	Male	Female
Cirebon	July-Dec 2014	1,333	718
Demak	Jan-Dec 2014	1,536	1,433
Rembang	Jan-Dec 2014	1,679	1,995
Sumenep	Jan-Dec 2014	2,360	2,156
Sampit	March-May 2014	302	253
Jakarta	Dec 2014-Jan 2015	315	311
Total		7,525	6,866

Data analysis

Carapace width to weight relationship

The carapace width to weight relationship was fitted using power regression for male and female BSC separately as follows: $W = a*CW^b$, where CW is the carapace width, a is the initial growth coefficient, and b is slope or growth coefficient (Ricker 1973). The relationship between carapace width to individual weight of males and females was tested using a t-test, to test whether isometric or allometric.

Growth

The growth parameters of BSC were determined by fitting the von Bertalanffy growth function $CW_t = CW_\infty * (1-e^{-k(t-to)})$ to the length frequency data using ELEFAN 1 incorporated in FiSAT II (Gayanilo *et al.*, 2005), where CW_t is the carapace width at the time t ;CW_∞ is the asymptotic carapace width; k is the growth coefficient; t_o is the theoretical carapace width at age 0. Maximum age (t_{max}) was calculated using empirical equation from Pauly (1980), $t_{max} \approx 3/k$, where k is the growth coefficient.

Natural Mortality

The natural mortality was estimated by the formula of Pauly (1980). Pauly mentioned there was water temperature influenced to natural mortality rate, as follows: $Log\ M = -0{,}0066 - 0{,}279\ log\ CW_\infty + 0{,}6543\ log\ k + 0{,}4634\ log\ T$, where M is natural mortality, CW_∞ is the asymptotic carapace width; k is the growth coefficient, T is mean of waters temperature.

Size at first maturity and at first captured

Size at first maturity (CW_m) was estimated by fitting a logistic curve to the relationship between the proportion of mature females in each size class, using the following formula $P = 1/ (1+\exp (-r (CW-CW_m))$, where P is the proportion of mature females BSC and r is a constant (King 1995). Determination of female crab gonads based on Sumpton et al. (1994), as follows: stage 1, no macroscopic mark of gonad; stage 2 immature of gonad, white or transparent, diameter of oocytes up to 0.14 mm; stage 3, maturing of gonad, light orange, not spreading into hepatic region, oocyte diameter 0.15-0.21 mm; stage 4, mature of gonad, bright orange, spreading into hepatic region, diameter of oocyte 0.22-0.40 mm.

Calculating the average size of crabs when first captured (CW_{50}) or CWc in the same gear as the trap trawl selectivity approach is to use the escape gap logistic function. The formula used is as follows: r (*CW*) = exp (a+b*CW*)/1+exp (a+b*CW*), where r (*CW*) is the opportunity for crabs of certain size to be retained, *CW* is carapace width of crabs that are captured, a and b are selectivity curve parameters (a<0 and b>0), so the carapace width where 50% retained, CW_{50} or CWc will be: $CW_{50} = -a/b$.

Spawning Potential Ratio (SPR)

An assessment of SPR was performed using the assessment software developed by Dr. Adrian Hordyk, Murdoch University (Hordyk et al. 2015), with the web-based interface. The SPR Reference Points used are as follows: (i) SPR 20%: Biological Limit Reference Point below which reproductive potential should be maintained before recruitment is impaired (Bunnel and Miller 2005b) and (ii) SPR 40%as the Biological Target Reference Point for ensuring sustainability (Hordyk et al. 2015b). Calculating SPR was needed some parameters life history by the ratios M/k, F/M, and CW_m/CW_∞ (Hordyk et al. 2015a).

Breeding season

The abundance and size of ovigerous crabs in Cirebon, Demak, Rembang, and Sumenep was recorded each month. Reproductive patterns for a breeding season were determined by a monthly distribution of mature female gonad and the proportion of ovigerous to the adult females (Rasheed and Mustaquim 2010, de Lestang et al. 2003a, 2003b, 2003c). Analysis of reproductive pattern was also completed by gonad condition of an ovigerous female. We propose that the spawning female population by estimating the relative percentage of adult females and ovigerous in each 5-mm CW size class, as well as their ratio as performed by Rasheed and Mustaquim (2010) and Zairion et al. (2015). Determination of ovigerous stages following Sumpton et al. (1994), as follows: stage 1, non-ovigerous; stage 2, ovigerous with egg mass paly to dark yellow, no eyespots outward in eggs; stage 3, ovigerous with yellow-grey of egg mass, present of eyespot; stage 4, ovigerous with grey egg mass, eyespots and chromatophores observable; stage 5, spent.

RESULTS AND DISCUSSION

Carapace width to weight relationship and width composition, growth, and size at first maturity and at first captured

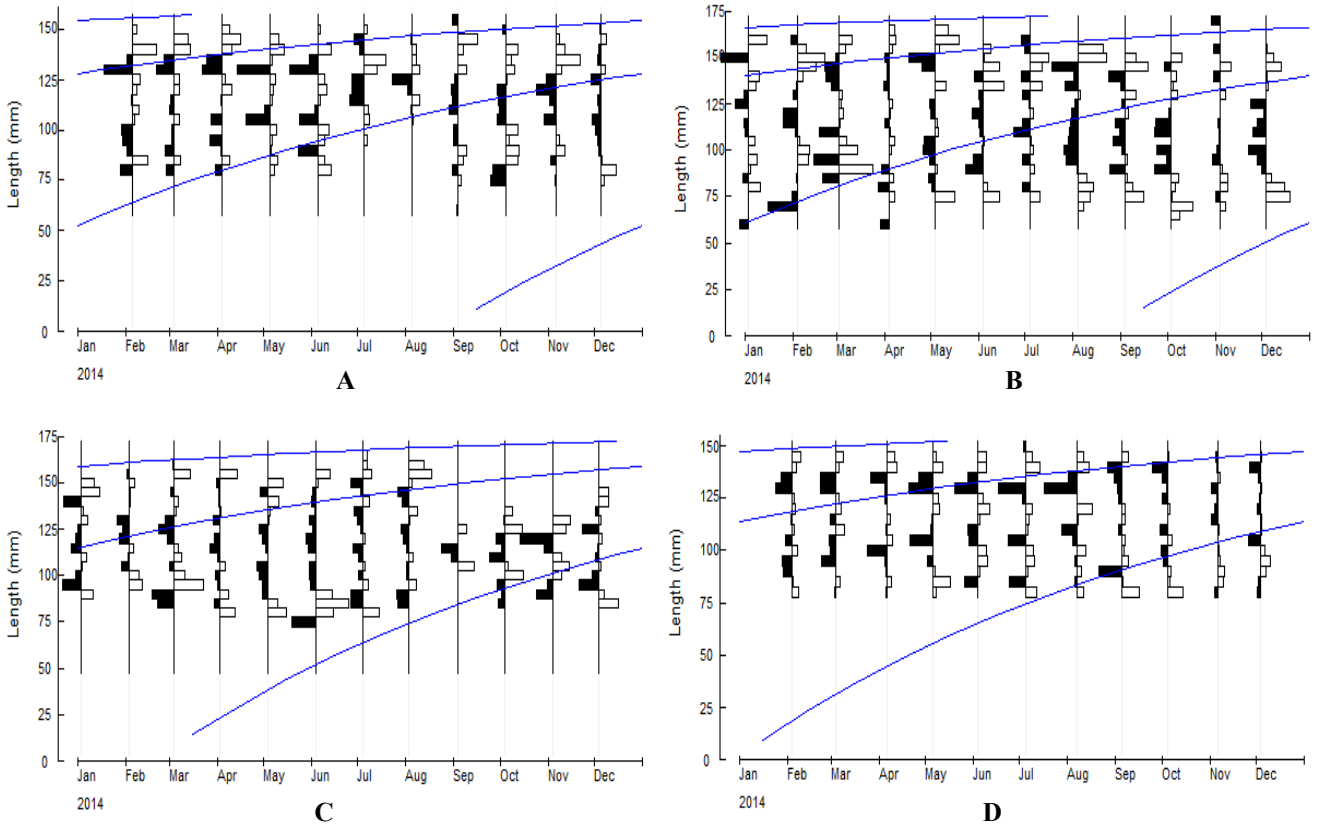
The males were heavier significantly than that of females as shown by a bigger *b* exponent for males (Table 2). The values for the exponent (b) in the present study (b<3 or b>3) indicated allometric growth. We found that the mean carapace width for both male and female in Jakarta was the smallest (males: 95.3 ± 10.7 cm, and females: 94.5 ± 11.4), and the highest at Sampit for both males (134.6 ± 14.7) and females (132.0 ± 14.3), compared with all other locations (Table 3). Our study found that males were heavier than females and growth were allometric. The mean width for male and female BSC in Jakarta was the smallest, while the largest carapace widths of both males and females were observed at Sampit.

Table 2. Carapace width to weight relationship for BSC at different landing sites (significance level, $p>0.05$ = n.s., $p<0.05$ = *)

Sex	Variable	Carapace width-weight relationship ($W = aL^b$)					
		Jakarta	Cirebon	Demak	Rembang	Sumenep	Sampit
Male	a	7.00E-05	4.00E-05	8.00E-06	2.00E-05	3.10E-03	2.00E-05
	b	2.995$^{n.s.}$	3.106*	3.449*	3.280*	2.220*	3.310*
	R^2	0.922	0.865	0.963	0.932	0.8303	0.8830
Female	a	1.20E-04	6.00E-05	1.80E-05	1.00E-05	3.23E-02	5.00E-05
	b	2.880$^{n.s}$	3.0234$^{n.s}$	3.2695*	3.394*	1.7118*	3.0722*
	R^2	0.8714	0.8668	0.935	0.947	0.8311	0.81
All	a	1.00E-04	4.00E-05	1.00E-05	2.00E-05	1.07E-02	2.00E-05
	b	2.9304$^{n.s}$	3.102*	3.347*	3.201*	1.953*	3.221*
	R^2	0.8955	0.8678	0.9443	0.928	0.8092	0.8512

Table 3. Carapace width size of male and female BSC at each landing sites during study period

Site	Male Size (mm)			Female Size (mm)		
	min	max	mean±SD	min	max	mean±SD
Jakarta	73.4	152.0	95.3±10.7	69.1	122	94.5±11.4
Cirebon	71.1	151.3	111.6±11.6	60.0	152.6	108.1±14.7
Demak	58.8	168.4	110.4±18.1	40.4	166.1	112.6±18.2
Rembang	70.4	159.2	115.3±15.5	75.4	165.6	116.4±15.8
Sumenep	75.6	148.0	116.0±15.6	77.2	143.8	114.9±17.0
Sampit	87.0	165.0	134.6±14.7	90.0	183	132.0±14.3



Figures 2. Growth of blue swimming crabs based on monthly carapace width distribution at four landing sites (A. Cirebon, B. Demak, C. Rembang, D. Sumenep) in 2014

Table 4. The growth parameters of combine male and female of BSC: CW_∞ (mm) = Carapace width at infinity, K (per year) = growth rate, t_o = age, t_{max} (year) = maximum age of BSC at each landing site

Site	Growth parameters			
	L_∞ (mm)	K (per year)	t_o	t_{max} (year)
Cirebon	168.60	1.05	-0.1040	2.86
Demak	179.35	1.11	-0.1083	2.70
Rembang	178.80	1.18	-0.1155	2.54
Sumenep	161.40	1.21	-0.1219	2.48

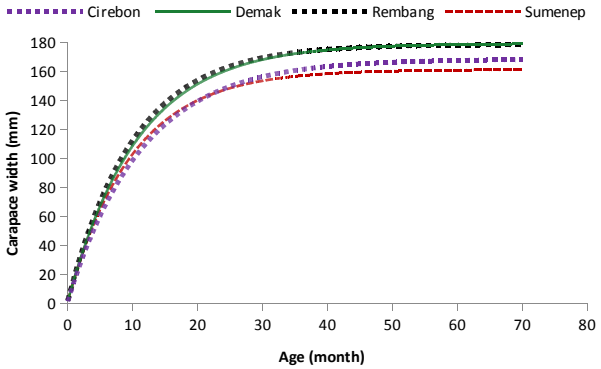


Figure 3. The growth curves for blue swimming crabs at different landing sites

Table 5. The mean carapace width (mm) at first maturity (CWm) and mean carapace width at first capture (CW_c) of BSC with different fishing gear used at each landing site

Site	CWm/ (mm)	CWc/ (mm)			
		Collapsible trap	Gill Net	Mini bottom trawl	Dredge Net
Jakarta	n/a	100.21	93.64	-	-
Cirebon	99.23	109.01	107.22	108.52	99.38
Demak	104.89	123.32	101.34	105.43	-
Rembang	101.06	115.72	108.84	-	-
Sumenep	101.86	114.13	-	-	-
Sampit	123.89	-	130.96	-	-

The growth parameters varied between the sampling locations. The growth of BSC based on monthly carapace width distribution at four landing sites is shown in Figure 2. The von Bertalanffy growth equation was used to determine the maximum age of BSC in each location. The infinite carapace width of combine male and female BSC at the four landing sites was ranged from 161.4 to 179.3; the maximum age was less than three year with fast growth (K >1) 1. (Table 4, Figure 3). The condition of gonad has been used to estimate of body size at sexual maturity for male and female (Castiglioni and Negreiros-Fransozo 2006) or allometric changes in growth of the body parts is related to functional maturity (Rasheed and Mustaquim 2010) or both (de Lestang et al. 2003a, 2003b).

The infinite carapace width at four landing sites was ranged from 161.4 to 179.3, and they can reach size up to 100mm in 10 months, with maximum age was less than 3 years and growth rate (K) has indicated a fast growth as shown by K value more than 1, showing that BSC in study area is a fast-growing crustacean with a short lifespan. This dynamic condition of growth parameter seems influenced by the differences of environment oceanography and exploitation rate. According to Beverton (1963) *in* Pauly (1980) that maximum carapace width (CW_{max}) is achieved approximately 95% of asymptotic carapace width (CW_∞).

The mean carapace width at first maturity (CW_m) in the Java sea at the sampled landing sites was 10.6 cm (Table 5), with the lowest CW_m of female crabs being landed in Cirebon and the highest in Sampit. The carapace width at first capture (CW_c) of BSC was varied at six landing sites. The lowest CW_c was obtained from bottom gillnet in Jakarta and followed by dredge net in Cirebon (Table 5). The highest CW_c was observed from Collapsible trap and gill net from Demak and Sampit, respectively. The mean carapace width at first maturity (CW_m) in the Java Sea at our study sites was 10.6±1.01 cm carapace width. Synchronous physiological and functional maturity was found in females when studied by both approaches, but some asynchrony was found for some studies of males. Therefore, physiological maturity is often used to determine the size at sexual maturity of females portunid crabs, since there is an actual capacity to release gametes, which corresponds to functional maturity (Castiglioni and Negreiros-Fransozo 2006). Also, morphometric maturity does not always indicate functional maturity (Oh and Hartnoll 1999) or may not coincide with physiological sexual maturity (Choy 1988). Previous studies on the northern coast of Java (Hermanto 2004; Sunarto 2012) and in East Lampung (Zairion et al. 2015) have found that the mean size at which 50% of females sexually mature (Lm50; judged by gonad condition) to be 105, 101 and 103 mm CW, respectively. Biometric data from ovigerous females *Portunus pelagicus* are not different by the embryonic development that characterized by the eggs color (Hamid et al. 2015).

This study found that mean CW_c was bigger than CW_m indicating that the caught crabs had already spawned. The lowest CW_c for BSC was obtained from bottom gillnet caught crabs landed in Jakarta, followed by crabs caught in dredge nets in Cirebon. The lowest CW_c for BSC was obtained from crabs caught by bottom gillnet landed in Jakarta, followed by dredge net caught crabs in Cirebon. This can be explained by the fishing area for bottom gillnet (Jakarta), and dredge net (Cirebon) in both locations are around near-shore areas such as estuaries. However, the mean L_c was bigger than L_m indicating that crabs caught in this study had spawned at least once. This situation must be maintained to ensure the long-term sustainability of the BSC stocks, especially in the Java Sea. Another factor that might influence the maturity size of BSC is fishing intensity because it may reduce the density of the species (de Lastang et al. 2003a, Dixon and Hooper 2011). Fishing area or sampling site (i.e., estuarine versus offshore) may also influence the mean size at maturity of female *P.*

pelagicus due to the emigration of mature females from estuaries to offshore habitats (de Lastang et al. 2003b, 2003c; Johnson et al. 2010).

Breeding season

The percentage distribution of ovigerous females crab in Cirebon, Demak, Rembang, and Sumenep was variable, and ovigerous females were recorded throughout the year at all sites (Figure 4). The peak spawning season occurred in different months at four sampling locations: Cirebon in March and August, Demak in March and October, Rembang in February and October, and Sumenep in March, May, and September. In general, the peak-spawning season in those locations had two peaks, between February-April and August-October.

Our analysis of *P. pelagicus* reproductive patterns was supported by supplementary evidence of the occurrence of ovigerous females and their ovarian conditions. The peak of ovigerous in each month identified the peak-spawning season. In this study, the ovigerous females were observed through the year, with two peaks, between February-April and August-October. The reproduction periodicity in the study sites was categorized as seasonal-continuous, due to the presence of an ovigerous female in all months of the year, but with a distinguishable peak in some months or seasons (Pinheiro and Fransozo 2002). In South Australia waters that the peak period for ovary maturation and spawning starts in October with a peak of ovigerous females occurring in November-December (Clarke and Ryan, 2004). In tropical regions, *Portunus pelagicus* breeds throughout the year (Batoy *et al.*, 1987). The peak of spawning or breeding season varied between locations and might relate to different environmental condition prevailing in that period, such as changes in temperature, salinity, rainfall, food availability, and predators (Pinhero et al. 2012).

Spawning Potential Ratio (SPR)

The life history parameters at each site were calculated and used to estimate the status of the BSC population at each landing site using the Length Based Spawning Potential Ratio (LB SPR) approach (Table 6). The estimated spawning potential ratio (SPR) for BSC was 11%, 15%, and 15% at Cirebon, Demak, and Rembang, respectively (Table 7). An SPR below 20% is below the Biological Limit Reference Point where reproductive potential and recruitment is impaired. While the SPR in Madura was 24%, it was below 30% meaning it was still below the Biological Target Reference Point target to ensure sustainability (Table 8). The length on the selectivity of the catch in the Java Sea was 123.5 mm (±1.4mm) and 158.7 mm (±2.04 mm) between 50% and 95% respectively. The output from the maturity and selectivity curves calculated by the LB SPR is shown in Figure 5.

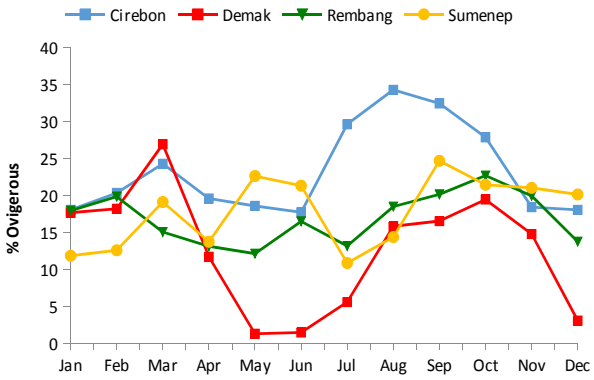


Figure 4. The monthly percentage of ovigerous females blue swimming crabs during 2014 at four landing sites

Table 6. The life history parameters used to estimate the stock status of the blue swimming crabs fishery in the study sites, using the length based spawning potential ratio approach

The life history paramenters	Cirebon	Demak	Rembang	Sumenep	Java Sea
Mortality/Growth Ratio (M/K)	1.392	1.393	1.394	1.394	1.393
Size on Maturity (50%) (L_{50})	99.23	104.89	101.06	101.86	101.57
Size on Maturity (95%) (L_{95})	110	121	118	144	125
CWm/CW∞ Ratio (CWm/CW∞)	0.588	0.585	0.565	0.628	0.534
Length Infinity (L_{∞})	168.6	179.35	178.8	161.4	190
B (egg production α length)	3	3	3	3	3

Table 7. Parameter Estimates (SD) from the Length Based Spawning Potential Ratio assessment of the blue swimming crabs fishery in the Java Sea for F/M (Fishing Mortality to Natural Mortality Ratio), SPR (Spawning Potential Ratio), S_{L50} (Length on 50% Selectivity) and S_{L95} (Length on 95% Selectivity).

Site	F/M	SPR	S_{L50}	S_{L95}
Cirebon	5.13 (0.765)	0.11 (0.006)	109.7 (2.805)	136.5 (4.314)
Demak	2.16 (0.135)	0.15 (0.008)	97.4 (1.264)	118.9 (2.419)
Rembang	3.92 (0.33)	0.15 (0.004)	114.2 (1.74)	141.3 (2.833)
Sumenep	10.97 (0.56)	0.24 (0.004)	144 (0)	182.7 (0.739)
Java Sea	5.24 (0.341)	0.15 (0.002)	123.5 (1.445)	158.7 (2.049)

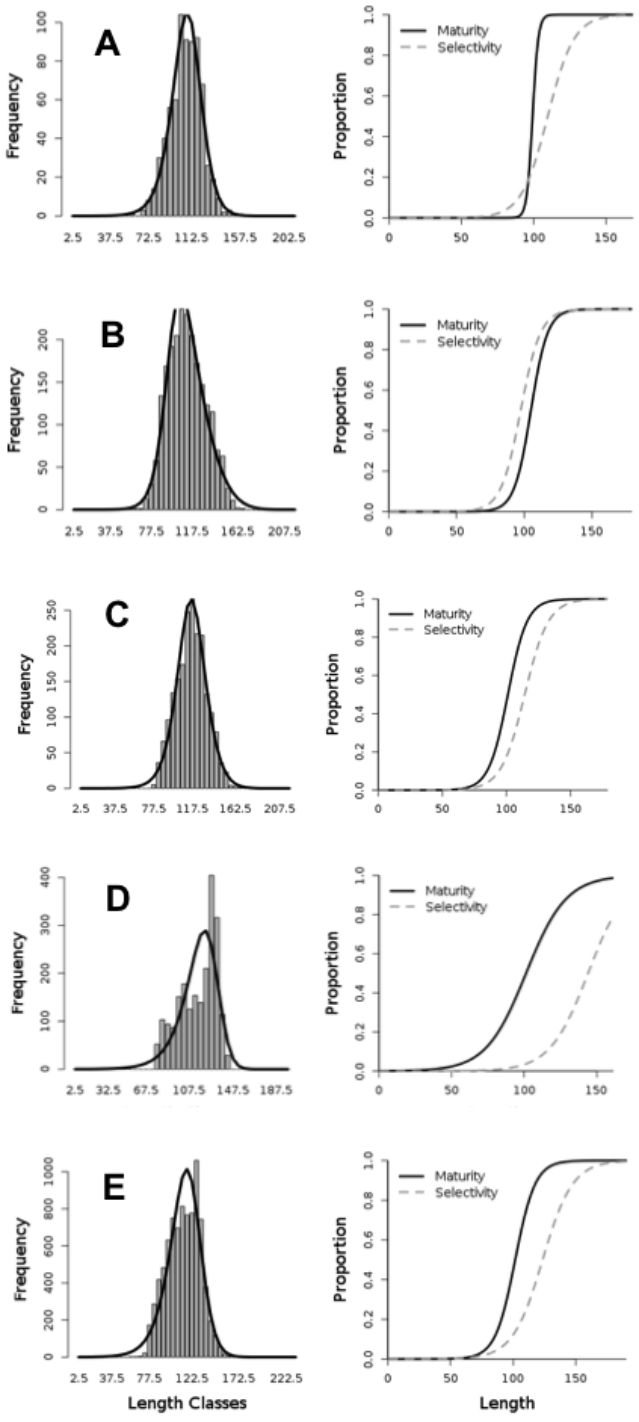


Figure 5. Frequency distribution of carapace length classes and proportion of different lengths at each site showing selectivity and maturity of captured crab at each site (A: Cirebon, B: Demak, C: Rembang, D: Madura, and E: Java Sea-Total)

The ratio of fishing mortality to natural mortality (F/M) was 5.24 (±0.34), with the highest value in Sumenep (10.97±0.56) and the lowest in Demak (2.16±0.13). The selectivity curves are shown in Figure 5.

Despite the mean Lm being 10.6 cm, the SPR ranged from 11 to 15% in Cirebon, Demak, Rembang, while in Madura it was 24%. To assist the recovery of the BSC stock in the Java Sea or to increase the SPR to the 20% level (the biological sustainability reference point) a minimum 12 cm carapace width is needed. Revisiting an earlier analysis (see Clark 1991), and after considering recruitment variability, Clark (1993) recommended SPR40% as maximum sustainable yield (MSY) proxies. More recently Clark (2002) expanded his analyses to consider lower values of slope (steepness) at the origin of the stock-recruitment-relationship, as might be expected for long-lived rockfish, but he still recommended a single level of SPR that would be sustainable for most fish without causing too much reduction in yield and to maintain more resilient stocks. He concluded that SPR>40% should meet current management needs, but noted that for very low productivity species, the appropriate fishing level could be F60% or even F70% (Clark 2002). However, the BSC is fast growing and has high productivity; the SPR40% could be used as target reference point. These analyses could become the basis for the SPR Reference Points widely recognized under Indonesian fisheries laws.

Implications for fishery management

Based on our findings we suggest the following. (i) A closed season for the fishery to conserve a brood stock. Plotting the number ovigerous or egg-berried females for each month would identify when this closed season should be implemented. The peak occurrence of ovigerous females in the Java Sea happens in September, October, and November. This assumes that after a couple of days the ovigerous females will hatch their eggs. We proposed that the closed season should be between March-May and August-September. (ii) We recommend a Minimum Landing Size for the *P. pelagicus* fishery in the Java Sea, which should be greater than Lm50 and we proposed 106 mm CW. This was the size of the mean ratio of ovigerous females to adult females, meaning that 21.38% of the spawning females in the population would have become part of the breeding population. This would be an appropriate pre-cautionary approach for maintaining a spawning and breeding female population and therefore stock productivity. This could be monitored through integrated traceability tools (see Madduppa et al. 2016). (iii) Concerning fishing gear selectiveness, the collapsible trap was the most selective gear, with an average size of BSC up to 112 mm at all landing sites, and the least selective was trawling. These results indicate that the ban on trawling as announced by the Ministry of Marine and Fishery decree No. 2/2015 could support the sustainability of BSC. The different gear types might need different forms of management to achieve the outcome of leaving smaller crabs in the water longer to grow. Boutson et al. (2009) mention that escapes gaps could be fitted to traps and that minimum size limits could apply to the catch of traps, dip-nets, and trammel nets, while mini-trawling could be kept to deeper water, where the larger crabs occur. Overall, optimal harvest control rules for any fishery resources bases on fishery management purposes, and keep maintaining the law enforcement of the MLS and fishing gear regulation.

In conclusion, the study observed that BSC is a fast growing (100 mm in months) and a short lifespan (less than three years). The most selective fishing was observed at the collapsible trap. The Spawning Potential Ratio (SPR) and the selectivity curve for the BSC fishery at each landing sites are positioned well to the right of the generic maturity curve, except in Demak, suggests that the operation enables almost all crabs to mature and spawn before entering the fishery. Therefore consideration needs to be given to the different gear types used to catch crabs to leave smaller crabs in the water longer to grow to maturity. Considering its fast growth and peaks season (February-April and August-October), a closed-period is recommended at some sites that have been over-exploited to rebuild a broodstock to support the long-term sustainability of this species.

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Biology and fisheries aspects of Western Longnose Spurdog, *Squalus edmundsi* from the Eastern Indian Ocean, Indonesia

FAHMI[1,♥], AGUS ARIFIN SENTOSA[2]

[1]Research Center for Oceanography, Indonesian Institute of Sciences. Jl. Pasir Putih I, Ancol Timur, Jakarta Utara 14430, Jakarta, Indonesia. Tel.: +62-21-64713850, Fax.: +62-21- 64711948, ♥email: fahmi.lipi@gmail.com

[2]Research Institute for Fish Enhancement and Conservation, Ministry of Marine Affairs and Fisheries. Jl. Cilalawi No.1 Jatiluhur, Jatimekar, Purwakarta 41152, West Java, Indonesia

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Abstract. *Fahmi, Sentosa AR. 2017. Biology and fisheries aspects of Western Longnose Spurdog,* Squalus edmundsi *from the Eastern Indian Ocean, Indonesia. Biodiversitas 18: 1714-1722.* A research has been done to determine some biological aspects of Western Longnose Spurdog (*Squalus edmundsi*) from the Eastern Indian Ocean Fishing Region in Indonesia (WPP 573). A total of 1797 samples were recorded from bottom longline fisheries at Tanjung Luar, Lombok from July 2015 to November 2016 by a trained enumerator. The study revealed that Selat Alas and the south west of Sumbawa waters are the most fished areas in the region for this species, with the peak fishing season may occur from January to March. The average catch rate of *S. edmundsi* was about 8 individuals per boat. The size of sharks varied from 470 mm to 1150 m total length (TL), with average size was 723.6±111.5 mm. Size distributions and sex ratio between females and males were significantly different, indicating a sexual dimorphism. This study revealed a fact that there was no seasonal pattern in the reproductive cycle of *S. edmundsi* in this region, which means the parturition may occur throughout the year. Analysis on its population status showed that this species was very susceptible to overfishing. This condition should be responded by the government through management actions for its fishery.

Keywords: *Squalus edmundsi*, Squalidae, biology, Indian Ocean

INTRODUCTION

Eastern Indian Ocean Fishing region (WPP573) is one of the most important fishing regions in Indonesia. It covers about a half of Indonesian's Indian Ocean area from south of Java to south of East Nusa Tenggara waters. This area is also considered as one of the most exploited waters in Indonesia, due to many fishing methods are involved, from traditional to industrial fisheries (Blaber et al. 2009; Fahmi and Dharmadi 2013a).

This area is also the most favorite spot for shark fishery, with the average shark catch more than 10,000 tons annually until 2007, but then there was a rapid decline in the following years to around 4,000 tons (Fahmi and Dharmadi 2013a). Deepwater shark fishery plays an important role at several fish landings in the region due to the high demand of shark liver oil in both domestic and international markets in which deepwater sharks such as squalids and centrophorids are target species (Fahmi and Dharmadi 2013a). There are several fishing methods to catch deepwater sharks, but the most common one is bottom longlining, which operates in the depth from 50 to more than 400 m (Dharmadi and Fahmi 2007; Fahmi and Dharmadi 2013a).

Dogfish shark or spurdog shark (Family Squalidae) is one of the most commonly caught in deepwater shark fishery in Indonesia (Fahmi and Dharmadi 2013a). At least five species of squalids are known to occur in this area, i.e. *Squalus edmundsi* White, Last and Stevens 2007, *S. hemipinnis* White, Last and Yearsley 2007, *S. montalbani* Whitley 1931, *S. nasutus* Last, Marshall and White, 2007, and *Cirrhigaleus barbifer* Tanaka, 1912 (White et al. 2006; Last et al. 2007; White et al. 2007). The Western Longnose Spurdog, *Squalus edmundsi,* is known as one of the most common squaloids in the deepwater shark longline fisheries operating in the eastern Indian Ocean fishing region, together with the Indonesian short snout spurdog, *Squalus hemipinnis* (Dharmadi and Fahmi 2007; White and Dharmadi 2010). This species is a member of the "*Mitsukurii*" group of the Family Squalidae, which is characterized by relatively large size, having a dark caudal bar through the base of lower caudal fin lobe, low dorsal fin spines, and first dorsal fin spine is shorter than the second (Last et al. 2007; White et al. 2007; Last and Stevens 2009). *Squalus edmundsi* was previously known as *Squalus* sp. C in previous kinds of literature (Last and Stevens 1994; White et al. 2006). This species is usually caught at a depth between 200 and 850 m and well distributed in the East Indian Ocean from Indonesia to Western Australia (White et al. 2007; Last and Stevens 2009).

The conservation status of this species in the IUCN red list for threatened species is categorized as Near Threatened (White 2009). Nevertheless, biological information of *S. edmundsi* is still very few. This article aims to provide updated information on the biology of this species including its fishery.

MATERIALS AND METHODS

A daily survey had been conducted to record shark catch landings at Tanjung Luar, East Lombok District, West Nusa Tenggara Province, Indonesia from July 2015 to November 2016 (Figure 1). Information on the total catch, species name, sex, and length of the shark's species was gathered through direct observation at landing site by a trained enumerator, while a number of boats operated, boat name and number of days' trip were recorded through interviews and using landing port data. Shark species were identified following identification guides by White et al. (2006) and Fahmi and Dharmadi (2013b). The total length for each shark was measured to the closest cm. Females and males were differentiated by the presence of claspers organ on males.

Length frequency of both sexes was compared and tested using two-samples Kolmogorov-Smirnov test (Motta et al. 2005) to see whether any differences in their size distributions. Sex ratio between females and males was also analyzed using Chi-Square test to determine the significant difference from the expected ratio 1: 1 (Gay 1996).

Length-weight relationship was calculated using the standard power equation $W=aL^b$ and ln transformation was applied to plot the linear regressions. Analysis of Covariance (ANCOVA) was used to test the difference of length-weight relationships between female and male. The maturity stages of females were not analyzed as specimens were not dissected. Maturity stages of males were classified based on the condition of the claspers. The non-calcified and not fully-calcified claspers were categorized as immature, while the full-calcified claspers were categorized as mature (White and Dharmadi 2010). Length at first maturity of male *S. edmundsi* was estimated using Spearman-Karber method (Udupa 1986).

The catch rate (*R*) of *S. edmundsi* from the bottom longline fishery operating from Tanjung Luar, Lombok was calculated using formula following Hoenig et al. (1997):

$$R = \frac{\sum_{j=1}^{n} Cj}{\sum_{j=1}^{n} Lj}$$

Where, *Cj* = number of catch per boat; *Lj*= number of the day at operation per boat; and *n* = number of boats.

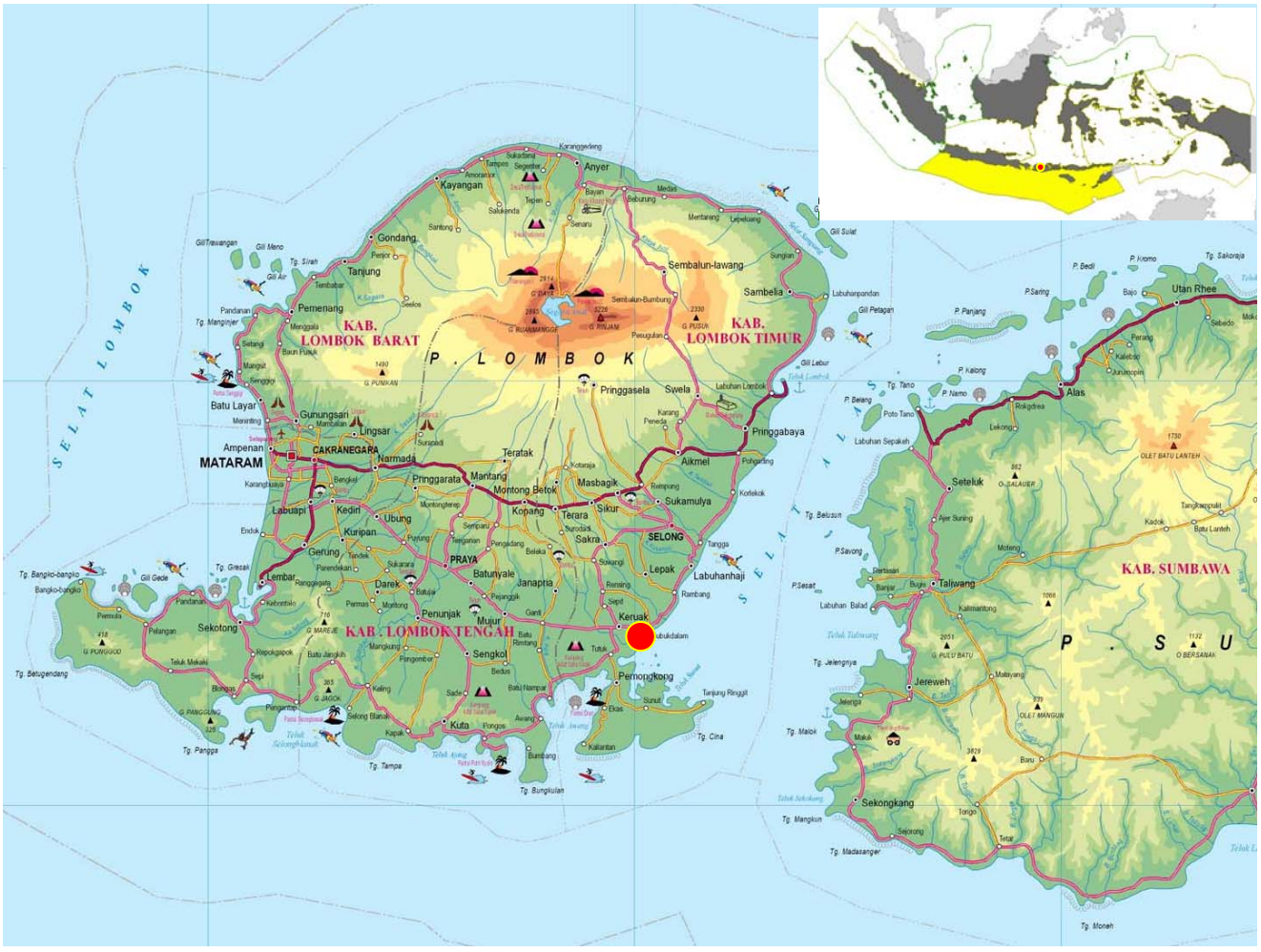


Figure 1. Study site at Tanjung Luar (●), Lombok Island, in Eastern Indian Ocean Fishing region (WPP573; yellow highlighted)

In order to determine the population structure of this species in Eastern Indian Ocean fishing region, the length-based frequency data was used to estimate the growth curve that "best" fits a set of length-frequency data in the growth parameters. The growth parameters were estimated using Von Bertalanffy model and natural mortality was estimated using Pauly's empirical equation from the regression line which extrapolated from the length-converted catch curve. Relative yield per recruit (Y′/R) was estimated using Beverton and Holt model that was modified by Pauly and Soriano (1986) and plotted with the exploitation level to estimate the maximum exploitation level (E_{max}). All analyses were performed using FISAT II software package (Gayanilo et al. 2005).

RESULTS AND DISCUSSION

Size distribution

A total of 1797 individuals of Western Longnose Spurdog, *Squalus edmundsi* were recorded from the deepwater longline fishery during the study period, with 997 of them were measured and weighed. The sharks were caught at size between 470 and 1150 mm Total length (TL) with average size at 723.6±111.5 mm TL and mode length was at 680 mm TL. According to their sex, length frequency for females was 470-1150 mm (n=802) and 490-990 mm for males (n=192). The mean and modal length of females were 746.67±107.81 and 700 mm, while males were 626.87±67.80 and 570 mm TL, respectively. The size class 670-719 mm was a predominance of females, while males were predominant at size class 620-669 mm (Figure 2 and 3). The maximum size of *Squalus edmundsi* in this study was higher than those stated in White et al. (2007) and Last and Stevens (2009).

The t-test showed a significant difference in the average of total length between females and males (t-test, $p<0.01$). While the Kolmogorov-Smirnov test (KS) for significant differences in the size distributions indicated that females and males were taken from populations with different distribution ($p< 0.05$). This result clearly showed that males are generally smaller in size comparing to females. The monthly averages of the total length of *S. edmundsi* also show that the size of females was always greater than males. The average total length of females varied from 694-834 mm TL, while males were from 583-702 mm TL. (Figure 4).

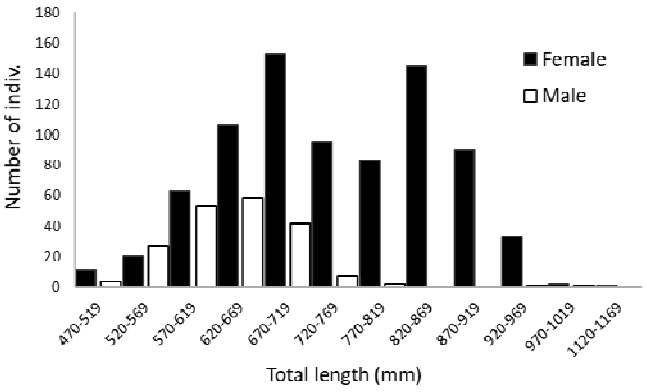


Figure 2. Length frequency of females and males of *Squalus edmundsi* from Tanjung Luar landing site from July 2015 to November 2016

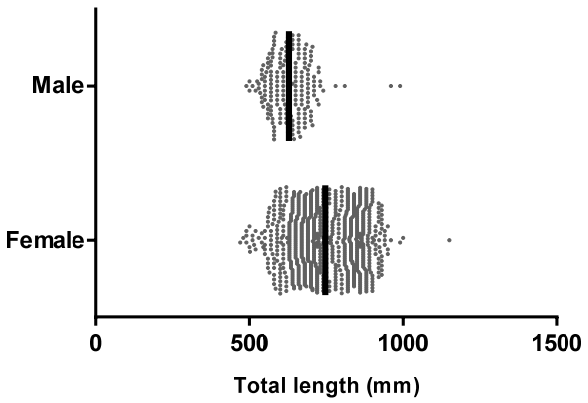


Figure 3. Scatter plot graph shows the size distribution of females and males of *Squalus edmundsi* from Tanjung Luar landing site. Black lines indicate the mean size

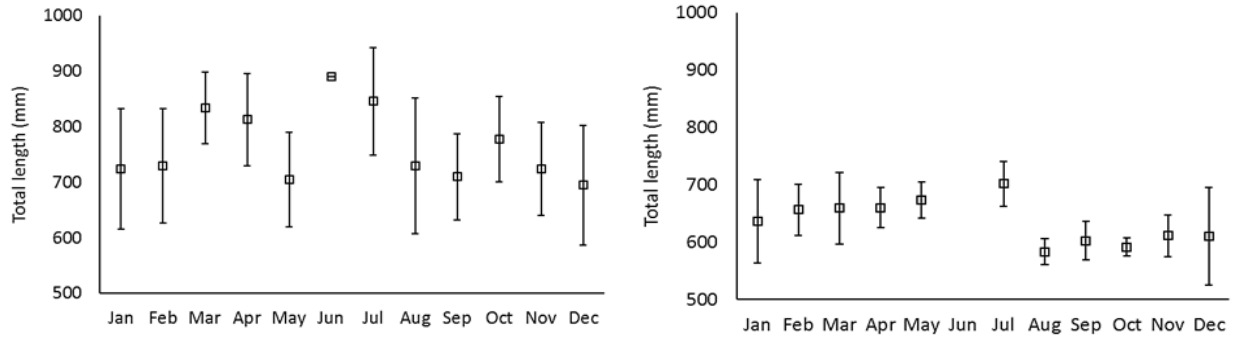


Figure 4. Monthly mean total length of females (left) and males (right) *Squalus edmundsi* from Tanjung Luar landing site. Error bars show standard of deviations

The greater size of females compared to males showed a clearly sexual dimorphism. The fact that females are generally larger than males also occurred on other *Squalus* species such as *S. acanthias* Linnaeus 1758 and *S. megalops* (Macleay 1881) (e.g. Hanchet 1988; Watson and Smale 1998), and also for most sharks (Compagno 1984; White et al. 2006).

Sex ratio

The total number of females *S. edmundsi* captured during the study period was much higher than males, reaching up to four times. A total of 802 females were recorded during the study comparing to 195 males. Overall sex ratio between males and females of *S. edmundsi* in this fishing region was 1: 4.11. This ratio indicated that there was a significant difference from the ratio 1: 1 (χ^2 = 369.56; P<0.05). In general, the monthly sex ratio between males and females were also significantly different throughout the year (Figure 5), except for those in July 2015 when the sex ratios were equal (χ^2 = 0 and 1; P>0.05).

The imbalance proportion between females and males throughout the year indicates that there are segregation patterns in the population of *S. edmundsi* in the Eastern Indian Ocean fishing region. This study revealed the segregation is not only by sex but also by body size or age (between young and adults, see Figure 8). Many authors noted that segregation patterns in elasmobranchs occur not only between females and males but also between size class and reproductive stages (Nammack et al. 1985; Rago et al. 1996; Ellis and Shackley 1997). The disparity in the sex ratio, which more females than males, commonly happen on several deepwater shark species, such as *Squalus megalops* (Macleay 1881), *S. acanthias* Linnaeus 1758, *Deania calcea* (Lowe 1839), *Etmopterus granulosus* (Günther 1880), *E. spinax* (Linnaeus 1758), *Mustelus antarcticus* Günther 1870, and other squalids (see Vince 1991; Lenanton et al. 1990: Wetherbee 1996; Graham 2005; Hazin et al. 2006; Gennari and Scacco 2007; Moura et al. 2014; Irvine et al. 2012).

The imbalanced sex ratio may give implication to the decrease of reproduction outputs. However, there is no evidence of this situation can lead to lower recruitment due to lack of information on the reproductive behavior of this species. The differences in the sex ratio are thought to be related to breeding behavior and are likely to result in unisexual aggregations in certain areas at different times of the year (Compagno 1984; Ellis and Shackley 1997). Several factors related to the sexual and size segregation patterns of a deepwater shark have been identified for particular species, and reproductive strategy could be the main reason (Main et al. 1996; Sims et al. 2005; Cabello et al., 2007). Main et al. (1996) suggested that females occupied an area where the food source is available. While Sims et al. (2005) suggested that males tend to swim more actively to seek females, while females may just stay in a hiding place such as sea canyons. These segregation patterns, therefore, should be put into consideration of the management plan of this species. Hanchet (1988) found that females *Squalus acanthias* in New Zealand waters move to shallower waters at about 50 m depth during their gestation period and swim back to deeper water (200-300m) where parturition, mating, and ovulation take place. This migrating behavior of females *S. acanthias* into shallower waters has also been identified in other areas such in British waters and the southern Black Sea (Compagno 1984; Avsar 2001). On the other hand, Graham (2005) found that the mature females of *S. megalops* tend to aggregate at the deeper water than the mature males.

The bias in sampling method was also suggested by several authors, especially for the sample collected by commercial fishers (Lenanton et al. 1990; Ellis and Shackley 1997; Watson and Smale 1998). Sharks may be taken only from particular areas where may not represent the entire range of the species distribution. The abundance of females in the catch in particular period of time may also indicate the seasonal variation in the reproduction cycle of several species such as the lesser spotted dogfish *Scyliorhinus canicula* (Linnaeus 1758) and the gummy shark *Mustelus antarcticus* (see Lenanton et al. 1990; Ellis and Shackley 1997).

Length-weight relationship

A total of 971 individuals of *S. edmundsi* were used to generate the length-weight relationship curve for combining sexes. The weight of this species ranged from 400 and 4800 g for females and from 400 to 2200 g for males. The relationships between total length (TL) and total weight (W) were expressed by the formulas: $W=0.00000017\ TL^{3.81}$ (r^2=0.85; n=971) for combining sexes; $W=0.00000018\ TL^{3.85}$ (r^2=0.83; n=781) for females; and $W=0.000000018\ TL^{3.95}$ (r^2=0.76; n=190). The ANCOVA test failed to detect significant differences in the slopes between regressions of the ln transformed TL and W for both females and males (F=3.6; p>0.05) (Figure 6). These relationships may indicate that the sampled sharks in this study were from one age group or in the same condition factor.

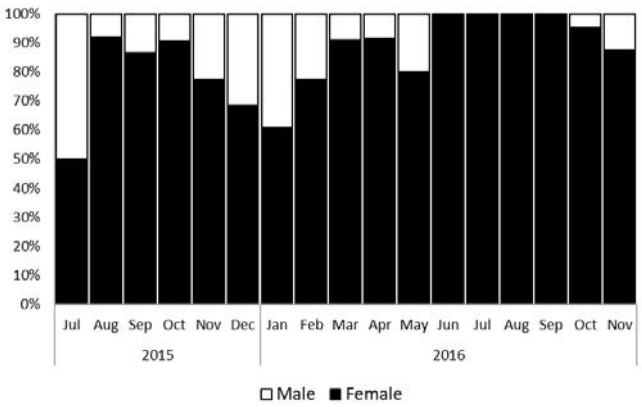


Figure 5. The sex ratio between males and females of *Squalus edmundsi* from the Eastern Indian Ocean fishing region at several occasions from July 2015 to November 2016.

The length-weight relationship analysis is essential to determine the fish condition in its environment (Safran 1992). The variations in length-weight relationship may indicate different sample size, unequal size distribution between males and females or age groups, and also ontogenetic differences (Stevens and Wiley 1986). The presence of high proportion of sampled pregnant females in the data set may lead to the variation in length-weight relationship between the sexes, as the weight of pregnant females is considerably heavier than non-pregnant ones (Motta et al. 2005).

Length at maturity

A total of 196 males were examined to determine the maturity level of males *S. edmundsi*. During the study period, about 75% of sampled males were categorized as mature, with the rests were immature. The smallest size of mature male recorded in this study was at 520 mm TL and the largest immature male was identified at 640 mm TL (Figure 7). The size at first maturity (L_{50}) of male *S. edmundsi* estimated using Spearman-Karber method was at 597.6 mm TL, with 95% confidence limits of class between 556.8 and 641.4 mm TL, respectively.

The size at first maturity of males in this study is similar to White et al. (2006) but higher than those are stated in Last and Stevens (2009) and White and Dharmadi (2010). While the smallest size of mature male recorded in this study was slightly shorter than stated in White et al. (2007). The size distribution and monthly mean total length of *S. edmundsi* showed that this species was mainly caught in the mature stage. The averages of the total length of both sexes were greater than the estimation size at first maturity. It means the sharks were caught from a population which consists of more than 50% mature animals.

The monthly catch proportion between immature and mature male sharks also showed an unfairness (Figure 8). Mature males dominated the catch for most of the year except from August to October. However, due to the low number of sample collected during the study, it is difficult to state that the seasonal reproduction cycle occurs on the male *S. edmundsi*. The occurrence of mature individuals in significant proportion in each month data indicated that there is no seasonal reproduction cycle for this species. Nevertheless, there is no information on the maturity stages of females that may lead to uncertainty whether the fishing areas of *S. edmundsi* are utilized as mating or breeding zones. The occurrence of mature individuals in relatively large numbers throughout the year indicated a non-seasonality of the reproductive cycle, which means that parturition occur throughout the year. A similar pattern is also shown by its sister species, the shortnose spiny dogfish (*Squalus mahia,* previously identified as *S. megalops*) from the Agulhas Bank, South Africa, that (Watson and Smale 1998; Viana et al. 2017).

Watson and Smale (1998) suggested that the absent of juveniles and young sharks in the sampling result was affected by the gear selectivity, such as using the trawl net with large mesh size. However, Graham (2005) and Rochowsky et al. (2015) found evidences that the gear selectivity was not one of key factors of the exclusion juveniles and young sharks from the catch.

Catch and efforts

According to interviews and landing port data, the fishing area of the deepwater longline fishery operating from Tanjung Luar is from the south of Lombok to the Sawu Sea with the number of days of operation varied from one to 20 days. Based on the catch data, *S. edmundsi* were caught at a larger number from Alas Strait and the south west of Sumbawa waters rather than any other fishing areas.

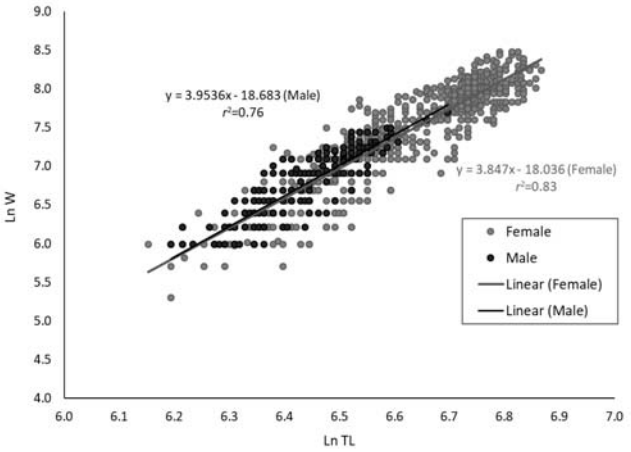


Figure 6. Length-weight relationships between ln transformed TL and ln W of females and males *Squalus edmundsi* in the eastern Indian Ocean fishing region

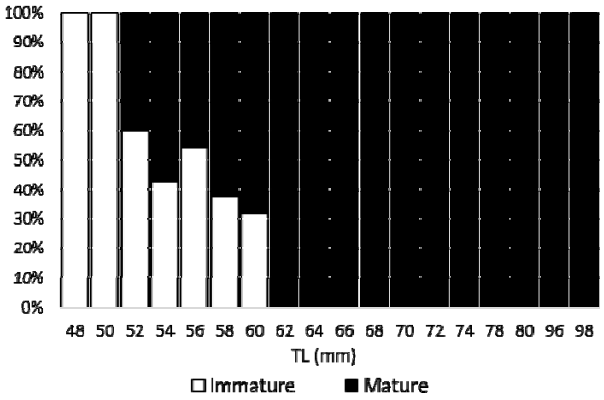


Figure 7. The proportion of immature and mature males of *Squalus edmundsi* based on their length class

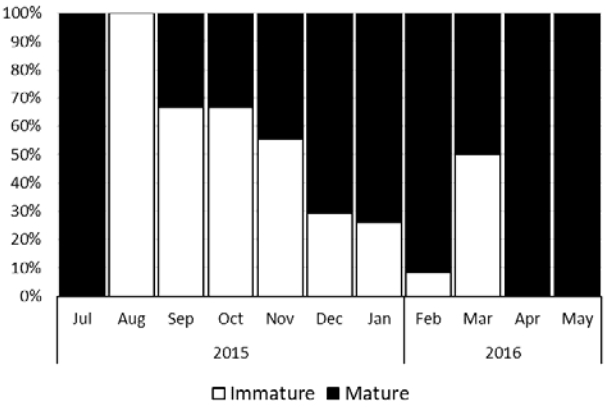


Figure 8. The proportion of immature and mature males of *Squalus edmundsi* at several occasions from July 2015 to May 2016

The numbers of the catch of *S. edmundsi* were fluctuated through time, with the peak season was identified from January to March (Figure 9). During the peak season, the catch reached up to more than 300 individuals per month. In contrast, the lowest catch occurred in June 2016 by only a single individual recorded during this time.

The estimation of daily catch rate of *S. edmundsi* by all fishers operating from Tanjung Luar was 2.66 individuals per boat. According to the number of days at sea, the short time fishing periods (1-2 days) had relatively higher catch rate rather than the longer periods of fishing (Figure 10). As the main fishing gear to target the deepwater sharks, bottom longline plays important role in the number of catch of *S. edmundsi.* The total catch per unit efforts (CPUEs) for catching the species using this single type of fishing gear during the period was 8.46±4.91 ind/boat and 2.95±2.89 ind/day of operation. The monthly CPUE of bottom longliners fluctuated throughout the year. Similar to the total catch, the period between January to March seemed to be the peak season for dogfish fishing, with the CPUE reached a peak in January (Figure 11).

The deepwater shark fishing session seemed different from the pelagic sharks fishing session in the same area. The pelagic shark fishing session mainly occurs from June to October each year, when sharks are usually caught in large numbers comparing to other months. This fishing period is convinced related to the east monsoon season when strong upwelling occurs along the coastline of the Eastern Indian Ocean bringing the high level of primary productivity and the abundance of fishes in the area (Fahmi and Dharmadi 2015). The relatively low productivity of the deepwater fishery in this period may be affected by most fishers from Tanjung Luar focused and attracted more to catch pelagic sharks. It is also shown by the fairly lower number of bottom longline boats operated during the period, leading to the lower CPUEs. On the other hand, a period between January and March seems to be the peak season for the deepwater shark fishery. There are two main reasons that make it happened. First, the west monsoon occurs from November to March, when air masses are flowing from Asia to Australia causing the rainy season in Indonesia. During this period, strong wind, high wave, and heavy rain, commonly happen in the eastern Indian Ocean and adjacent areas (Kurniawan et al. 2011). This situation leads to the difficulty for fishermen, especially who own relatively small fishing boats to go further and make a long trip for fishing. Alternatively, they tend to make a short fishing trip and catch the sharks in the nearby areas such as around Alas Strait, south Lombok, and south-west Sumbawa. As a result, the CPUEs of squalid fishing in the period from January to March was significantly higher than other months.

Population status

The growth parameters of *S. edmundsi* were calculated based on the class-length frequency data with sex combined, due to the low numbers of individuals recorded in each month. The results of the Von Bertalanffy's growth curve parameters were $L\infty$ =1176mm, k=0.16 year^{-1},t_o= -0.39 year, respectively. The asymptotic length ($L\infty$) value was fairly close to the largest observed shark, which was 1150 mm with the estimated age at 26 years, while the shark is estimated to get its first maturity at 4-5 years old (Figure 12).

Mortality rates in length-based method generated by ELEFAN I routine in FISAT, showed that instantaneous total mortality coefficient (Z) was 0.69 year^{-1}, natural mortality (M) 0.19 year^{-1}, fishing mortality (F) 0.5 year^{-1}, and current exploitation ratio was 0.73year^{-1}. Based on the plot of relative yield per recruit (Y′/R) and exploitation ratio (E), the maximum exploitation level (E_{max}) was estimated to be 0.68 year^{-1} (Figure 13).

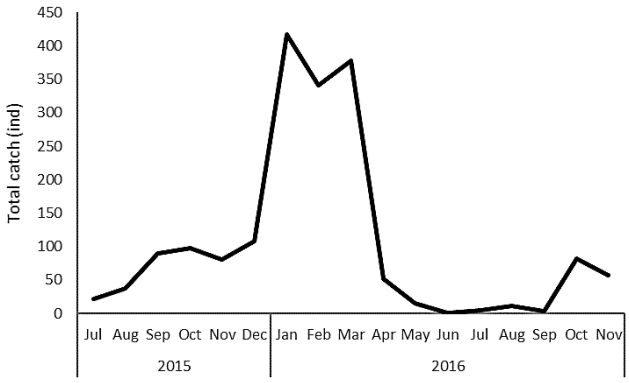


Figure 9. Total catch of the *Squalus edmundsi* at Tanjung Luar landing site from July 2015 to November 2016.

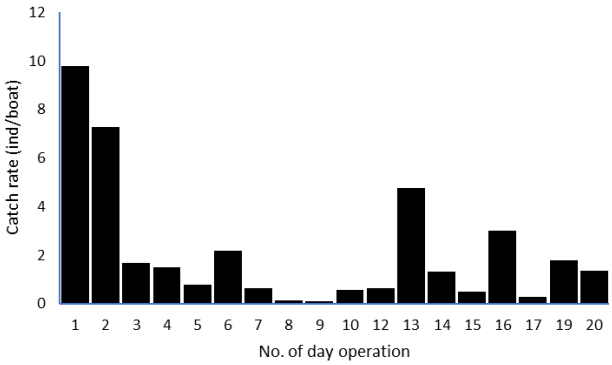


Figure 10. Catch rates of *Squalus edmundsi* based on the number of days operation

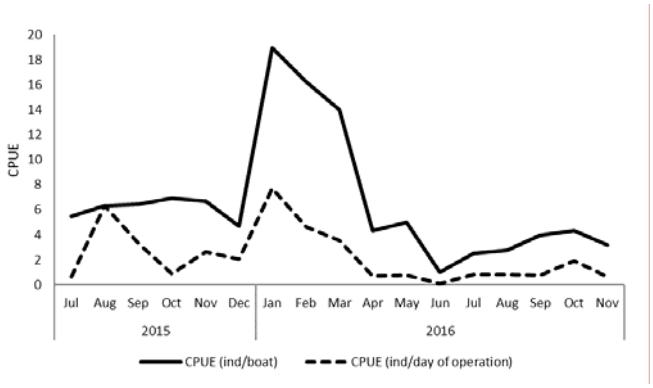


Figure 11. Catch per Unit Effort of *Squalus edmundsi* based on the number of boat and number of days at operation

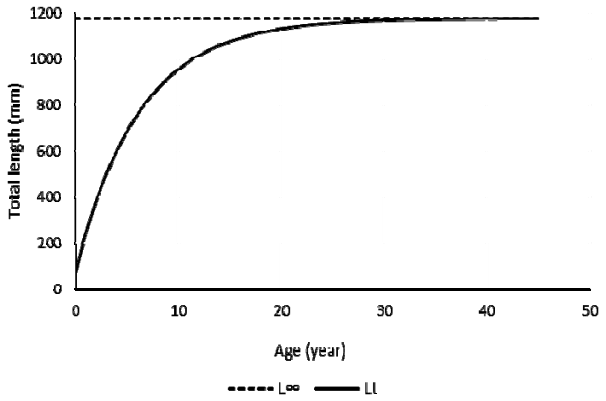


Figure 12. Von Bertalanffy growth curve from length frequency data for *Squalus edmundsi*

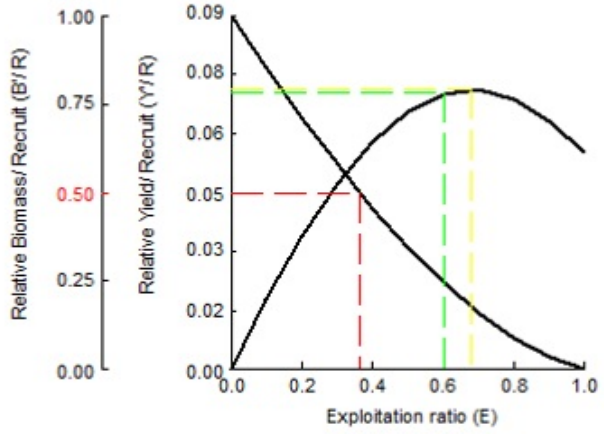


Figure 13. The relationship between relative yield per recruit (Y′/R) and relative biomass per recruit (B′/R) using selective ogive of *Squalus edmundsi*

Understanding the age and growth parameters of sharks is important to determine the population status and to predict the change of populations through time (Cailliet et al. 1986; 2006). The using of the ELEFAN I method to estimate the Von Bertalanffy growth curve based on length-frequency distribution data may underestimate the values of growth coefficient (k) and overestimate the asymptotic length (L∞) due to the difficulty to determine modes when the cohorts are overlap, linearization of the model and assumption of individual variability of growth parameters (Isaac1990; Tsangridis and Filippousis 1991; Sahinler and Can 2003). The bias of k value is still adequate if the coefficient of variation of the parameters L∞ and k not exceed 10% (Isaac 1990).

The underestimation of age parameter can lead to a serious impact on overexploitation of a population or species due to overly optimistic estimates of growth and mortality ratio. Therefore, methods for quantifying aging accuracy and age validation should be implemented (Campana 2001). The most common known method to verify age and growth parameters of sharks is using marginal increment analysis, following by centrum edge analysis and back-calculation method, while size frequency analysis is the least due to the costly and time-consuming (Campana 2001; Cailliet and Goldman, 2004).

Previous studies on estimation of age and growth parameters on squaloids were mostly using dorsal spines (Machado and Figueiredo 2000; Avsar 2001; Clarke et al. 2002a; 2002b; Irvine et al. 2006a; 2006b).The age and growth estimation of *S. edmundsi* in this study was relatively higher than its congeners from other areas. The growth coefficient (k) of *Squalus acanthias* from the Black Sea was estimated 0.17 for both sexes with estimation of maximum age at 13-14 years (Avsar 2001). This species was estimated to get its first maturity at age 12-13 years old based on specimens from Swedish waters (Stenberg 2005). Another congener species, *Squalus mahia*, from South Africa was estimated to have k values 0.033 and 0.089 for females and males, attaining its first maturity at 15 years (females) and 9 years (males) with maximum age at 27 and 18 years, respectively (Watson and Smale 1999).

The closely related species with *S. edmundsi* in "*Mitsukurii*" group, *Squalus mitsukurii*, has variously estimated k-values depending on its area. The estimated k-values for species from central North Pacific Ocean were 0.041 for females and 0.155 for males (Wilson and Seki 1994), while those from Japan were varied from 0.039 to 0.103 for females and from 0.06 to 0.252 for males (Taniuchi and Tachikawa 1999). Females of *S. mitsukurii* were estimated to have their first maturity at a later age compared to males, which were 15 years compared to 4 years in North Pacific, and 9-16 years compared to 5-7 years in Japan (Wilson and Seki 1994; Taniuchi and Tachikawa 1999).

The exploitation rate (E) of *S. edmundsi* in this study was estimated to be higher than its maximum exploitation (E_{max}), which means there is an indication of overfishing on its population in the area. Dogfish sharks and most of the deepwater sharks are living in low productivity environment, having slow growth and high longevity, which makes them highly susceptible to overexploitation and stock depletion, especially if they are targeted (Clarke et al. 2002a; Daley et al. 2002; Irvine et al. 2006a; 2006b). Norse et al. (2012) stated that deep-sea fishes can be sustainably fished at very low catch rates if maintained by respectable fisheries manager.

In conclusion, analyses on the population status of *Squalus edmundsi* showed that this species was susceptible to overfishing, as indicated by the high value of its exploitation rate. The threat on this species population is worsened by none of the fishery-related management to the deep-sea shark fishing in Indonesia. As squaloid shark liver oil has high demands and high prices in the international markets, this product has become a target in the deep-sea fishery. Currently, there is no species-specific catch data of *S. edmundsi* in Indonesian fishery statistics, as they are compiled together with all other squaloid sharks. This condition makes it difficult to assess the conservation status of the species for its fishery management. This study accordingly can be a consideration for the government to start paying attention to the *S. edmundsi* and other deep-sea sharks by implementing a sustainable deep-sea fisheries management.

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Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

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Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com

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