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Melissopalynological analysis of honey produced by two species of stingless bees in Lombok Island, Indonesia

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Abstract. *Jayadi LZ, Susandarini R. 2020. Melissopalynological analysis of honey produced by two species of stingless bees in Lombok Island, Indonesia. Nusantara Bioscience 12: 97-108.* Honey is a natural product with a variety of benefits that commonly used as food sweeteners, health supplements, and traditional medicine. There has been no comprehensive publication regarding the diversity of pollen contained in honey produced by stingless bee *Tetragonula laeviceps* and *Heterotrogona itama* from Lombok. This study aimed to reveal the diversity of pollen content in honey through melissopalynological analysis of honey samples produced by two species of stingless bees *T. laeviceps* and *H. itama* in Lombok Island. This melissopalynological study was performed on honey samples obtained from nine villages from nine sub-districts representing the three districts on Lombok Island. Pollen extraction from honey was carried out using standard methods for melissopalynological analysis. The diversity of pollen recovered from honey varied from 15 to 41 pollen types. Total number of plant species whose pollen was recovered from honey samples was 127 which consisted of 61 families. The occurrence of predominant pollen type in particular unifloral honey samples indicated their botanical origin and presumed geographical origin of honey. Results of this study are useful in confirming botanical origin of honey and generating information on plants potential as food source for sustainable beekeeping in Lombok Island.

Keywords: *Heterotrigona itama*, Lombok Island, melissopalynology, pollen diversity, *Tetragonula laeviceps*

INTRODUCTION

Honey is a popular natural product throughout the world which generally tastes sweet with a pleasant aroma. The content of fructose, glucose, maltose, sucrose, and other polysaccharides makes honey taste sweet (Sakac et al. 2019). The high nutrition content in honey makes it not only used as a natural sweetener, but also as a health supplement. Honey has been used food sweetener and supplement to improve general health and enhance immunity (Abdiniyazova et al. 2016; Nguyen et al. 2018).

Honey is produced by honey bees from various species including stingless bees, such as *Tetragonula laeviceps* and *Heterotrigona itama*. There are 46 species of stingless bees recorded in Indonesia with two of them, *T. laeviceps* and *H. itama*, which have regional distribution in Asian countries (Engel et al. 2018). Honey produced by stingless bees has the potential to be promoted as a natural product with various medicinal properties. Publications on honey produced by stingless bees were generally on their physicochemical characteristics, such as those by Biluca et al. (2016), Bakar et al. (2017), and Omar et al. (2019).

The main raw material for honey production is nectar collected by honey bees from flowers. In addition to nectar, honeybees also collect pollen as a source of protein, vitamins and minerals for their health (Di Pasquale et al. 2013) which affects their growth and development (Danner et al. 2017). The nutrition content of pollen varies between different plant species which differ significantly in protein content which influences the growth of bee colonies, such as reported by Liolios et al. (2015) that pollen of *Polygonum aviculare* has 13.91%, *Rubus ulmifolius* has 25.52%, and *Zea mays* have 14.78% protein in 1 g pollen. The relationship between the nutritional value of pollen on reproductive development and productivity of bee colonies has been reported by Campos et al. (2010) and Di Pasquale et al. (2013). Analysis of nutritional composition of pollen collected by *Tetragonula laeviceps* complex, *T. testaceitarsis, Lepidotrigona terminata,* and *L. flavibasis* showed differences in both macronutrient and micronutrient content (Chuttong et al. 2018).

The presence of pollen in honey provides information on plant species visited by honey bees during their search for nectar as major material on honey production. The study of pollen content in honey is known as melissopalynology. Melissopalynology or analysis of pollen diversity in honey is a common method for determining the originality, botanical origin, and geographical origin of honey (von der Ohe et al. 2004; Bryant 2018). Melissopalynological analysis is a suitable approach to study the interactions between plants and bees by identifying the diversity and number of pollen grains extracted from honey (Dhawan et al. 2018). A number of melissopalynological studies on honey produced by different honey bees from Indonesia have been conducted on honey samples from different regions. Lestari and Susandarini (2019) reported the diversity of pollen in honey produced by *Apis cerana* from Java which found variations on pollen diversity from 21 to 69 pollen types recovered from 12 samples of honey that were all classified as multifloral honey. Meanwhile, a melissopalynological study by Rasyiid and Susandarini (2020) revealed the diversity in pollen content of honey produced by *Apis dorsata binghami* from Central Sulawesi, ranging from 30 to 57 pollen types extracted from four samples of multifloral honey, and the variation was due to differences in vegetation from where the honey samples were collected.

Melissopalynological studies on honey produced by nine species of stingless honey bees consisted of *Cephalotrigona capitata, Melipona bicolor, M. marginata, M. mondury, M. quadrifasciata, M. scutellaris, M. seminigra, Scaptotrigona xanthotricha*, and *Tetragonisca angustula* have been reported from Brazil (do Nascimento et al. 2015). Similar study was for honey produced by *Tetragonula iridipenni*s from India (Vijayakumar and Jeyaraaj 2016), honey of *Tetragonula pagdeni* from Thailand (Thakodee et al. 2018), and honey produced by *Heterotrigona itama* from Malaysia (Majid et al. 2019). However, publications on the diversity of pollen in honey produced by stingless bees from Lombok Island are still very limited. It is, therefore, until now there is no melissopalynological study to reveal the diversity of pollen in honey produced by *T. laeviceps* and *H. itama* from Lombok Island. Until now the published reports on honey from Lombok Island were on physicochemical properties of stingless bee honey of unspecified place of origin was reported by Wahyuni and Anggadhania (2020). Meanwhile, melissopalynological reported by Anggadhania et al. (2020) was based on a study conducted on 2016 using honey samples from four areas of Lombok without specifically mentioning the precise locations which found 27 pollen types from 12 plant families without any predominant pollen type was existed among samples.

The objective of this study was to reveal the diversity of pollen in honey samples produced by *H. itama* and *T. laeviceps* originated from nine different locations in Lombok Island. These two species of stingless bees were the most common species reared by beekeepers in Lombok for producing honey. The importance of this study is providing scientific information on the diversity of pollen in honey which is one of the indicators of the botanical origin of honey, and that the concentration of pollen in honey could be used as indicator of originality and authenticity of honey. Information on diversity of plants visited by honeybees revealed from this melissopalynological study is useful as scientific basis in formulating recommendations for the conservation of habitat and plant species as sources of nectar and pollen for honey bees.

MATERIALS AND METHODS

Honey samples

Honey samples were obtained from stingless bee farms in nine villages from nine districts representing West Lombok, Central Lombok, and North Lombok Regencies (Table 1, Figure 1). Sample collection was conducted in August and September 2019. The honey samples were obtained manually squeezed the honeycomb freshly harvested from the hives (Ponnuchamy et al. 2014). Honey samples were put in the bottles and stored in a refrigerator before the process of microscopic preparations in the laboratory.

Figure 1. Village locations of honey samples in Lombok Island, East Nusa Tenggara Province, Indonesia. 1. Batu Putih, 2. Sedau 3. Karang Bayan, 4. Sesela, 5. Bengkaung, 6. Lempenge, 7. Montong Alung, 8. Genggelang, 9. Karang Bajo. (Wikipedia & Google map)

Table 1. Locations of honey samples origin in Lombok Island, West Nusa Tenggara, Indonesia

No.	Village	District	Regency	Altitude* (m asl.)	General condition
1.	Batu Putih	Sekotong	West Lombok	10	rice field area, close to the beach
2.	Sedau	Narmada	West Lombok	136	rice field and open areas
3.	Karang Bayan	Lingsar	West Lombok	97	settlement
4.	Sesela	Gunung Sari	West Lombok	19	settlement, close to rice field
5.	Bangkaung	Batu Layar	West Lombok	8	open areas, home yard gardens
6.	Lempenge	Pringgarata	Central Lombok	340	settlement
7.	Montong Alung	Batukliang Utara	Central Lombok	350	settlement, close to community plantation
8.	Genggelang	Gangga	North Lombok	5	settlement, close to rice field
9.	Karang Bajo	Bayan	North Lombok	7	rice field and open areas

Note: *source: https://lombokbaratkab.bps.go.id, https://lomboktengahkab.bps.go.id https://lombokutarakab.bps.go.id

Procedures for preparing pollen microscopic slides and observation of pollen morphology

Preparation of pollen microscopic slides from honey was carried out following the procedure of von der Ohe et al. (2004) with some modifications. Five mL of honey was put into a glass tube, and then 20 mL of warm (40-60°C) distilled water was added. The mixture was stirred manually using a glass rod until honey sample was dissolved thoroughly. The solution was centrifuged at 1700 rpm for 10 minutes, and the supernatant was decanted. This procedure was repeated once to ensure the sugar component was completely dissolved and eliminated from the sample. The pellet was stained with 1 mL of 0.5 % safranin and left for 24 hours at room temperature. The next step was washing off the stain by adding 10 mL of distilled water and centrifuged at 1700 rpm for 10 minutes. After decanting the water, 2-3 mL 40% glycerin was added to the pellet and mixed well using a vortex. A drop of this mixture was placed onto a microscope slide and secured with a coverslip.

Observation of pollen morphology was done using Boeco BM-180 light microscope at magnification of 10 x 10 or 10 x 40 equipped with OptiLab digital camera for microscopy for measurement and photographic documentation of pollen grains. Pollen identification was carried out using pollen reference from Huang (1972) and the Australian Pollen and Spore Atlas database (http://apsa.anu.edu.au).

Data analysis

Quantitative analysis to determine the presence of predominant pollen was done by taking random photographs containing 500 pollen grains from each sample, and then calculating the percentage of occurrence for each pollen type from particular honey sample.

RESULTS AND DISCUSSION

Variation in pollen diversity among honey samples

Honey samples obtained from nine areas in Lombok Island were produced by two species of stingless bee, *Heterotrigona itama*, and *Tetragonula laeviceps*. These two stingless bee species actually have wide distribution areas in Indonesia (Engel et al. 2018), and based on the information gathered from the beekeeper Lombok, these two species are easy to care for and they will not leave the hive or escape to other places when food sources are low or times of food scarcity. This was in line with the statement of Thakodee et al. (2018) that stingless bees might change their foraging strategy from economic plants to weed plants if the presence of agricultural or plantation crop plants becomes scarce. Results on the analysis of pollen diversity showed variation on the number of pollen types from 15 in honey sample from Lempege Village to 41 in honey sample from Sedau Village (Table 2). Based on the composition of pollen types and the presence of predominant pollen, samples from eight locations were classified as unifloral honey, while the one from Sedau Village was classified as multifloral honey. The classification of honey category refers to the criteria of von der Ohe et al. (2004) in which unifloral honey is characterized by the presence of dominant pollen type, which occurs in the percentage of ≥ 45%. Accordingly, multifloral honey is characterized by the absence of predominant pollen found among pollen types.

Total number of pollen types identified from all nine honey samples was 127 which came from 61 plant families (Table 3). Representatives of pollen grains recovered from honey samples were shown in Figure 2. Variation in the diversity of pollen types found in honey samples was due to differences in diversity of plants visited by honey bees during their foraging activities, and this becomes the essential aspect of melissopalynology. Melissopalynological studies provide information on pollen grains collected by honey bees either intentionally or unintentionally during their foraging activities (Ponnuchamy et al. 2014). The presence of pollen with a certain percentage is the basis for honey classification, either as unifloral or multifloral honey. Quantitative analysis of pollen in honey provide strong and reliable basis for of assuring originality of honey by identifying botanical origins of unifloral honey, such as manuka honey which refers to honey from manuka trees (*Leptospermum scoparium*) (Alvarez-Suarez et al. 2014), longan honey from *Dimocarpus longan* (Wanjai et al. 2012), or acacia honey from *Acacia* spp. (Mohammed et al. 2017). The majority of unifloral honey found in this study might result from the local abundance of certain plant taxa surrounding the beehives, the coincidences of flowering time of certain plant taxa with the active period of beekeeping, and preferences of bee species (Njokuocha 2019).

Variation in number and composition of plant species and families represented by the occurrence of pollen in honey

The diversity of plants as represented by their pollen recovered from honey samples showed that there was a remarkable difference in the number and composition of plant families among the nine samples. The diversity plant families and percentage number of species on each family as represented by their pollen in honey samples indicated that the source of pollen was coming from various plant taxa (Table 3). Brief descriptions of each location where honey samples were collected along with explanations pollen diversity extracted from honey in connection to their plant families were given in the following section.

Honey from Batu Putih Village was collected from beekeeping farm near the beach and rice fields. The beehives were placed in rice fields where a number of flowering plants intentionally planted nearby such as *Solanum lycopersicum*, *Carica papaya*, *Capsicum annuum*, *Datura metel*, and some species of Myrtaceae family. The predominance of *S. lycopersicum* pollen was due to the abundance of this species found around the hives. Even though there was high diversity of plants grow near the hives, the dominant occurrence of *S. lycopersicum* pollen indicated that this plant the most visited species by bees in searching for food. This result was in line with the study by Cholis et al. (2019) which found that *T. laeviceps* showed consistency in collecting pollen from Solanaceae plants as much as 70.76%. Another pollen type found in considerable percentage was from coconut tree (*Cocos nucifera*) which grows around the nest. The occurrence of *C. nucifera* pollen at notable amount was due to the preference of stingless bee to forage on flowers with high sugar concentration which are available throughout the year (Selvaraju et al. 2019). The pollen composition in honey indicated that *T. laeviceps* forage for foods from plants close to the hives as long as they remain available. This result was in line with the finding of Thakodee et al. (2018) which indicated that stingless bees forage for foods from flowers that are not far from their hives. Pollen grains found in honey were originated from 17 plant families with Myrtaceae and Solanaceae plants became two dominant families represented by five and four species, respectively.

Sedau Village is a tourist destination area with a cooler climate than other research areas. The beekeeping farms were located in the rice fields with some flowering plants found near the beehives including *Durio zibethinus, Garcinia mangostana, Fragaria x ananassa, Muntinga calabura, C. nucifera, M. paradisiaca*, and *Sesbania grandiflora*. Pollen grains found in large quantities in honey were from *Phyllanthus warnockii* and *C. nucifera*, but their percentage did not reach 45% honey from this village was classified as multifloral honey. *P. warnockii* flowers have both intrastaminal and extrastaminal nectary glands (WFO 2020) and the presence of more than one source of nectar might be the reason why this plant species is attractive for the bees. This multifloral nature was obviously seen from composition pollen recovered from honey which consisted of 22 families. Three families were dominated, the Arecaceae, Asteraceae, and Anacardiaceae. Pollen composition in honey showed that the stingless bee species producing honey in this village forage from various plant taxa. This result was in line with the report from Jaapar et al. (2018) on the foraging behavior of *H.itama* which showed that this species visited plants from forest and agricultural areas, and thus supporting the statement of de Novais et al. (2014) and do Nascimento et al. (2015) that stingless bees have generalist habit in foraging for foods.

Honey from Karang Bayan Village was collected from beehives placed in the middle of densely populated settlements with only limited vegetation in the area. Pollen grains found in honey came from 20 plant families but each family was only represented by a few species. *Arenga pinnata* (Arecaceae) pollen which was dominant in honey was not directly obtained by bees from *A. pinnata* trees, but from the harvested inflorescences which were deliberately placed by farmers near the bee hives of *H. itama*. Flowers of *A. pinnata* contain high concentrations of sugar, and this match with the behavior of *H. itama* that tends to forage for flowers close to the hives and prefers to visit flowers having nectar concentrations of 30-50% (Basari et al. 2018). Moreover, Majid et al. (2020) noted that flowers from Arecaceae plants, such as *Elaeis guineensis*, *Cocos nucifera*, and *Nypa fruticans* are attractive sources of foods for bees by providing both nectar and pollen.

Table 2. Variations on diversity of pollen recovered from honey, honey type, and predominant pollen

No.	Village	Number of pollen type	Honey category	Predominant pollen type*	Honey bee species
1.	Batu Putih	31	Unifloral	*Solanum lycopersicum*	*T. laeviceps*
2.	Sedau	41	Multifloral	(none)**	*H. itama*
3.	Karang Bayan	27	Unifloral	*Arenga pinnata*	*H. itama*
4.	Sesela	37	Unifloral	Fabaceae	*T. laeviceps*
5.	Bengkaung	33	Unifloral	*Arenga pinnata*	*H. itama*
6.	Lempenge	15	Unifloral	Myrtaceae	*T. laeviceps*
7.	Montong Alung	28	Unifloral	*Schefflera avensis*	*T. laeviceps*
8.	Genggelang	18	Unifloral	*Polygonum* sp.	*T. laeviceps*
9.	Karang Bajo	21	Unifloral	*Polygonum* sp.	*T. laeviceps*

Note: *predominant pollen was those present in ≥ 45% based on pollen count, characteristic for unifloral honey. ** multifloral honey is characterized by the absence of predominant pollen

Figure 2. Representatives of pollen types recovered from honey samples. 1. *Acalypha scandens* 2. *Acer* sp. 3. *Adenia poggei* 4. *Amaranthus spinosus* 5. *Arenga pinnata* 6. *Bombax ceiba* 7. *Bredia oldhamii* 8. *Capsicum annuum* 9. *Carica papaya* 10. *Citrus* sp. 11. *Cleome rutidosperma* 12. *Cocos nucifera* 13. *Elepanthopus scaber* 14. *Eucalyptus* sp. 15. *Ficus mollis* 16. *Hypericum nagasawae* 17. *Ilex* sp. 18. *Camonea vitifolia* 19. *Mabea* sp. 20. *Mangifera indica* 21. *Mimosa pudica* 22. *Muntingia calabura* 23. *Nephelium* sp. 24. *Peltophorum pterocarpum* 25. *Phyllanthus urinaria* 26. *Huberantha forbesii* 27. *Polygonum* sp. 28. *Psidium guajava* 29. *Rinorea sylvatica* 30. *Schefflera avenis* 31. *Solanum lycopersicum* 32. *Syzygium malaccense* 33. *Tabernaemontana arborea* 34. *Trifolium* sp. 35. *Viburnum* sp. 36. *Ziziphus mauritiana* 37. Asteraceae type 38. Cyperaceae type 39. *Euphorbia* type 40. Malvaceae type 41. Myrtaceae type 42. Poaceae type

Table 3. List of plant families and species whose pollen was found in honey samples

Families and species/pollen type*	Location								
	BPH	SDU	KBY	SSL	BKG	LPG	MTA	GGL	KBJ
Acanthaceae									
Ruellia sp.				+					
Acoraceae									
Acorus gramineus Aiton			+						
Actinidiaceae									
Saurauia sp.				+					
Adoxaceae									
Viburnum sp.			+			+			
Alismataceae									
Sagittaria platyphylla (Engelm.) J.G.Sm.						+			
Amaranthaceae									
Amaranthus spinosus L.	+	+	+	+				+	+
Amaranthaceae *undifferentiated*		+							
Amaryllidaceae									
Crinum sp.				+					
Anacardiaceae									
Ozoroa insignis Delile		+							
Rhus sp.		+							
Mangifera indica L.			+	+	+			+	+
Anacardiaceae *undifferentiated*		+							
Annonaceae									
Huberantha forbesii (F.Muell. ex Diels) Chaowasku	+								
Apiaceae									
Eryngium foetidum L.		+							
Apocynaceae									
Tabernaemontana arborea Rose		+			+		+		
Leuconotis sp.							+		
Apocynaceae *undifferentiated*			+		+		+		
Aquifoliaceae									
Ilex aquifolium L.			+				+		
Araceae									
Colocasia sp.				+					
Pinellia ternata (Thunb.) Makino					+				
Araliaceae									
Schefflera avenis (Miq.) Harms							+		
Arecaceae									
Cocos nucifera L.	+	+	+		+		+		
Arenga pinnata (Wurmb) Merr.		+	+		+		+		
Pinanga coronata (Blume ex Mart.) Blume				+					
Adonidia merrillii (Becc.) Becc.						+			
Oncosperma sp.		+		+					
Arecaceae *undifferentiated*	+	+	+	+			+		+
Asteraceae									
Acmella sp.		+							
Pterocaulon alopecuroides (Lam.) DC.		+							
Circium sp.		+							
Jungia sellowii Less.			+						
Tridax procumbens L.				+	+				
Elephantopus scaber L.					+				
Zinnia elegans Jacq.					+				
Ageratum conyzoides L.					+				
Sphagneticola trilobata (L.) Pruski					+				
Bidens sp.					+				
Eclipta prostrata (L.) L.						+			
Cosmos caudatus Kunth									+
Asteraceae *undifferentiated*	+				+				
Balsaminaceae									
Impatiens balsamina L.	+						+		
Bombacaceae									
Bombax ceiba L.								+	+
Bombax sp.		+	+						

Brassicaceae									
Brassica napus L.	+								
Brassica juncea (L.) Czern.				+					
Brassica sp.					+				
Arabis sp.	+		+						
Roripa sp.				+					+
Bromeliaceae									
Alcantarea sp.								+	+
Capparaceae									
Cleome rutidosperma DC.					+				
Caricaceae									
Carica papaya L.	+						+	+	
Caryophyllaceae									
Uebelinia rotundifolia Oliv.	+								
Celastraceae									
Tripterygium wilfordii Hook.f.	+								
Convolvulaceae									
Camonea vitifolia (Burm.f.) A.R.Simões & Staples			+						
Cunoniaceae									
Eucryphia sp.					+				
Cuscutaceae									
Cuscuta polygonorum Ces.	+								
Cyperaceae									
Bolboschoenus maritimus (L.) Palla	+							+	
Carex vesicaria L.					+				+
Cyperus sp.					+				+
Euphorbiaceae									
Mallotus sp.	+								
Croton churumayensis Croizat		+							
Acalypha hellwigii Warb.			+						
Mabea sp					+				
Euphorbia sp.			+						+
Euphorbiaceae *undifferentiated*			+	+					+
Fabaceae									
Peltophorum pterocarpum (DC.) Backer ex K. Heyne			+						
Onobrychis sp.				+					
Neonotonia wightii (Wight & Arn.) J.A. Lackey				+					
Leucaena leucocephala (Lam.) de Wit					+				
Glycine max (L.) Merr.							+		
Trifolium sp.							+		
Fabaceae *undifferentiated*				+			+		
Gesneriaceae									
Stauranthera grandifolia Benth.				+					
Gnetaceae									
Gnetum gnemon L.					+				
Hypericaceae									
Hypericum nagasawae Hayata			+	+					
Lamiaceae									
Scutellaria sp.		+							
Loranthaceae									
Helixanthera sp.		+					+		+
Malvaceae									
Waltheria indica L.	+								
Sida rhombifolia L.					+				
Theobroma cacao L.					+				
Hibiscus rosa-sinensis L.						+	+		
Alcea rosea L.							+		
Malvaceae *undifferentiated*	+			+					
Melastomaceae									
Tibouchia sp.			+						
Bredia oldhamii Hook.f.				+					
Menyanthaceae									
Nymphoides peltata (S.G. Gmel.) Kuntze				+					
Molluginaceae									
Trigastrotheca pentaphylla (L.) Thulin						+			

Moraceae									
Ficus mollis Vahl			+				+		
Ficus sp.		+							
Mimosaceae									
Mimosa pudica L.					+			+	
Moringaceae									
Moringa oleifera Lam.				+					
Muntingiaceae									
Muntingia calabura L.		+		+					
Myrtaceae									
Eucalyptus sp.1	+	+	+						
Eucalyptus sp.2	+								
Psidium guajava L.	+					+		+	
Syzygium cumini (L.) Skeels	+				+	+	+		
Syzygium aqueum (Burm.f.) Alston	+			+					
Syzygium malaccense (L.) Merr. & L.L. Perry					+				
Rhodomyrtus tomentosa (Aiton) Hassk.		+							
Myrtaceae *undifferentiated*					+	+			
Nyctaginaceae									
Bougainvillea spectabilis Willd.								+	+
Passifloraceae									
Adenia poggei (Engl.) Engl.							+		
Phyllanthaceae									
Phyllanthus warnockii G.L.Webster		+							
Phyllanthus urinaria L.		+							
Phyllanthus warburgii K. Schum.				+		+			
Bridelia leichhardtii Baill. Ex Müll.Arg.			+			+			
Synostemon bacciformis (L.) G.L. Webster			+						
Piperaceae									
Piper retrofractum Vahl									+
Piper sp.					+				
Poaceae									
Cenchrus purpureus (Schumach.) Morrone		+							
Sporobolus mobberleyanus P.M. Peterson & Saarela		+							
Paspalum scrobiculatum L.			+						
Zea mays L.								+	+
Poaceae *undifferentiated*	+							+	
Polygonaceae									
Polygonum aviculare L.		+		+					
Polygonum sp.				+				+	+
Persicaria acuminata (Kunth) M. Gómez					+				+
Polypodiaceae									
Campyloneurum sp.		+							
Rhamnaceae									
Ziziphus mauritiana Lam.				+					
Ranunculaceae									
Ranunculus sp.		+	+						
Rosaceae									
Rosa canina L.							+		
Rosa sp.							+		
Malus sylvestris (L.) Mill.								+	
Rutaceae									
Citrus sp.						+			
Salicaceae									
Flacourtia jangomas (Lour.) Raeusch.		+							
Sapindaceae									
Acer sp.	+						+		
Nephelium sp.				+					
Solanaceae									
Solanum lycopersicum L.	+								
Solanum americanum Mill.							+		
Solanum sp.	+	+				+	+		
Capsicum annuum L.	+				+			+	
Datura metel L.	+								
Cestrum nitidum M. Martens & Galeotti		+		+					
Nicotiana tabacum L.							+		

Typhaceae									
Typha sp.		+							
Violaceae									
Viola arvensis Murray		+					+		
Rinorea sylvatica (Seem.) Kuntze				+					
Xanthorrhoeaceae									
Aloe vera (L.) Burm.f.	+	+		+		+			
Unidentified 1	+			+				+	+
Unidentified 2	+	+			+		+		+
Unidentified 3	+	+		+	+				
Unidentified 4		+	+						
Unidentified 5			+	+			+		+
Unidentified 6		+			+	+			
Unidentified 7		+		+			+		
Unidentified 8		+		+			+		
Total	31	41	27	37	33	15	28	18	21

Note: *pollen type indicated the presence of typical pollen for the corresponding family, in palynological terminology it was marked by family name followed by the word "undifferentiated". BPH: Batu Putih, SDU: Sedau, KBY: Karang Bayan, SSL: Sesela, BKG: Bengkaung, LPG: Lempenge, MTA: Montong Alung, GGL: Genggelang, KBJ: Karang Bajo.

Honey from Sesela Village was collected from beehives placed in a narrow house yard close to the rice fields. Flowering plants found around the hives and considered as a source of food for bees include *M. calabura*, *Syzygium aqueum*, *Portulaca* sp., and *Dimocarpus longan*. The dominance of Fabaceae pollen which reaches 49% was likely due to the insufficient food source provided by flowering plants around the hives so that the bees were looking for food sources from other parts of the village. In this case, many factors were identified in affecting the behavior of bees in looking for food source, including the availability of food at certain season, the distance between the hive and the food source, and competition between bee's colonies (Fidalgo and Kleinert 2010; León et al. 2015). The number of plants as source of pollen in honey was 26 families, the highest number of families among all samples in this study. Arecaceae had the highest number of species compared to other families, consisted of *Arenga pinnata*, *Pinanga coronata*, and *Oncosperma* sp. However, even though Arecaceae has the highest number of species, the dominant pollen in honey was Fabaceae, represented by *Onobrychis* sp. and *Glycine wightii*. This fact was possibly due to the nature of Fabaceae as entomophilous plants with flower size larger than Arecaceae so they provide more food and attract honey bees to visit. Previous studies revealed that stingless bees collect considerable amount of Fabaceae pollen (do Nascimento et al. 2015; Vijayakumar and Jeyaraaj 2016; Mohammad et al. 2020). The high diversity of plant families visited by *T. laeviceps* from this village might indicate that this species has no specific preferences in certain types of flowers in finding food sources. In regard to this behavior, Gadhiya and Pastagia (2015) noted that *T. laeviceps* visited flowers from wide range of plants, including vegetable crops, fruit crops, oilseed crop, pulse crop, ornamental plants, and even from weeds.

In Bengkaung Village, the beekeepers placed *H. itama* hives in the house yard surrounded by *A. pinnata*, which become the major source of pollen in the honey. The predominant occurrence of *A. pinnata* pollen showed that the bees forage for food sources in the area close to the hives. The behavior *H. itama* which prefers to forage on flowers in a short distance from their hives has been reported by Basari et al. (2018) and Thakodee et al. (2018). In addition, *H. itama* prefers to forage pollen from small flowers of white or yellow colors (Mohammad et al. 2020), and obviously, *A. pinnata* flowers have these two characteristics. The diversity of pollen sources in this area was high, consisted of 19 plant families. Although *A. pinnata* pollen was dominant in honey, only two plant species of this family (*A. pinnata* and *Cocos nucifera*) were found in the area as shown by the occurrence of their pollen. This situation was most likely due to the nature of Arecaceae that is entomophilous plants flowering throughout the year with abundance flowers in its massive inflorescences (Perera et al. 2010), and the fowers are containing high sugar concentration (Selvaraju et al. 2019), and thus provide plentiful source of food for honey bees.

Lempenge Village is located in the center of Lombok Island where farmers placed the beehives in the middle of their settlement. The diversity of pollen in honey from this village was the lowest among the nine samples, with only 15 pollen types found, with Myrtaceae (*Psidium guajava* and *Syzygium cumini*) pollen type being predominant. Plant diversity as indicated by the presence of their pollen extracted from honey was also the lowest one which only comprised of 10 plant families. The occurrence of predominant Myrtaceae pollen type resulted from the flowering season of the plants during rearing period of the bees until the honey was harvested. Myrtaceae plants, especially *S. cumini* have massive flowers and thus provided abundance food source for bees either in the form of nectar or pollen. Myrtaceae plants are known to be visited by many stingless bees as noted by Antonini et al. (2006), and this phenomenon was emphasized by do Nascimento et al. (2015) who mentioned that pollen spectrum of honey produced by stingless bees from Brazil showed significant representation of Myrtaceae pollen. The presence of Myrtaceae pollen in honey samples produced by stingless bees from melissopalynological studies in neighboring countries has also been reported such as in Thailand by Thakodee et al. (2018) who found pollen of *Melaleuca quinquenervia* and *Syzygium jambos* and from West Coast of Malaysia by Selvaraju et al. (2019) who found pollen of *Baeckea crassifolia* and *Psidium guajava.*

The beekeeping in Montong Alung Village is located in the middle of settlements adjacent to the plantation. Some of the plants found in farmers' house yards were *Theobroma cacao*, *Musa paradisiaca*, *C. papaya*, *C. nucifera* and *Mangifera indica.* Meanwhile, *Schefflera avenis* (Araliaceae), plant species contributing to predominant pollen in honey was not found in settlements area, but it was found in a community plantation area at a distance of 10 m from the beehives. The morphology of pollen grains of *S. avenis* has been described by Jones and Pearce (2015) as circular shape in polar view, prolate spheroidal shape in equatorial view, 3-colporate, tectate-perforate, finely reticulate exine sculpture, size 20–26 x 23–28 µm. This fact indicated that the bees were looking for food sources not only around the farmer's yard. *S. avenis* was actually found in plantation area within a short distance from the beehives. This finding was in line with the statement of Basari et al. (2018) that the closer the bees to the food, the more colonies will visit the food sources. Pollen grains found in honey came from 18 families, with Arecaceae, Malvaceae, Fabaceae, Rosaceae, Apocynaceae, Solanaceae were represented by two species, and other families were only consisted of one species. The fact that *T. laeviceps* forage diverse plant taxa was in line with the statement of de Novais et al. (2014) that stingless bees might show polylectic foraging behavior or considered as generalist foragers. Although the diversity of plants as food sources was high, but *S. avensis* pollen was predominant in honey samples. The fact that the distance from beehives to a place where this species grows was relatively close was possibly the main reason. Another possible reason was that *Schefflera* has plentiful flowers in big inflorescences offering both nectar and pollen that makes it possible for bees to collect as much food as possible from this plant (Nuraliev et al. 2014). In this case, Leonhardt et al. (2007) explained that mixed foraging strategy, in which the bees collect both nectar and pollen at a single foraging trip, is an efficient way to gather foods.

Honey from Genggelang Village was obtained from beekeeping farms with dozens of beehives placed in the middle of settlements near the rice fields. Flowering plants commonly found around the farms were *T. cacao, M. paradisiaca, C. papaya, C. nucifera,* and *M. indica.* Aside from being dominated by *Polygonum* sp. pollen type, the number of *Bombax ceiba* and *Bougainvillea spectabilis* pollen grains was quite high, indicating that stingless bees forage on flowers from wide range of plants including ornamental flower plants (Gadhiya and Pastagia 2015). The fact that pollen from plants near the beehives was only found in small number could happen because the flowering season of plants near the hives did not coincide with the honey harvest season. This is one of the factors affecting the pollen profile recovered from the honey (Aronne and De Micco 2010). Another possible cause was the competition between bee's colonies for the food sources around the beehives which forced them to look for food sources located at some distance from the hives. In this case, León et al. (2015) noted that competition affected the foraging activity and explained that the bees might forage on plants at farther distance which offer better reward for them. The number of plant families represented by their pollen in honey was 12. Although Poaceae had the highest number of pollen types, the number of their pollen grains were not dominant in honey. This result could be explained by the fact that flowers from Poaceae are nectarless so that bees cannot obtain nectar from this plant. In addition, Poaceae is an anemophilous plant whose pollination is mediated by the wind (Wolowski and Freitas 2015). Meanwhile, the predominant occurrence of *Polygonum* sp. pollen in line with the report of Cholis et al. (2019) that *T. laeviceps* showed flower constancy in Polygonaceae as part of its foraging behavior.

Honey from Karang Bajo Village was unifloral honey dominated by *Polygonum* pollen type. In addition to the dominance of *Polygonum* pollen which reached 78%, *Bombax ceiba* pollen grains were found in a fairly high percentage. Based on direct observation in the location, this situation was most probably due to the occurrence of plentiful flowers of *B. ceiba* produced by a big tree near the sites of beehives. Moreover, the flower of B. ceiba is big, cup-shaped, have nectar, with approximately 100 anthers per flower, and the average number of pollen per anther is 88,630 grains (Raut et al. 2017). The constancy of *T. laeviceps* in collecting pollen from Polygonaceae has been pointed out by Cholis et al (2019) who found bee species collected as much as 78.32% Polygonaceae based on the analysis of pollen load. In this village, the beehives were placed in rice fields where some flowering plants were intentionally planted such as *Cosmos caudatus, Iris pseudacorus, Euphorbia* sp., *Artocarpus heterophyllus, C. papaya, M. indica,* and *C. nucifera*. Pollen in honey from this village came from 14 plant families. The high diversity of pollen found was likely to occur because of competition among bee colonies originating from 40 beehives in obtaining food sources close to their hive, and consequently, force the bees to collect foods in wider areas far from the hives. This kind of situation was mentioned by Putra et al. (2014) that Asiatic stingless bees such as *T. laeviceps* tend to utilize food sources close to the hive in the absence of competitors. The similarity on the occurrence of predominant *Polygonum* pollen type, and the notable representation of *Bombax* pollen between honey from this village to honey from Genggelang was most probably due to relative closeness of these two locations which were in the northern part of Lombok Island (Figure 1). Such similarity in pollen profile showed by these two honey samples indicated the role of melissopalynological analysis in determining not only botanical origin but also in detecting geographical origin, as has been mentioned in many previous studies (von der Ohe et al. 2004; Shubharani et al. 2012; Chatterjee 2016; Soares et al. 2017).

A number of studies to uncover the foraging behavior of *T. laeviceps* and *H. itama* have shown that the two species have different plant preferences. A study by Pangestika et al. (2017) conducted at Ecology Park of Cibinong, West Java indicated that *T. laeviceps* showed flower constancy for plants from the Poaceae family, while *H. itama* showed flower constancy for Solanaceae plants. A similar study by Cholis et al. (2019) on flower constancy conducted in Bogor showed that *T. laeviceps* mainly collected pollen from plants of Polygonaceae, Amaranthaceae, and Solanaceae families, while the pollen collected by *H. itama* was dominated by plants from Polygonaceae. According to Md Zaki and Abd Razak (2018), on rubber smallholding in Trengganu Malaysia showed that *H. itama* showed a preference for small flowers with little or no petals such as *Ixora coccinea*, *Caudatus sulphureus*, *Mimosa pudica*, and *Pereskia sacharosa*.

Based on the analysis of each honey sample, especially those classified as unifloral honey, it was clear that pollen composition and the presence of predominant pollen type could be used as a basis in determining the botanical origin of honey. Melissoplaynologial study on unifloral honey from stingless bees among others, was reported by Shamsudin et al. (2019) on *Melaleuca cajuputi* honey produced by *H. itama* and *Acacia mangium* honey produced by *Geniotrigona thoracia*. The important role of melissopalynological analysis in determining botanical origin and geographic origin of honey might be accomplished either by identifying composition of floral sources (Vijayakumar and Jeyaraaj 2016) or by identifying the existence of specific pollen markers (Selvaraju et al. 2019). Such cases were found in this study by comparing unifloral honey samples showing similarity in their predominant pollen type. The occurrence of *A. pinnata* pollen which was dominant in unifloral honey samples from Karang Bayan and Bengkaung could be considered as pollen marker on both botanical and geographical origin of the honey where a large number of *A. pinnata* were found in the surrounding area, with approximately 20 trees within the distance of 6 m from the hives. The same case could be deducted for honey from Montong Alung with predominant *S. avenis* pollen coming from the area of a short distance of 7-10 m from the beehives. Results of this study, therefore, provide confirmation on the role of melissopalynology in the verification of botanical and geographical origin of honey, as well as identifying the pollen marker for honey authentication as has been mentioned by Selvaraju et al. (2019) and Njokuocha (2019). Pollen diversity found in honey samples from Genggelang and Karang Bajo which showed the dominance of *Polygonum* sp. pollen was in line with the results of research on flower constancy shown by *T. laeviceps* and *H. itama* (Cholis et al. 2019). Honey from Batu Putih which had the predominant pollen of Solanaceae, honey from Lempenge with predominant pollen from Myrtaceae, and Honey from Sesela with predominant pollen from the Fabaceae family were all monofloral honey, clearly showed a preference for stingless bees towards the three plant families as reported by de Novais et al. (2014) that stingless bees collect large amount of Fabaceae and Solanaceae pollen, and those from do Nascimento et al. (2015) that Fabaceae and Myrtaceae pollen was found at considerable amount in honey produced by stingless bees. Meanwhile, the multifloral nature of Sedau honey indicated that in conditions of availability of many plants from various families, stingless bee showed generalist foraging behavior by maximizing the collection of all available resources as mentioned by Kaluza et al. (2017).

The plant diversity profiles presented in the distribution of plant families in this study (Fig. 3) offer valuable information for local beekeepers and stakeholders in the study area. The diversity of pollen found in honey serves as indicator of the diversity of plants providing food sources for honey bee. This information might be used as a reference for farmers in determining plants and landscape structuring for proper ecosystems functioning suitable for honey farming practices (Ponnuchamy et al. 2014). The characteristics of honey that were represented in the diversity of pollen composition come from combination of flora composition, flowering phenology, and flower selection by honey bees (Aronne and De Micco 2010), and thus underlining the importance of melissopalynological studies. The results of this melissopalynological study notably contributed to defining the efforts that need to be made in structuring the landscape that supports the sustainability of beekeeping practices, as pointed out by Md Zaki and Abd Razak (2018). In this case, knowledge on the diversity of plants as nectar and pollen sources might serve as a scientific basis for conservation of habitats that support the life and productivity of honey bees. Based on results of this study a recommendation on plants as potential sources of nectar and pollen for sustainable honey production in the area of study could be made accordingly. *Cocos nucifera* and *Arenga pinnata* are suitable plants for lowland area since these two species produce flowers throughout the year. Seasonal fruit plants recommended are *Mangifera indica* dan *Syzygium cumini* that produce massive flowers of small size and attractive color for stingless bees. Meanwhile, a wild and weedy plant species recommended for open areas surrounding beehives are *Amaranthus spinosus* and *Mimosa pudica*.

This study revealed the diversity of pollen recovered in honey samples collected from nine locations in Lombok Island. The diversity of pollen in honey indicated plant species visited by two species of stingless bee, i.e. *Tetragonula laeviceps* and *Heterotrigona itama* in their foraging activities to collect nectar and pollen as their food and materials for producing honey. Melissopalynological analysis carried out in this study showed that pollen diversity in honey varied greatly among the samples. Composition of pollen types in each honey sample indicated that *T. laeviceps* and *H. itama* forage flowers from various plant families, and thus emphasizing the generalist characteristic of these two stingless bee species. Since most of honey samples were classified as unifloral, this melissopalynological analysis is useful to determine the botanical origin of honey from Lombok Island.

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