

Diversity of sponge-associated fungi from a mangrove forest in Kemujan Island, Karimunjawa National Park, Indonesia

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Abstract. *Sibero MT, Pribadi R, Larasati SJH, Calabon MS, Sabdono A, Subagiyo S, Frederick EH. 2021. Diversity of sponge-associated fungi from a mangrove forest in Kemujan Island, Karimunjawa National Park, Indonesia. Biodiversitas 22: 5695-5605.* Plenty of studies have reported interesting biological properties and bioactive compounds from sponge-associated fungi in Indonesia. However, only a few studies consider their biodiversity. Most of the studies collected the sponge from the coral reef ecosystem and barely reported from the mangrove ecosystem. This study aimed to discover the biodiversity of sponge-associated fungi from a mangrove ecosystem using a DNA barcoding approach. Five mangrove-associated sponges were collected from a tidal mangrove forest in Kemujan Island, Karimunjawa National Park, Indonesia. In total, we isolated 56 sponge-associated fungi, which consisted of seven genera in the phylum Ascomycota (77.78%), one genus in the phylum Basidiomycota (11.11%), and one genus in the phylum Mucoromycota (11.11%). *Trichoderma* (n: 15, 26.79%), *Aspergillus* (n: 13, 23.21%) and *Fusarium* (n: 12, 21.45%) were the top three dominant genera. In addition, based on the association of fungi to its sponge hosts, *Aspergillus*, *Aureobasidium*, *Fusarium*, and *Trichoderma* are sponge-generalists; *Absidia* and *Pestalotiopsis* are sponge-associates; while *Lasiodiplodia*, *Montagnula*, and *Wallemia* are sponge-specialist.

Keywords: DNA barcoding, marine facultative fungi, marine mycology, phylogeny, tropical mycology

INTRODUCTION

The marine sponge is an important invertebrate that helps the establishment of marine ecosystems since they provide shelter for other organisms, including microorganisms (Becking et al. 2013; Calcinai et al. 2017; Ismet et al. 2020). They also contribute to an enormous process in marine ecosystems (González-Rivero et al. 2011). Many studies have reported their role in reef ecosystems (González-Rivero et al. 2011; Bell et al. 2019), however, either sponge's biodiversity or ecological function in mangrove ecosystem is less reported, especially in Indonesia (Becking et al. 2013; Calcinai et al. 2017; Setiawan et al. 2019; Sibero et al. 2019). Vahidi et al. (2020) stated the physicochemical condition in a mangrove ecosystem significantly impacts the dispersion and diversity of associated invertebrates. They emphasized that the benthic fauna in coastal zone mangroves had the lowest diversity rather than deltaic and island zones. This fact is very interesting since several mangrove forests in Indonesia are located in the coastal zone, such as mangrove forests in Kemujan Island, Karimunjawa National Park. In addition, this location faces stresses from the fluctuating salinity, pH, wave, tidal, current, and anthropogenic activity (Kamal et al. 2016; Puryono and Suryanti 2019; Hapsari et al. 2020). It leads to an understanding that

mangrove sponges from the coastal zone are supposed to have a unique resilience strategy; thus, only particular genera or species can survive (Setiawan et al. 2018; Chakraborty 2019).

Further, sponge holobiont has a prominent function in maintaining sponge resilience in its habitat. Due to the filter-feeding behavior, the sponge hosts fungi and other microorganisms to establish its holobiont (Pita et al. 2018). Sponge-associated fungi are commonly reported as marine-derived fungi. It means the fungi might originate from freshwater or terrestrial environments brought by the wind or water into the ocean then grow and possibly sporulate in seawater (Nguyen and Thomas 2018; Calabon et al. 2019; Sibero et al. 2019). These fungi are well known as potential producers of unique bioactive compounds with diverse biological properties (Imhoff 2016; Indraningrat et al. 2016). Our previous works successfully isolated some interesting novel compounds from Indonesia's sponge-associated fungi, such as karimunone A-B, karimanone, and penicitrinone G (Sibero et al. 2019; Sabdaningsih et al. 2020; Sibero et al. 2020). In addition to the potential for biotechnology, the study of biodiversity of sponge-associated fungi from Indonesia has been neglected and has not been explored. Nonetheless, biodiversity is correlated with its chemodiversity for biotechnological purposes (Bovio et al. 2019).

The diversity of sponge-associated fungi is strongly connected to host species and their environmental conditions (Yang et al. 2019). This idea is supported by Nguyen and Thomas (2018) that found some sponges were host-specific to particular marine fungi. The diversity of sponge-associated fungi from the mangrove environment is rarely reported. Calabon et al. (2019) discovered *Aspergillus* and *Penicillium* were the most dominant genera in four mangrove-associated sponges from New Washington, Aklan in the Philippines. Nevertheless, the study did not perform molecular identification to confirm the species and lead to the description of plenty of morpho-species of sterile mycelium. In our previous study, molecular identification through DNA barcoding was conducted to verify the species of sponge-associated fungi from a mangrove sponge from Mangkang, Indonesia (Sibero et al. 2019). It was the first and the latest study of sponge-associated fungi from Indonesia's mangrove forest; however, there is no report about the diversity yet.

Due to the lack of information and high potential of sponge-associated fungi from the mangrove ecosystem in Indonesia, this research was conducted to isolate, identify and determine fungal diversity of mangrove-associated fungi from Kemujan Island, Karimunjawa National Park Indonesia. This research is fundamental to understanding the diversity and ecology of the neglected microbial biodiversity in Indonesia.

MATERIALS AND METHODS

Sampling

Sponge collection was conducted in April 2019 in Kemujan Island, Karimunjawa National Park, Central Java Indonesia (Figure 1). A purposive sampling method was applied to collect sponges attached to the living mangrove roots and bark along the coastal zone. Environmental parameters (temperature, pH, salinity, dissolved oxygen), coordinate, and mangrove hosts were recorded to describe the habitat of the sponge.

Fungal isolation

Sponge-associated fungi from mangrove habitats were isolated according to the protocol that has been published in our previous work (Sibero et al. 2019). Potato dextrose agar (PDA) was prepared in natural seawater with 2% Chloramphenicol to eliminate bacterial growth. Sponges were washed using sterilized natural seawater followed by 70% alcohol, then rewashed using sterilized natural seawater. The mesohyl part was cut into three pieces, then put onto isolation media and incubated at 27°C. Fungal growth was observed every day. Each mycelium grown on samples was transferred into new agar media as a single isolate. During fungal isolation, environmental control was prepared to decrease untargeted microbes, such as airborne fungi. Unfortunately, most of the collected sponges had a small size (<4 cm) due to the restriction of sampling in Karimunjawa National Park; hence sponge identification was not performed.

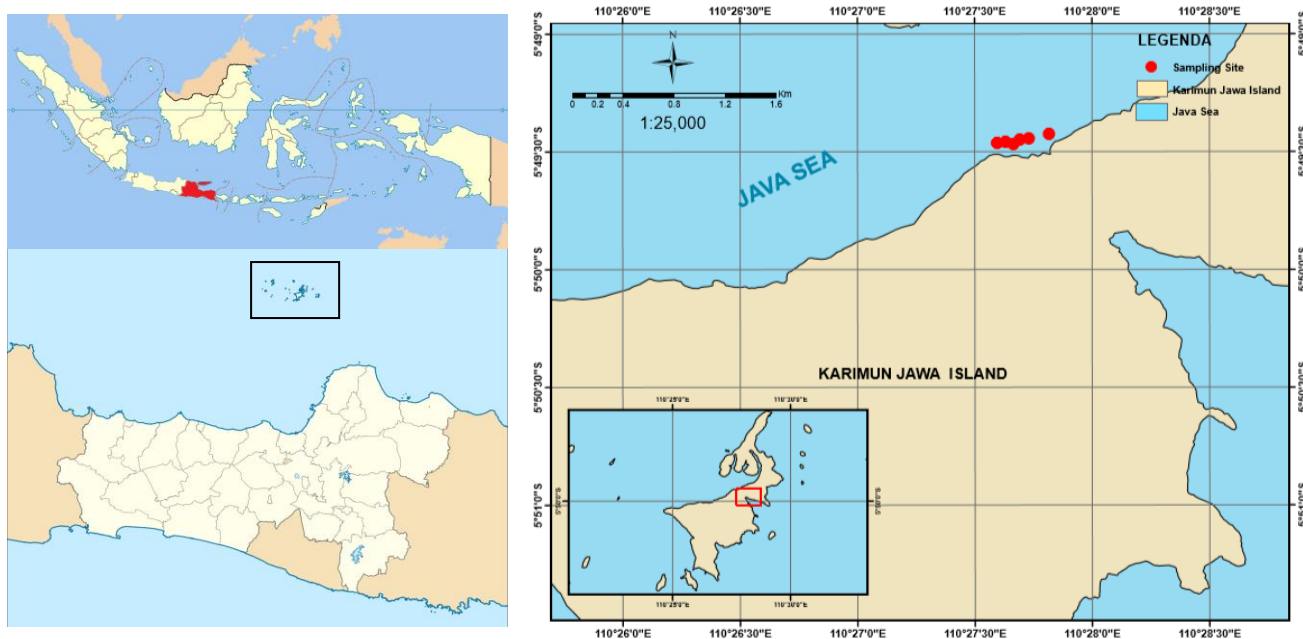


Figure 1. Location of sponge collection in Kemujan island, Karimunjawa National Park, Central Java, Indonesia

Fungal macro-microscopic characterization

All filamentous fungi from mangrove-associated sponges were characterized according to their macroscopic (colonial) and microscopic characteristics. The macroscopic (colonial) characteristics included colonial color and texture, a soluble pigment in agar media, presence of exudate and sclerotia. On the other hand, the microscopic feature was observed by slide culture (Riddell 1950; Sibero et al. 2018) and tape touch methods (Harris 2000) with the addition of lactophenol cotton blue (HiMedia). Some microscopic descriptions included conidiophore, spore, phialides, hyphae, and vesicle (for *Aspergillus* spp.) were observed under a microscope. Every isolate with the same macroscopic and microscopic characteristics was co-cultured on the same Petri to observe the difference. Afterward, isolate with the same growth pattern and colonial characteristics without inhibition zone were expected as the same species. Furthermore, the isolate was expected as different species then identified through DNA barcoding to confirm the species.

Fungal identification through DNA barcoding

The isolates were cultivated for 4-7 days on PDA for DNA extraction using Quick-DNATM Fungal/Bacterial Miniprep Kit (D6005, Zymo Research). PCR protocol for DNA amplification was conducted according to Sibero et al. (2019). The PCR mixture consisted of 12.5 µL PCR mix master (Promega), 1 µL primer ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') (Macrogen), 1 µL primer ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Macrogen), 1 µL DNA template and 9.5 µL of ddH₂O with a final volume of 25 µL. The DNA in the PCR mixture was amplified using a thermal cycler with the following condition: denaturation at 95°C for 1 min, annealing for 1 min, and extension at 72°C for 1 min. We highlighted that the annealing temperature for PCR protocol was various; therefore, temperature optimization for annealing was conducted. The quality of PCR products was checked using gel electrophoresis in 1% agarose gel then sent to 1st BASE DNA Laboratories Sdn Bhd, Malaysia for sequencing. The phylogenetic tree was reconstructed following Dissanayake et al. (2020).

Data analysis

Data analysis was conducted according to (Calabon et al. 2019). The total frequency of occurrence (FOC), fungal diversity, species dominance, evenness, and species similarity was analyzed.

The total frequency of occurrence (FOC) was calculated using these formulas:

$$\text{Total FOC (\%)} = \frac{\text{Number of presence}}{\text{Total sponge}} \times 100$$

Frequency of occurrence (FOC) of species A (%) per sponge species:

$$\text{FOC per species (\%)} = \frac{\text{No. of collections of species A}}{\text{Number of samples examined}} \times 100$$

The fungal diversity was calculated according to Ludwig and Reynolds (1988).

Shannon Index (H')

$$\text{Shannon Index (H')} = -\sum (p_i \ln p_i)$$

Where, p_i refers to the proportion of individuals that species i contribute to the total number of individuals. p_i value was obtained by using this following formula:

$$p_i = n_i/N$$

Where, n_i refers to the number of individuals $i_1, i_2, i_3, i_4, \dots, i_x$; while N is the total number of individuals (records).

Simpson Index of Dominance (D)

$$D = \sum p_i^2$$

Where, p_i : proportion of individuals in the i th species.

The value of D is the opposite of diversity; when D score increases, the diversity decreases. This following calculation obtains the Simpson index of dominance:

$$1 - D \text{ or } \frac{1}{D}$$

Shannon Evenness (J')

$$J' = \frac{H'}{H_{\max}}$$

Where, H_{\max} refers to the maximum value of diversity for the number of species present.

Simpson's Evenness ($E_{1/D}$)

$$E_{1/D} = (1/D)/S$$

Where, D refers to Simpson' index of diversity while; S refers to species richness.

Jaccard Index of Species Similarity

$$J = \frac{a}{(a + b + c)}$$

Where, a refers to the number of fungal species occurring in both hosts; b refers to the number of fungal species unique to the; c refers to the number of fungal species unique to the second host.

RESULTS AND DISCUSSION

The mangrove forest in Kemujan Island, Karimunjawa National Park, Central Java, Indonesia, is a conservation area to protect the coastal ecosystem and an ecotourism site due to its floral and faunal diversity (Puryono and Suryanti 2019; Winata et al. 2020). Previous studies reported mangrove zonation, diversity, and carbon stock from this location (Nehren and Wicaksono 2018; Winata et al. 2020). Some mangrove species such as *Avicennia alba*, *A. marina*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Rhizophora apiculata*, *R. mucronata*, *Sonneratia alba*, and *Xylocarpus*

granatum have been previously reported (Hapsari et al. 2020; Winata et al. 2020). Nonetheless, no study was ever conducted to report mangrove-associated sponges in the tracking mangrove Kemujan Island. Table 1 shows the mangrove-associated sponge, host species, and environmental conditions during the sampling, whereas specimen morphology is provided in Figure 2.

Interestingly, the current study found all sponge specimens associated with *R. mucronata* and grew on the root (KMS 1, 2, 5, and 13) or at the edge of the root to establish the lifeform (KMS 15). Apart from sample KMS 1, other sponges were submerged during the low tide. Various factors such as currents, tides, and winds influenced the larval settlement in the marine ecosystem (Indrayanti et al. 2019). *Rhizophora mucronata* was noted as the outer species in the tracking mangrove Kemujan Island, Karimunjawa National Park. Due to the position, *R. mucronata* is directly exposed by the wind, current, and tides suggested to drive the larval settlement on its root. The number of mangrove-associated sponges is minimal.

Setiawan et al. (2019) also stated that sponge diversity in mangrove habitat was generally lower than in the shallow-water habitat. Unfortunately, sponge identification was not performed in this study because of the maximum sample size restriction since the sampling site is a conservation area.

Fungi produce spores, conidia, and aerial mycelia to help them scatter into the environment through the air (Després et al. 2012). The wind, raindrops, or other biological agents bring these vegetative cells then land in the aquatic environment include the marine ecosystem. According to their needs for salinity, there are two categories of marine fungi, namely marine facultative and marine obligate fungi. Fungi that grow and sporulate in marine and other environments without salinity are defined as marine-derived or marine facultative fungi. Meanwhile, fungi that strictly grow and sporulate only in the marine environment with salinity are defined as a marine obligate (Kohlmeyer and Kohlmeyer 1979; Imhoff 2016; Raghukumar 2017).

Table 1. Mangrove-associated sponge, host species, and environmental condition from tracking mangrove Kemujan Island, Karimunjawa National Park, Central Java, Indonesia

Sample	Host	DO (mg/L)	Water temperature	Salinity (ppm)	TDS (mg/L)	Depth (cm)
KMS 1	<i>Rhizophora mucronata</i>	5.25	32.3°C	20	4.70	3.50
KMS 2	<i>Rhizophora mucronata</i>	6.53	34.5°C	25	6.53	12.50
KMS 5	<i>Rhizophora mucronata</i>	5.76	32.4°C	21	5.66	5.00
KMS 13	<i>Rhizophora mucronata</i>	5.85	34.8°C	23	7.07	7.10
KMS 15	<i>Rhizophora mucronata</i>	6.53	34.5°C	25	6.53	12.50

Note: KMS: Kemujan Sample

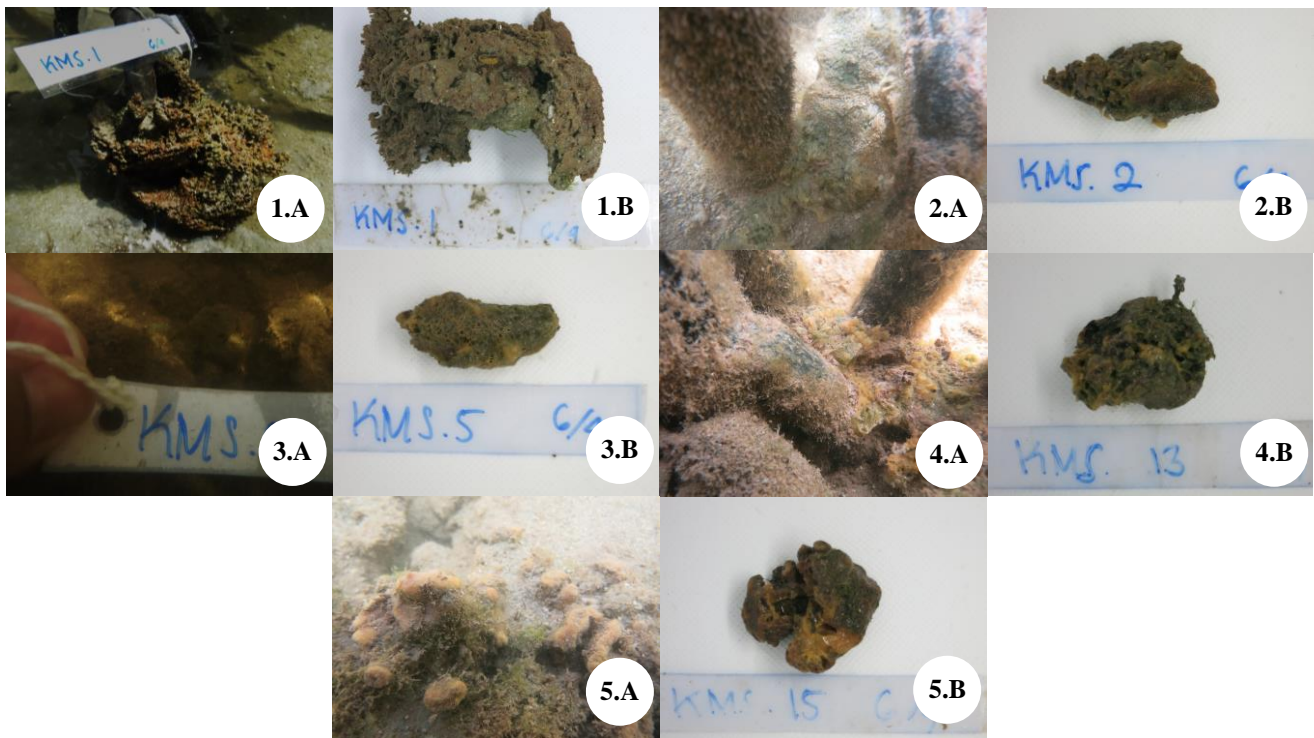


Figure 2. Habitat and morphology of mangrove-associated sponge in tracking mangrove Kemujan island, Karimunjawa National Park, Indonesia

The sponge as a filter feeder collects the vegetative cells from the water; therefore, various fungal species have been discovered from this marine invertebrate. Some might be consumed as food, while others might stay to establish a symbiotic relationship with the sponge (Kohlmeyer and Kohlmeyer 1979; Raghukumar 2017; Nguyen and Thomas 2018). Nevertheless, only a few studies successfully discovered the symbiotic relationship between sponge and its fungi (Maldonado et al. 2005; Calabon et al. 2019). In this study, a total of 56 fungi were isolated from five mangrove-associated sponges from Kemujan Island, Karimunjawa National Park. Colonial and morphological identifications led to 19 isolates for molecular identification (in Supplementary Data). Furthermore, the phylogenetic tree of these isolates is presented in Figure 3.

Molecular identification was performed through a fungal DNA barcoding approach. The isolates are members of nine genera, namely *Absidia*, *Aspergillus*, *Aureobasidium*, *Fusarium*, *Lasiodiplodia*, *Montagnula*, *Pestalotiopsis*, *Trichoderma*, and *Wallemia*, which share 93.61-100% percent identity similarity with fungal species in GenBank (NCBI) according to ITS analysis. Furthermore, they belong to 7 genera from the phylum Ascomycota (77.78%), one genus from the phylum Basidiomycota (11.11%), and one genus from the phylum Mucoromycota (11.11%). The diversity and abundance of sponge-associated fungi from Indonesia are still barely reported. Figure 4 shows that our works discovered that *Trichoderma* (n: 15, 26.79%) was the most dominant genus, followed by *Aspergillus* (n: 13, 23.21%) and *Fusarium* (n: 12, 21.43%). These three genera have been well known as cosmopolitan fungi (Gal-Hemed et al. 2011; Benoit et al. 2013; Imhoff 2016; Abdel-Azeem et al. 2019). They produce an abundance of aerial vegetative cells easily brought by the wind, water, and other biological agents. Further, *Aureobasidium* (n: 5, 8.93%) and *Pestalotiopsis* (n: 5, 8.93%) had a same relative abundance value, followed by *Absidia* (n: 3, 5.36%). It was noted that *Lasiodiplodia*, *Montagnula*, and *Wallemia* only had one species for each with a relative abundance value of 1.79%. Several genera have been reported as sponge-associated fungi such as *Aspergillus*, *Aureobasidium*, *Fusarium*, *Lasiodiplodia*, *Pestalotiopsis*, *Trichoderma* (Calabon et al. 2019; Sibero et al. 2019). However, *Absidia* and *Wallemia* are less reported as sponge-associated fungi (Liu et al. 2010; Bovio et al. 2018; Chalearmsrimuang et al. 2019).

The overall frequency of occurrence (FOC) is shown in Table 2. Moreover, this study noted that *Fusarium* sp. MGKMS 13.4.1 was the most dominant species with a total FOC of 10.71%, followed by *A. pullulans* PKMS 2.2 (FOC 8.93%), *P. microspora* MKMS 2.5.1 (FOC 8.93%), *T. longibrachiatum* MKMS 15.1 (FOC 8.93%), and *T. reesei* MKMS 5.1 (FOC 8.93%). Interestingly, all genera of the isolated sponge-associated fungi are noted as marine-derived fungi, and none of them was reported as marine obligate fungi. Marine-derived fungi adapt to the saline environment by modifying their cell wall, controlling gene

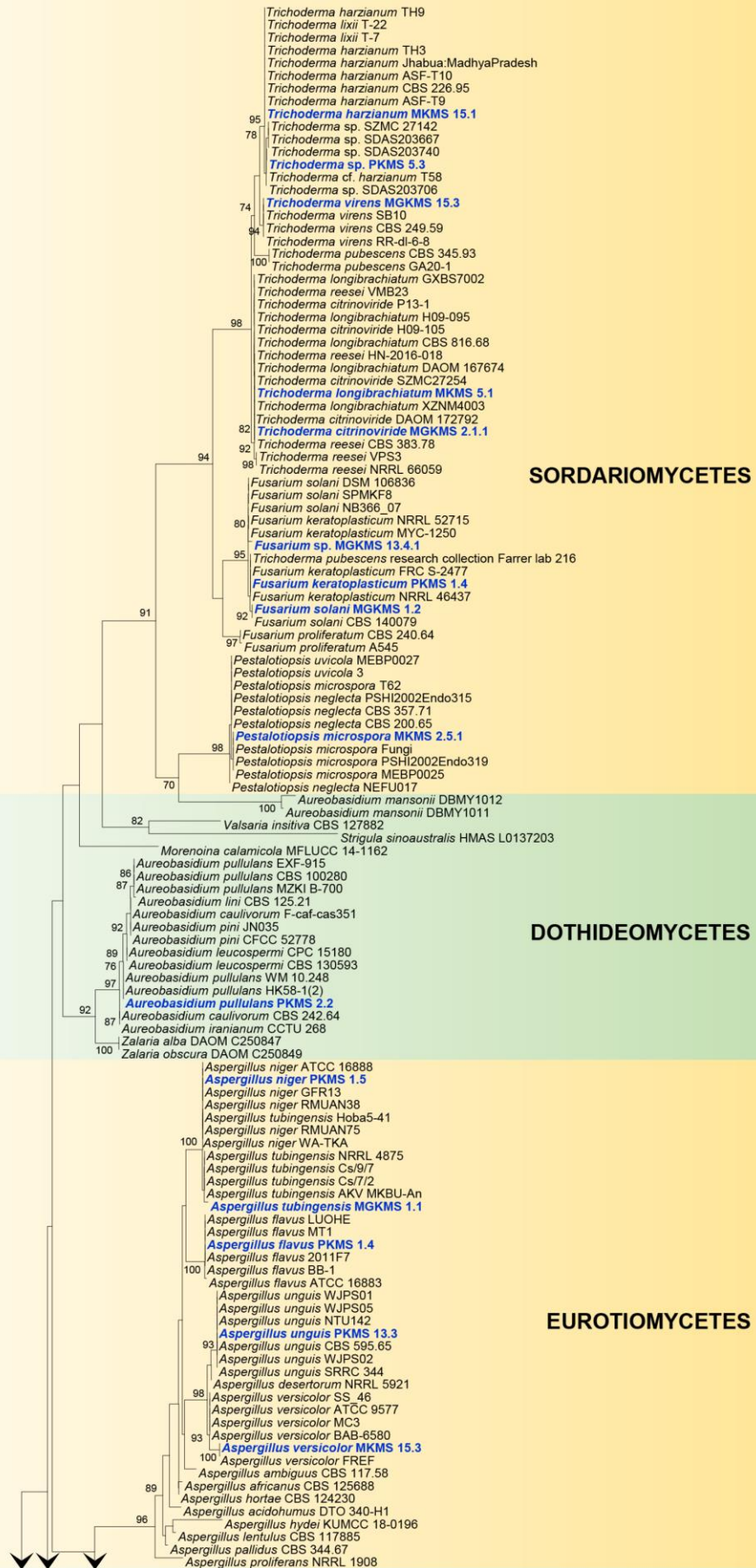
expression (e.g., hydrophobin genes) to control the osmotic pressure, and regulating the water homeostasis during the increase of salinity (Fang et al. 2014; Pang et al. 2020; Pérez-Llano et al. 2020).

The diversity of sponge-associated fungi was calculated using the Shannon index (H') and Simpson index of dominance ($1/D$) (Calabon et al. 2019). The Simpson index of dominance ($1/D$) is significantly related to species evenness, while the Shannon index (H') is related to species richness (Johnson and Burnet 2016). It was noted that mangrove-associated sponge KMS 2 possessed the highest Shannon index value of 0.35 (H') and lowest Simpson index of dominance with a value of 14.67 (Table 2). In contrast, sponge KMS 5 had the lowest H' value and highest $1/D$ value. Calabon et al. (2019) stated that a higher H' value means a higher diversity while a higher $1/D$ value means a lower diversity. Therefore, the sponge KMS 2 had the highest fungal diversity and the lowest fungal diversity owned by sponge KMS 5. Furthermore, the Shannon index of evenness (J') calculation gave the highest value to KMS 2 (1.0), then followed by KMS 1 (0.93), KMS 15 (0.90), KMS 13 (0.87), then KMS 5 (0.78) had the lowest value. On the other hand, Simpson index of evenness ($E1/D$) calculation gave a different result due to the highest value belonging to KMS 5 (6.87), followed by KMS 13 (3.42), KMS 15 (2.54), KMS 1 (1.94), and the lowest was possessed by KMS 2 (0.98). The lower J' value indicates less evenness in communities between the species (Pielou 1966). Further, Smith and Wilson (1996) stated that evenness calculation using $E1/D$ had some advantages, such as it has a strong relation with diversity and can reach the minimum of zero in any number of species. The higher $E1/D$ value means the lower evenness in communities between species. Jaccard index of similarity was calculated to understand the species similarity between two sponge hosts (Table 3). Our data suggested that the highest similar fungal species were found between sponge KMS 1 and KMS 15 with a value of 0.73. It means fungi isolated from KMS 1 were highly similar to the fungi hosted by sponge KMS 15. On the other hand, fungi from KMS 5 were very distinct from the fungi isolated from sponge KMS 2.

There are three categories of cultivable fungi in sponges: sponge-generalist, sponge-associated, and sponge-specialist (Li and Wang 2009). Table 4 presents the association analysis of cultivable sponge-associated fungi. Our data suggested that *Aspergillus*, *Aureobasidium*, *Fusarium*, and *Trichoderma* could be defined as sponge-generalist since they were isolated from all mangrove-associated sponges. This result is in line with several studies that also emphasized *Aspergillus* as a sponge-generalist; however, *Aureobasidium* has never been reported as a sponge-generalist, while *Trichoderma* and *Fusarium* have been known as sponge-associates in a previous study (Li and Wang 2009; Menezes et al. 2010; Calabon et al. 2019). *Absidia* and *Pestalotiopsis* were isolated from multiple mangrove-associated sponges; hence, they were classified as sponge-associates.

Table 2. The overall frequency of occurrence (FOC) of sponges-associated fungi from mangrove habitats in Kemujan Island, Central Java, Indonesia

Species	KMS1		KMS2		KMS5		KMS13		KMS15		Total no. of occurrence	Total FOC (%)
	No. of occurrence	FOC (%)	No. of occurrence	FOC (%)	No. of occurrence	FOC (%)	No. of occurrence	FOC (%)	No. of occurrence	FOC (%)		
<i>Absidia repens</i> MGKMS 2.3	1	25	1	25	0	0	0	0	1	25	3	5.36
<i>Aspergillus flavus</i> PKMS 1.4	1	25	0	0	0	0	0	0	1	25	2	3.57
<i>Aspergillus niger</i> PKMS 1.5	1	25	0	0	1	25	1	25	1	25	4	7.14
<i>Aspergillus tubingensis</i> MGKMS 1.1	0	0	1	25	0	0	0	0	0	0	1	1.79
<i>Aspergillus unguis</i> PKMS 13.3	1	25	1	25	0	0	0	0	1	25	3	5.36
<i>Aspergillus versicolor</i> MKMS 15.3	1	25	0	0	0	0	2	50	0	0	3	5.36
<i>Aureobasidium pullulans</i> PKMS 2.2	1	25	1	25	1	25	1	25	1	25	5	8.93
<i>Fusarium keratoplasticum</i> PKMS 1.4	2	50	1	25	1	25	0	0	0	0	4	7.14
<i>Fusarium solani</i> MGKMS 1.2	0	0	1	25	0	0	1	25	0	0	2	3.57
<i>Fusarium</i> sp. MGKMS 13.4.1	1	25	2	50	1	25	1	25	1	25	6	10.71
<i>Lasiodiplodia theobromae</i> MKMS 2.1.2	0	0	0	0	1	25	0	0	0	0	1	1.79
<i>Montagnula scabiosae</i> PKMS 13.1	0	0	1	25	0	0	0	0	0	0	1	1.79
<i>Pestalotiopsis microspora</i> MKMS 2.5.1	0	0	1	25	2	50	0	0	2	50	5	8.93
<i>Trichoderma citrinoviride</i> MGKMS 2.1.1	0	0	0	0	0	0	1	25	0	0	1	1.79
<i>Trichoderma harzianum</i> MKMS 15.1	1	25	2	50	0	0	1	25	1	25	5	8.93
<i>Trichoderma longibrachiatum</i> MKMS 5.1	1	25	1	25	0	0	1	25	2	50	5	8.93
<i>Trichoderma</i> sp. PKMS 5.3	0	0	1	25	0	0	0	0	0	0	1	1.79
<i>Trichoderma virens</i> MGKMS 15.3	1	25	0	0	1	25	1	25	0	0	3	5.36
<i>Wallemia</i> sp. MKMS 2.1	0	0	1	25	0	0	0	0	0	0	1	1.79
Total per sponge	12		15		8		10		11		56	
Shannon index of diversity (H')	0.33		0.35		0.27		0.30		0.31			
Simpson index of diversity (D)	0.04		0.07		0.02		0.03		0.03			
Simpson index of diversity (1/D)	23.33		14.67		55.00		34.22		28.00			
Shannon index of evenness (J')	0.93		1.00		0.79		0.87		0.90			
Simpson index of evenness (E1/D)	1.94		0.98		6.87		3.42		2.54			



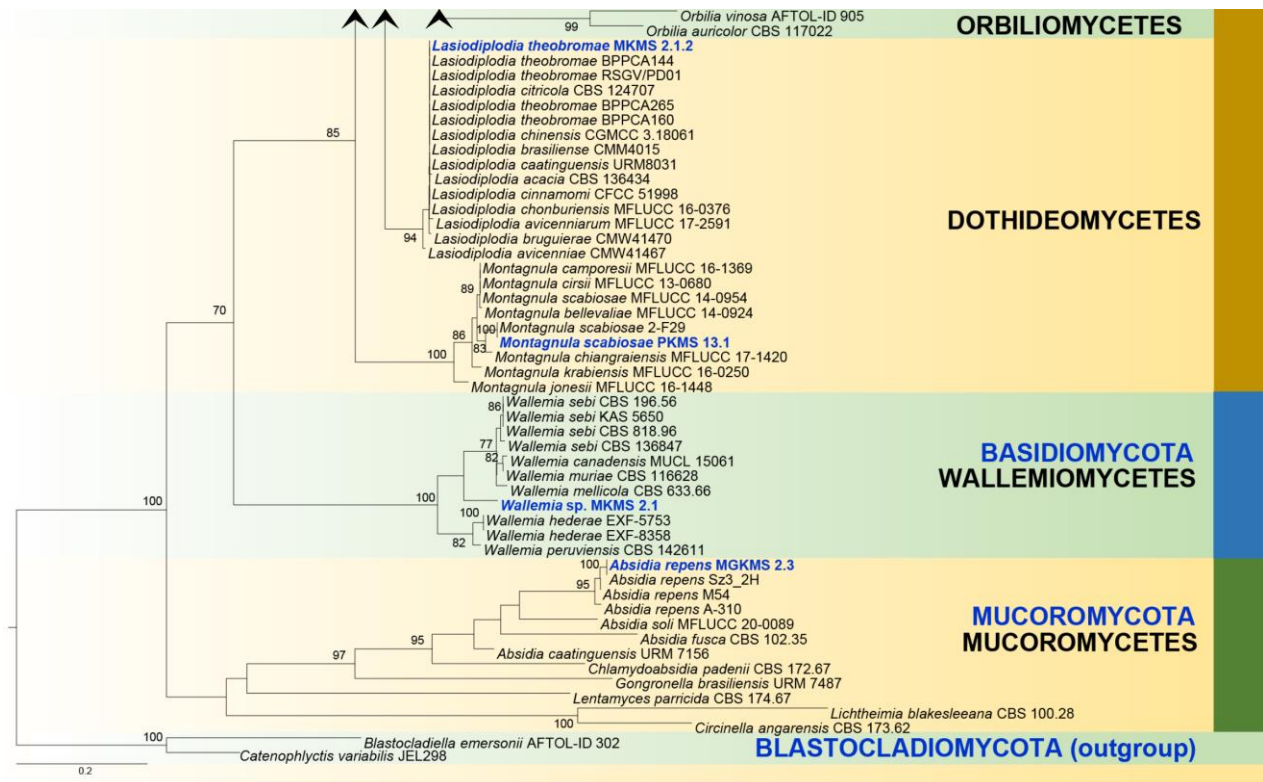


Figure 3. Phylogenetic tree generated from maximum likelihood (ML) analysis based on ITS sequence data for the species from Ascomycota, Basidiomycota, and Mucoromycota. *Catenophlyctis variabilis* (JEL298) and *Blastocladiella emersonii* (AFTOL-ID 302) were used as outgroup taxa. Bootstrap support values for ML equal to or greater than 75% are given above the nodes. The newly generated sequence is indicated in blue

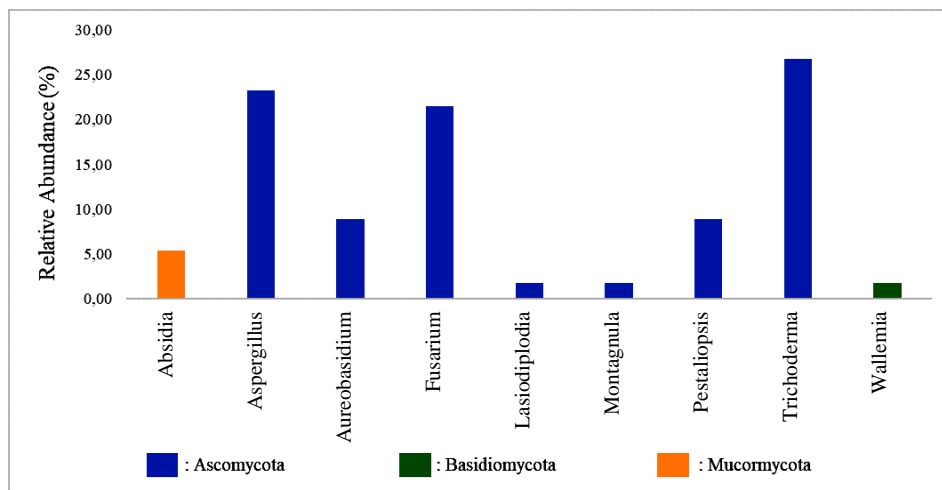


Figure 4. Relative abundance of fungal genera from mangrove-associated sponge collected from Kemujan island, Karimunjawa National Park, Central Java, Indonesia

Table 3. Jaccard index of similarity species among mangrove-associated sponge

Sponge	KMS 1	KMS 2	KMS 5	KMS 13	KMS 15
KMS 1		0.41	0.36	0.54	0.73
KMS 2	0.41		0.25	0.29	0.47
KMS 5	0.36	0.25		0.33	0.33
KMS 13	0.54	0.29	0.33		0.38
KMS 15	0.73	0.47	0.33	0.38	

On the other hand, the sponge-specialist would include *Lasiodiplodia*, *Montagnula*, and *Wallemia* since they only presented in one specific host. Our previous work has reported *Lasiodiplodia theobromae* as a sponge-associated fungus from a Semarang mangrove forest (Sibero et al. 2019) and Jones et al. (2015, 2019) listed this as filamentous marine fungus. Nonetheless, *Wallemia* is infrequently reported as sponge-associated fungi (Liu et al. 2010), and *Montagnula* is never reported from the marine environment.

Table 4. Association analysis of cultivable sponge-associated fungi

Fungal genera	Mangrove-associated sponge					Proposed association
	KMS 1	KMS 2	KMS 5	KMS 13	KMS 15	
<i>Absidia</i>	×	×			×	Sponge-associates
<i>Aspergillus</i>	×	×	×	×	×	Sponge-generalist
<i>Aureobasidium</i>	×	×	×	×	×	Sponge-generalist
<i>Fusarium</i>	×	×	×	×	×	Sponge-generalist
<i>Lasiodiplodia</i>			×			Sponge-specialist
<i>Montagnula</i>		×				Sponge-specialist
<i>Pestalotiopsis</i>		×	×		×	Sponge-associates
<i>Trichoderma</i>	×	×	×	×	×	Sponge-generalist
<i>Wallemia</i>		×				Sponge-specialist

Note: “×” indicates the presence of particular genera

Montagnula spp. has been widely reported as plant-associated fungi, where *M. scabiosae* was first isolated from *Scabiosa* sp. flower (Hongsanan et al. 2015; Tennakoon et al. 2016). The fact that *M. scabiosae* is a plant-associated fungus leads to a presumption that it lived inside the mangrove, then the spores fell into the water and were filtered by the sponge. We can also assume that *M. scabiosae* can be found in terrestrial and aquatic habitats and thrives on different hosts.

Moreover, previous studies have proven that the sponge and the seawater surrounding the sponge share several similar microorganisms, including the fungi (de Mares et al. 2017; Nguyen and Thomas 2018). Although three genera were isolated as a sponge specialists, further study is needed to support this result since there is no direct evidence to validate these fungi interaction with the host. In addition, the role of fungi in the sponge is still not clearly understood. Nguyen and Thomas (2018) stated that fungal communities in the sponge holobiont have less roles rather than the bacterial and archaeal symbionts. de Mares et al. (2017) stated that the fungal diversity in a sponge is determined by the host species and genus. Therefore, some fungi can only live in a specific species while others live as cosmopolitan fungi. In addition, the study of fungal recruitment in the sponge is barely understood. Yet, a previous study stated that unicellular fungus could be vertically transmitted via the oocytes in the Porifera (Maldonado et al. 2005).

In summary, this study successfully isolated 56 sponge-associated fungi from a mangrove forest in Kemujan Island, Karimunjawa National Park. Morphological characterization led to 19 fungal species that were expected as different species. DNA barcoding discover *Trichoderma* (n: 15, 26.79%), *Aspergillus* (n: 13, 23.21%) and *Fusarium* (n: 12, 21.45%) were the top three dominant genera among all isolates. This study also reported that *Aspergillus*, *Aureobasidium*, *Fusarium*, and *Trichoderma* could be defined as sponge-generalist; *Absidia* and *Pestalotiopsis* as sponge-associates; while *Lasiodiplodia*, *Montagnula*, and *Wallemia* as sponge-specialist. Mangrove-associated sponge KMS 2 possessed the highest Shannon index value of 0.35 (H') and lowest Simpson index of dominance with a value of 14.67.

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