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Melissopalynology and Vegetation Analysis Surrounding Sunggau of Giant Honey Bee *Apis dorsata* in Belitung Regency

Dwika Bramasta[1], Ibnul Qayim[1], Nina Ratna Djuita[1*], Rika Raffiudin[1], Ramadhani Eka Putra[2], RC Hidayat Soesilohadi[3], Hery Purnobasuki[4]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia*
[2]*School of Life Sciences and Technology, Bandung Institute of Technology (ITB), Bandung 40132, Indonesia*
[3]*Faculty of Biology, Gadjah Mada University, Yogyakarta 55281, Indonesia*
[4]*Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia*

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ABSTRACT

The forest conversion into oil palm plantations in Belitung impacts the plant source for pollen and nectar to support the honey bee *Apis dorsata*. This study aimed to identify the plants used by *A. dorsata* as pollen sources in honey and bee bread in honey bee nests and to analyze the vegetation composition and structure surrounding the sunggau (artificial nesting site) in Belitung Regency. Honey from *A. dorsata* was collected from bee nests in sunggau on Kampak Island and Tanjung Rusa. The pollen grains from 20 ml honey were acetolysed and counted until 1,200 grains for each honey sample. In Kampak Island, we found eight pollen types in honey dominated by *Rhizophora mucronate* mangrove pollen and eleven pollen types in bee bread dominated by *Melaleuca cajuputi*. The pollen type in honey in Tanjung Rusa was similar to those in Kampak Island, and nine pollen types were found in the bee bread dominated by *Elaeis guineensis*. The vegetation analysis revealed that mangrove and heath forests in Kampak Island were dominated by *Lumnitzera littorea* and *Melaleuca cajuputi*, respectively. The results of this study confirm the bees' notable use of the mangrove ecosystem, which adds conservation value, especially in supporting bee management efforts in Belitung.

1. Introduction

Despite the accelerating local economy in Belitung Island, the oil palm plantation has significantly impacted the pre-existing forest ecosystems (Hermon 2016). Consequently, various unique habitats in Belitung, including flora and fauna, are threatened (Hilwan 2015).

Bee farmers in Belitung construct the giant honey bee *Apis dorsata* artificial nesting site known as sunggau (Hadisoesilo and Kuntadi 2007). The existence of plants is essential for *A. dorsata*. Thus, the diversity of plants surrounding the sunggau affects the availability of bee pollen (Rosmarlinasiah *et al.* 2015).

Plants and honey bees have mutualistic interactions; bees pollinate plants, while plants provide nectar and pollen for bees (Noor *et al.* 2016). The pollen grains adhere to the hairy body of bees during their visitation to flowers (Thorp 2000). Bee pollen was carried in the corbicula (pollen basket) structure in their hind tibia while nectar was stored within their honey stomach (Bohart and Nye 1956). Most nectars are then stored in the bee hive, which gradually thickens as the water content evaporates and eventually forms honey (Jones and Bryant 2014). Honey also contains pollen, aside from the nectar as a significant component. Melissopalynology was developed to study pollen in honey, which was later used to pinpoint honey's botanical and geographical origin (Corvucci *et al.* 2015). Melissopalynology derives from melissa, which means the bee, and palynology for the study of pollen grains (Jones and Bryant 1992). Pollen has distinctive two-layer wall structures: exine and intine (Edlund *et al.* 2004). The intine layer is less resistant to chemical substances, opposite to the exine layer. The exine layer has

* Corresponding Author
E-mail Address: ninadj@apps.ipb.ac.id

variable shapes depending on plant species, hence used in pollen taxonomy studies (Weber 1998).

Melissopalynology of honey collected from *Heterotrigona itama* and *Tetragonula laeviceps* stingless bees in Belitung Regency revealed that the stingless bees forage to *Macaranga tanarius* and *Ageratum conyzoides* (Priambudi *et al.* 2021). However, there is a lack of data regarding melissopalynology from the honey of the giant honey bee *A. dorsata* in Belitung Regency. Therefore, this study aimed to examine plant species used as the pollen source by *A. dorsata* and the composition and structure of vegetation surrounding the sunggau in Belitung Regency.

2. Materials and Methods

2.1. Sample Collection

Honey samples from *A. dorsata* colonies in sunggau were conducted at two locations in Belitung Regency, i.e., Kampak Island (4° 53' 25.3" S, 107° 50' 41,2" E, 11 meters above sea level (masl) and Tanjung Rusa (4° 5' 59,7" S, 107° 49' 28,5" E, 8 masl). Vegetation analysis was conducted in two habitats, namely mangrove and heath forest in Kampak Island, whereas in Tanjung Rusa was conducted in the swamp and secondary forest. Honey and bee bread of *A. dorsata* were sampled in November 2020. A total of 250 ml honey was extracted from the honeycomb, while the bee bread was collected from 15 cells in a honeycomb of each colony and stored in a 15 ml tube.

2.2. Acetolysis and Pollen Identification

Acetolysis was conducted following the method of Louveaux *et al.* (1978). Pollen samples were observed with a compound microscope (CX-23) and then photographed using an OptiLab camera. Pollen identification was based on morphological characters such as pollen class, polar and equatorial shape, exine ornamentation on the outer wall (Paul and Chowdhury 2020), and pollen size based on the Australasian Pollen and Spore Atlas (http://apsa.anu.edu.au/).

2.3. Pollen Percentage Analysis

Pollen types were categorized into predominant (> 45%), secondary pollen (16-44%), important minor (3-15%), and pollen minor (<3%) by calculating the amount of a type of pollen in the sample from the total of all pollen (Louveaux *et al.* 1970). When pollen counting exceeds 1,200 grains, the percentage is calculated by comparing each pollen type with the total pollen types recorded (Louveaux *et al.* 1978).

2.4. Vegetation Analysis

Measurements were made at four levels of plant growing phases (seeding, sapling, poles, and trees) in the plots using the plotted transect method. The transect was established following the flight direction of the bees that were observed using binoculars with the sunggau as the center point of the transect. Based on the flight direction of the bees in Kampak Island, three plots were made to the southwest (mangrove vegetation) and four plots to the northeast (heath forest). In Tanjung Rusa, four plots were made toward the northeast (swamp area) and three plots toward the southwest (secondary forest). Measurement plots were conducted along the transect line, i.e., trees: 20 × 20 m, poles: 10 × 10 m, saplings 5 × 5 m for, and seedlings 2 × 2 m (Susilowati *et al.* 2020). Each plot of seedlings, saplings, poles, and trees phase was recorded for species name, species number, and tree trunk diameter.

Vegetation data were analyzed to determine the important value index (IVI), thus providing an overview of the dominant plant species in a specific area based on relative density, relative frequency, and relative dominance values. Differences in the important value index of vegetation indicate competition in each species for nutrients (Zhang *et al.* 2013). It was determined by calculating using the following formulas:

$$\text{Relative density (RD)} = \frac{\sum \textit{species i}}{\sum \textit{all species}} \times 100\%$$

$$\text{Relative frequency (RF)} = \frac{\textit{Frequency species i}}{\sum \textit{ frequency all species}} \times 100\%$$

$$\text{Relative dominance (RDo)} = \frac{\textit{Basal area species i}}{\sum \textit{ basal area all species}} \times 100\%$$

$$\text{Important value index (IVI)} = \text{RD} + \text{RF} + \text{RDo}$$

3. Results

3.1. Pollen Identified from Honey and Bee Bread *A. dorsata*

Fourteen pollen types were identified from honey and bee bread samples from Kampak Island and Tanjung Rusa (Figure 1). Eight pollen types were identified

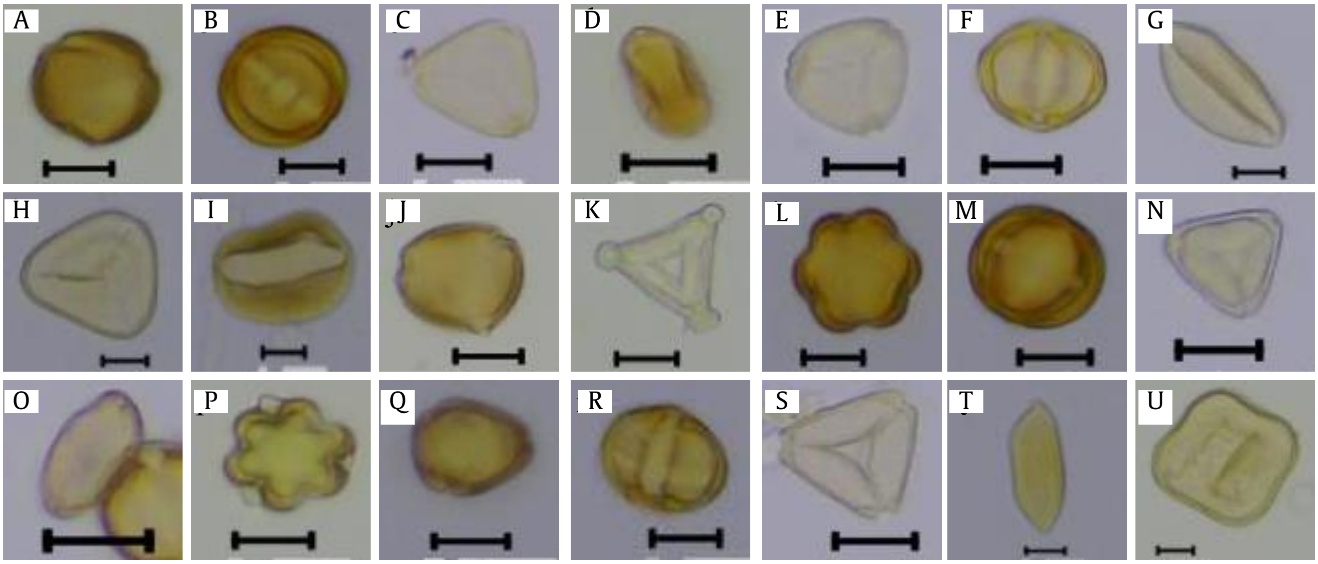

Figure 1. Pollen morphology: (A) *Avicennia alba* polar, (B) *A. alba* equatorial, (C) *Beackea frutescens* polar, (D) *B. grutescens* equatorial, (E) *Bruguiera gymnorrhiza* polar, (F) *B. gymnorrhiza* equatorial, (G) *Cocos nucifera* equatorial, (H) *Elaeis guineensis* polar, (I) *E. guineensis* equatorial, (J) *Heritiera littoralis* polar, (K) *Leptospermum polygalifolium* polar, (L) *Lumnitzera littorea* polar, (M) *L. littorea* equatorial, (N) *Melaleuca cajuputi* polar, (O) *M. cajuputi* equatorial, (P) *Melastoma malabathricum* polar, (Q) *Rhizophora mucronata* polar, (R) *R. mucronata* equatorial, (S) *Syzygium zeylanicum* polar, t. sp. 1, u. sp. 2. Scale: 10 µm

from the honey samples from Kampak Island (Table 1) and Tanjung Rusa (Table 2). Pollen from *R. mucronata* (Rhizophoraceae) showed the highest percentage in either Kampak Island (43.3%) or Tanjung Rusa (28.1%); both are categorized as secondary pollen types (16-45%).

Eleven and nine pollen types from bee bread were identified from Kampak Island (Table 3) and Tanjung Rusa (Table 4), respectively. *Melaleuca cajuputi* (Myrtaceae) pollen in bee bread revealed the highest percentage (28.8%) in Kampak Island and was categorized as a secondary pollen type. While in the bee bread of *A. dorsata* in Tanjung Rusa, *E. guineensis* (Arecaceae) pollen became the highest (58.2%) and was categorized as the predominant pollen type. Our research found that the triangular pollen form from the polar view was dominated in the honey and bee bread samples of *A. dorsata*. In contrast, the oblate form was dominated by equatorial views (Table 1-4).

3.2. Vegetation Structure

The composition and structure of vegetation surrounding the sunggau of *A. dorsata* were determined by identified plant species, individual number per species, and the diameter of the plant trunk. Based on the plant growth phase, we found 21, 18, 13, and 12 for seedling, sapling, pole, and tree species in Kampak Island. The vegetation in Tanjung Rusa comprised 27, 15, 19, and 13 seedling, sapling, pole, and tree species. The seedlings, poles, and trees were higher in Tanjung Rusa, while the growth rate of saplings was higher on Kampak Island (Figure 2).

Lumnitzera littorea dominated mangrove vegetation in Kampak Island at the seedling, sapling, and tree phases, while *Pandanus tectorius* was dominant at the pole phase (Table 5). *Melaleuca cajuputi* dominates the heath vegetation in Kampak Island at the seedling, pole, and tree phases, and *Syzygium urceolatum* dominates at the sapling phase (Table 6).

The swamp in Tanjung Rusa was dominated by *M. cajuputi* at seedling, sapling, and pole levels, with *Antidesma cuspidatum* dominating the tree level (Table 7). Meanwhile, the secondary forest on this island showed there was no domination at each phase: those were *Syzygium bankense, Commersonia bartramia, H. brasiliensis*, and *Acacia mangium* at seedling, sapling, pole, and tree level (Table 8).

Table 1. Diversity of pollen from honey *A. dorsata* sunggau Kampak Island

Species/family	Aperture	Polar shape	Equatorial shape	Exine ornamentation	Polar/equatorial size (µm)	Percentage (%)
A. alba/Acanthaceae	3-colporate	Cir	O	Reti	14.09±1.09/18.54±1,17	6.9
B. gymnorhiza/Rhizophoraceae	3-colporate	Cir	P	Reti	16.22±1.06/15.7±0.45	26.1
C. nucifera/Arecaceae	Monocolpate	-	COC	Sca	-/34.74±1.17	0.3
E. guineensis/Arecaceae	Syncolpate	ST	CO	Psi	27.69±2.02/30.22±1.91	13.9
L. littorea/Combretaceae	3-colporate	Poly	Cir	Gra	18.13±0.54/23.09±3.35	7.6
M. cajupati/Myrtaceae	3-colporate	Tri	O	Psi	12.9±0.7/10.65±3.13	1.8
R. mucronata/Rhizophoraceae	3-colporate	Tri	SP	Reti	13.61±1.28/16.92±1.07	43.3
Sp. 1	Dicolpate	-	E	Psi	-/37.6±2.3	0.2

Cir: circular, ST: semi triangular, Poly: polygonal, Tri: triangular, O: oblate, P: prolate, SP: subprolate, E: elliptic, COC: constricted oval circular, CO: circular oval, Reti: reticulate, Sca: scabrate, Psi: psilate, Gra: granulate

Table 2. Diversity of pollen from honey *A. dorsata* sunggau Tanjung Rusa

Species/family	Aperture	Polar shape	Equatorial shape	Exine ornamentation	Polar/equatorial size (µm)	Percentage (%)
A. alba/Acanthaceae	3-colporate	Cir	O	Reti	14.82/19.5±1.07	4.3
B. frutescens/Myrtaceae	3-colporate	Tri	O	Psi	16.4±1.1/15.7±1.5	7.5
B. gymnorhiza / Rhizophoraceae	3-colporate	Cir	P	Reti	15.3±1.7/15.5±1.4	21.5
E. guineensis/Arecaceae	Syncolpate	ST	CO	Psi	311±1.1/33.5±1.6	15.1
L. littorea/Combretaceae	3-colporate	Poly	Cir	Gra	19.2±1.2/20.7±0.6	7.5
M. cajupati/Myrtaceae	3-colporate	Tri	O	Psi	13.2±0.4/14.7±1.2	9.8
R. mucronata/Rhizophoraceae	3-colporate	Tri	SP	Reti	14.3±1.5/18.3±1.4	28.1
S. zeylanicum/Myrtaceae	3-colporate	Tri	-	Reti	16.1±0.9/-	6.3

Cir: circular, Tri: triangular, ST: semi triangular, Poly: polygonal, O: oblate, P: prolate, SP: subprolate CO: circular oval, Reti: reticulate, Psi: psilate, Gra: granulate

Table 3. Pollen diversity from bee bread of *A. dorsata* sunggau Kampak Island

Species/family	Aperture	Polar shape	Equatorial shape	Exine's ornamentation	Polar/equatorial size (µm)	Percentage (%)
A. alba/Acanthaceae	3-colporate	-	O	Reti	-/18.2±0.7	8.5
B. frutescens/Myrtaceae	3-colporate	Tri	O	Psi	15.9±0.97/16.1±0.4	1.6
B. gymnorhiza/Rhizophoraceae	3-colporate	Cir	P	Reti	15.8±0.2/15.5±0.9	12.8
C. nucifera/Arecaceae	Monocolpate	-	COC	Sca	-/35.7±1.7	1.1
E. guineensis/Arecaceae	Syncolpate	ST	CO	Psi	29.3±1.3/33.3±2.3	24.4
L. polygalifolium/Myrtaceae	3-colporate	Tri	-	Psi	18.48/-	0.1
L. littorea/Combretaceae	3-colporate	Poly	Cir	Gra	17.6±0.4/26.8±1.2	9.3
M. cajupati/Myrtaceae	3-colporate	Tri	O	Psi	12.2±0.4/13.4±0.3	28.8
M. malabathricum/ Melastomataceae	Heterocolpate	Poly	-	Pro	14.9±1.2/-	0.3
Rhizphora mucronata/ Rhizophoraceae	3-colporate	Tri	SP	Reti	14.9±0.3/17.5±0.7	13.2
Sp. 2	Inaperturate	Quad	-	Psi	37.54/-	0.1

Tri: triangular, Cir: circular, Poly: polygonal, Quad: quadrangular, ST: semi triangular, O: oblate, P: prolate, SP: subprolate, COC: constricted oval circular, CO: circular oval, Reti: reticulate, Psi: psilate, Sca: scabrate, Gra: granulate

Table 4. Diversity of pollen from bee bread of *A. dorsata* sunggau Tanjung Rusa

Species/family	Aperture	Polar shape	Equatorial shape	Exine's ornamentation	Polar/equatorial size (µm)	Percentage (%)
A. alba/Acanthaceae	3-colporate	Cir	O	Reti	16.8±0.7/18.7±1.2	0.4
B. frutescens/Sapu-Sapu	3-colporate	Tri	O	Psi	15.2±1.3/14.8±1.99	8.6
B. gymnorhiza/Rhizophoraceae	3-colporate	Cir	P	Reti	13.9±0.2/15.6±1.04	2.3
E. guineensi/Arecaceae	Syncolpate	ST	CO	Psi	28.2±2.01/32.8±1.7	58.2
H. littoralis/Malvaceae	3-colporate	Cir	-	Reti	18.26/-	0.1
L. littorea/Combretaceae	3-colporate	Poly	Cir	Gra	18.3±0.7/25.25±0.8	0.8
M. cajupati/Myrtaceae	3-colporate	Tri	O	Psi	11.2±1.2/10.5±1.08	26.5
R. mucronata/Rhizophoraceae	3-colporate	-	SP	Reti	-/17.2±1.4	0.6
S. zeylanicum/Myrtaceae	3-colporate	Tri	-	Reti	15.2±0.3/-	2.7

Cir: circular, Tri: triangular, ST: semi triangular, Poly: polygonal, O: oblate, P: prolate, SP: aubprolate, CO: circular oval, Reti: reticulate, Psi: psilate, Gra: granulate

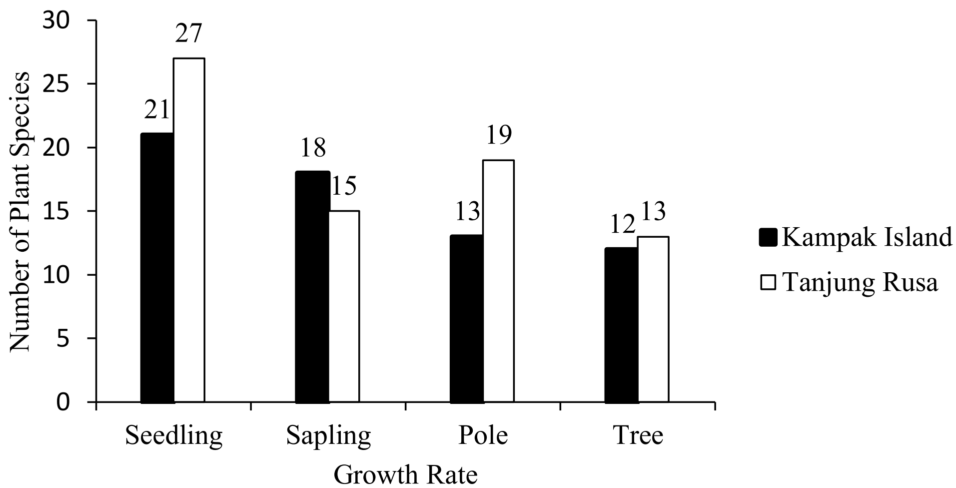

Figure 2. Number of plant species at various growth rates at the two observation sites

Table 5. The three highest Important Value Index (IVI) in mangrove vegetation of Kampak Island

Local name	Scientific name	Relative density (%)	Relative frequency (%)	Relative dominance (%)	IVI (%)
		Seedling			
Teruntum merah	*L. littorea*	68.32	27.25	-	95.57
Api-api	*A. alba*	7.92	18.17	-	26.09
Sembong laut	*Scaevola taccada*	7.92	9.08	-	17.00
		Sapling			
Teruntum merah	*L. littorea*	23.08	10.01	35.14	68.23
Api-api	*A. alba*	15.38	20.02	20.65	56.05
Waru	*H. tiliaceus*	23.08	20.02	9.96	53.06
		Pole			
Pandan laut	*P. tectorius*	36.36	24.97	15.91	77.24
Teruntum merah	*L. littorea*	18.18	12.48	19.02	49.68
Empenai laut	*Ardisia lanceolata*	9.09	12.48	17.99	39.56
		Tree			
Teruntum merah	*L. littorea*	73.68	42.92	93.31	209.91
Tumu	*R. mucronata*	21.05	42.92	5.71	69.68
Pandan laut	*P. tectorius*	5.26	14.31	0.97	20.54

Table 6. The three highest important value index (IVI) in the heath forest of Kampak Island

Local name	Scientific name	Relative density (%)	Relative frequency (%)	Relative dominance (%)	IVI (%)
		Seedling			
Gelam	*M. cajuputi*	44.78	11.76	-	56.54
Sapu-sapu	*B. frutescens*	9.57	11.76	-	21.33
Karemunting	*Rhodomyrtus tomentosa*	7.83	11.76	-	19.59
		Sapling			
Samak	*S. urceolatum*	20	16.67	35.69	72.36
Gelam	*M. cajuputi*	20	8.33	14.79	43.12
Sapat	*Syzygium acuminatissima*	10	8.33	8.20	26.53
		Pole			
Gelam	*M. cajuputi*	45	33.33	46.01	124.34
Nudong	*A. cuspidatum*	25	11.11	28.11	64.22
Pelempang hitam	*Adinandra dumosa*	10	11.11	13.05	34.16
		Tree			
Gelam	*M. cajuputi*	53.33	23.08	49.50	125.91
Samak	*S. urceolatum*	13.33	23.08	14.61	51.02
Arang-arang	*Syzygium calophyllifolium*	6.67	15.38	9.11	31.16

Table 7. The three highest Important Value Index (IVI) in the swamp vegetation of Tanjung Rusa

Local name	Scientific name	Relative density (%)	Relative frequency (%)	Relative dominance (%)	IVI (%)
		Seedling			
Gelam	*M. cajuputi*	57.52	23.53	-	81.05
Kletak	*M. malabathricum*	14.38	5.88	-	20.26
Mensira	*Ilex cymosa*	9.15	5.88	-	15.03
		Sapling			
Gelam	*M. cajuputi*	47.37	27.27	46.23	120.87
Pelawan	*Tristaniopsis obovata*	15.79	9.09	12.62	37.50
Pelangas	*Aporosa frutescens*	5.26	9.09	9.98	24.33
		Pole			
Gelam	*M. cajuputi*	30.77	21.43	8.78	60.98
Seru	*Schima walichii*	7.69	28.57	16.15	52.41
Mampat	*Cratoxylum formosus*	15.38	7.14	12.41	34.93
		Tree			
Nudong	*A. cuspidatum*	63.16	16.67	77.62	157.45
Gelam	*M. cajuputi*	21.05	33.33	12.87	67.25
Seru	*S. walichii*	5.26	16.67	5.06	26.99

Table 8. The three highest important value index (IVI) in Tanjung Rusa secondary forest vegetation

Local name	Scientific name	Relative density (%)	Relative frequency (%)	Relative dominance (%)	IVI (%)
		Seedling			
Pelanduk	*S. bankense*	42.37	4.55	-	46.92
Betor	*Calophyllum lanigerum*	5.93	9.1	-	15.03
Ncirit	*S. grandifolia*	10.17	4.55	-	14.72
		Sapling			
Mentenu	*C. bartramia*	16.67	11.11	20.81	48.59
Akasia	*A. mangium*	16.67	11.11	19.38	47.16
Pelawan	*T. obovata*	16.67	11.11	17.68	45.46

Table 8. Continued

Local name	Scientific name	Relative density (%)	Relative frequency (%)	Relative dominance (%)	IVI (%)
		Pole			
Karet	*H. braciliensis*	23.53	15.4	19.54	58.47
Ncirit	*S. grandifolia*	11.76	15.4	16.83	43.99
Leban	*Vitex pinnata*	11.76	7.7	19.99	39.45
		Tree			
Akasia	*A. mangium*	21.05	11.11	19.85	52.01
Ncirit	*S. grandifolia*	10.53	11.11	23.66	45.3
Seruk	*S. walichii*	15.79	11.11	12.97	39.87

4. Discussion

Pollen in honey and bee bread from Kampak Island and Tanjung Rusa has various morphology (Figure 1). Psilate exine ornament was dominantly found in honey and bee bread from Kampak Island (Table 1 and 3). In contrast, the reticulate type was dominantly found in honey and bee bread samples from Tanjung Rusa (Table 2 and 4). Pollinators such as bees generally prefer pollen with ornaments with rougher surfaces, such as reticulate, that help in collecting pollen, and also pollen grains with a deeply sculptured exine, such as psilate, that the sculptures would enhance the adherence of the pollen grains to the body (Sannier *et al.* 2009). Tricolporate pollen aperture was dominantly observed in honey and bee bread from both locations (Table 1-4). Tricolporate aperture is commonly found in pollen preferred by *A. dorsata* in Chandrapur District, India (Laxmikant and Devandra 2018).

Kampak Island has fewer plant species than Tanjung Rusa (Figure 2). The ecosystem in Kampak island consist of mangrove (Table 5) and heatth forest (Table 6), while swamp and secondary forest ecosystem were found in Tanjung Rusa (Table 7 and 8). As in other coastline habitats, the mangrove ecosystem in Kampak Island is characterized by high salinity, and only a few plant species tolerate this condition. Plants should have adapted to high salinity (e.g., using salt glands, salt exclusion, and vivipary) to grow well in this habitat (Noor *et al.* 2015).

Rhizophora mucornata was revealed as the second highest important index value (IVI) at tree level in the mangrove of Kampak Island (Table 5) and also was observed to have the highest percentage of pollen in honey sampled from this island (Table 1). This indicated that *R. mucronata* is essential for *A. dorsata* as a pollen source. The plants provide a considerable amount of pollen and nectar during the flowering period, serving as a potential food source for bees (Seethraman and Kandasamy 2011). The heath forest in Kampak Island was dominated by *M. cajuputi* (Table 6) and was also shown in the highest percentage of pollen in the bee bread of *A. dorsata* (Table 3).

We found a high percentage of mangrove species pollens recorded from honey in Tanjung Rusa, regardless of the long distance between the source and the sunggau of *A. dorsata* (Table 2). This indicated that mangrove vegetation is crucial as a pollen source for *A. dorsata.* Mangrove species such as *Rhizophora* sp., *Bruguiera* sp., and *Avicennia* sp. were previously reported as pollen and nectar sources for *A. dorsata* bees in West Bengal, India (Kamble *et al.* 2013).

Although oil palm *E. guineensis* is not a dominant species based on vegetation analysis in Tanjung Rusa (Table 7 and 8), *E. guineensis* pollen dominated the bee bread collected from this island (Table 2). As a significant component in monoculture plantations, *E. guineensis* was excluded from vegetation analysis. The same phenomenon was also found in Kampak Island, where *E. guineensis* has a high percentage of pollen in honey and bee bread (Table 1 and 3). However, the nearest oil palm plantation is 2 km from Kampak Island (personal observation).

Bees need both pollen and nectar to feed their colonies. Based on this study, *M. cajuputi, R. mucronata, B. gymnorhiza*, and *L. littorea* composed significant components in the pollen of *A. dorsata.* Therefore conservation efforts should be taken for these plants.

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