

PALYNOLOGY OF THE STINGLESS BEES, *Heterotrigona itama* (HYMENOPTERA: APIDAE) IN THE COASTAL FOREST OF TERENGGANU

**Roziyah Ghazi¹, Abd Jamil Zakaria¹, Wahizatul Afzan Azmi²,
Fahimee Jaapar³ & Norhayati Ngah^{1,4*}**

¹Faculty of Bioresources and Food Industry,
Universiti Sultan Zainal Abidin, Besut Campus,
22200 Besut, Terengganu, Malaysia.

²Faculty of Science and Marine Environment,
Universiti Malaysia Terengganu,
21300 Kuala Nerus, Terengganu, Malaysia.

³Biological Control Programme,
Agrobiodiversity and Environmental Research Centre,
MARDI Headquarters,
43400 Serdang, Selangor, Malaysia.

⁴East Coast Environmental Research Institute,
Universiti Sultan Zainal Abidin, Gong Badak Campus,
21300 Kuala Nerus, Terengganu, Malaysia

*Corresponding author: norhayatingah@unisza.edu.my

Received: 30 January 2024; Accepted: 21 August 2024

ABSTRACT

Stingless bee, *Heterotrigona itama* (Apidae, Meliponini) is one of the few social bee species naturally occurring in the coastal forests of Terengganu, where a wide variety of flowering plants are available throughout the year. Studies on the botanical sources of honey and bee bread produced by stingless bees in Malaysia are well-documented. However, data pollen load of stingless bees varies based its species-specific characteristics, behavioral factors and various environmental condition at certain location are lacking. Therefore, palynological investigation is important to explore the floral collection of *H. itama*, especially in the coastal forests. Six colonies of *H. itama* were selected for sampling pollen grains in the Taman Rimba Ilmu Tanah Beris (TRIBE), located at Universiti Sultan Zainal Abidin, Besut Campus, Terengganu, Malaysia. Pollen grains were collected from the pollen baskets of *H. itama* foragers. The data was analyzed based on the following categories: dominant pollen (>45%), accessory pollen (15-45%), isolated pollen (3-15%), and occasional pollen (<3%). Two-way Cluster Analysis on grouped pollen types was done to classify their similarity by hive and month, while Detrended Correspondence Analysis (DCA) to illustrate pollen assemblage ordination across different months and hives was done. Overall, 43 plant species belonging to 20 families were visited by *H. itama*, as observed directly and through palynological analysis. However, only 21 plant species from 20 families were successfully identified. The most abundant families collected by *H. itama* were Fabaceae (*Acacia* spp.) and Lamiaceae (*Orthosiphon aristatus*), followed by Passifloraceae (*Passiflora edulis*) and Asteraceae (*Bidens pilosa* and *Cosmos caudatus*). Other types of pollen grains were considered as accessory, isolated, or occasional

pollen grains. Highest amounts of pollen types were collected in May 2016, as it was the flowering season for most plant species in the TRIBE. The findings of this study can help us develop more effective strategies for the conservation of coastal forest ecosystems, and ensuring the sustainability of stingless bee habitats that are crucial for plant pollination.

Keywords: Stingless bee, coastal forest, palynology, pollen

ABSTRAK

Lebah kelulut, *Heterotrigona itama* (Apidae, Meliponini) merupakan spesies lebah sosial yang hidup secara semulajadi di hutan pantai Terengganu, yang ditumbuhi dengan pelbagai jenis tumbuhan berbunga sepanjang tahun. Walaupun kajian mengenai sumber botani madu dan roti lebah kelulut di Malaysia telah banyak dilaporkan, namun data debunga yang dikumpul oleh lebah kelulut berbeza berdasarkan ciri-ciri spesifik spesiesnya, faktor tingkah laku dan pelbagai keadaan persekitaran di lokasi tertentu masih kurang. Oleh itu, kajian palinologi adalah penting untuk mengkaji koleksi flora oleh *H. itama*, terutama di hutan pantai. Enam koloni *H. itama* telah dipilih untuk mengumpulkan butir debunga di Taman Rimba Ilmu Tanah Beris (TRIBE), yang terletak di Kampus Besut, Universiti Sultan Zainal Abidin, Terengganu, Malaysia. Butir debunga telah dikumpulkan dari bakul debunga lebah pekerja *H. itama*. Data dianalisis berdasarkan kategori berikut: debunga dominan (>45%), debunga aksesori (15-45%), debunga terpencil (3-15%) dan debunga sesekali (<3%) telah dijalankan. Analisis Kluster Dua Hala mengelompokkan jenis debunga untuk mengklasifikasikan kesamaan mereka mengikut sarang dan bulan, manakala Analisis Korespondensi Tidak Berterusan (DCA) menggambarkan susunan himpunan debunga merentas bulan dan sarang yang berbeza telah dijalankan. Secara keseluruhan, 43 spesies tumbuhan daripada 20 famili telah dilawati oleh *H. itama*, seperti yang diperhatikan secara langsung dan melalui analisis palinologi. Walau bagaimanapun, hanya 21 spesies tumbuhan daripada 20 famili yang berjaya dikenalpasti. Famili yang paling banyak dikumpulkan oleh *H. itama* adalah Fabaceae (*Acacia* spp.) dan Lamiaceae (*Orthosiphon aristatus*), diikuti oleh Passifloraceae (*Passiflora edulis*) dan Asteraceae (*Bidens pilosa* dan *Cosmos caudatus*). Jenis-jenis debunga lain dianggap sebagai debunga aksesori, terasing atau sementara. Jumlah debunga yang paling tinggi telah dikumpulkan pada bulan Mei 2016, kerana ia adalah musim berbunga bagi kebanyakan spesies tumbuhan di TRIBE. Hasil daripada kajian ini dapat membantu kita merangka strategi yang lebih efektif untuk konservasi ekosistem hutan pantai, sekaligus memastikan kelestarian habitat lebah kelulut yang penting bagi pendebungaan tumbuhan.

Kata kunci: Lebah kelulut, hutan pantai, palinologi, debunga

INTRODUCTION

Stingless bees of the species *Heterotrigona itama* (Hymenoptera: Apidae) are frequently found in the coastal forests of Terengganu, Malaysia (Lob et al. 2017). This bee species plays a crucial role in the functioning of coastal forest ecosystems, particularly in pollination services. Their actions contribute to the reproduction and propagation of numerous plant species, ultimately enhancing the overall biodiversity and functioning of coastal ecosystems. This diverse foraging behavior promotes cross-pollination, leading to increased genetic diversity within plant populations. As they collect nectar and pollen, these bees inadvertently transfer pollen anthers to the stigma of flowers, facilitating plant reproduction (Forster et al. 2023). Stingless bee is also uniquely adapted to coastal forest environments. Due to their small size, they can access and pollinate small, inconspicuous flowers that larger pollinators may not be able to reach

(Bueno et al. 2023). Additionally, their ability to fly through dense vegetation and their efficient navigation skills allow them to access various microhabitats within the coastal forest, increasing the chances of encountering different plant species.

Coastal forests are unique ecosystems that occur in the narrow zone between land and sea. This type of forest is rich in biodiversity and provide vital ecological services such as carbon sequestration, erosion control, and habitat provision for numerous plant and animal species (Friess et al. 2020). The flora species in the coastal forest is vary depending on the specific location and environmental conditions, but there are some common plant species and general features of these ecosystems. One of the prominent species of the coastal forest in Terengganu is the presence of mangrove trees. Malaysia's coastal forest is home to several mangrove species such as *Melaleuca cajuputi*, *Rhizophora apiculata* and *Alstonia spatulate* (Pesiou et al. 2022). Apart from mangroves, the coastal forest is characterized by a mix of shrubs, herbs, and grasses. These plant species are adapted to the low light conditions prevalent beneath the canopy of the larger trees. As an example, *Pandanus tectorius* (screw pine) is commonly found in coastal regions of Malaysia (Hamdan et al. 2020). It has long, slender, spiky leaves and an extensive root system that helps anchor the soil and sand, making it useful for stabilizing coastal areas. The coastal forest in Terengganu is also home to a wide array of ferns and epiphytes. These plants attach themselves to the trunks and branches of trees and absorb moisture and nutrients from the air and rain. The epiphytic orchids, such as *Dendrobium crumenatum* (pigeon orchid), and epiphytic ferns are quite common in coastal forest.

Within this complex web of life, stingless bees have evolved to become important players in maintaining the health and functioning of these coastal ecosystems. Many coastal forest plant species depend on these bees for pollination, as they have coevolved specific adaptations to attract and interact with stingless bees. Some plants even have flower structures perfectly sized for the bees, enabling an efficient exchange of pollen. Stingless bees are particularly adept at pollinating specific types of flowers, such as those with tubular shapes or with hidden nectar (Roubik et al. 2018). While other pollinators, such as butterflies or birds, might struggle to access the nectar, stingless bees are able to access it with their specialized mouthparts and body structures. As a result, they promote genetic diversity within plant populations and ensure the survival of various plant species in coastal forest ecosystems. Additionally, the fruits and seeds that result from successful pollination are vital food sources for a wide range of animal species, including birds, mammals, and insects, further supporting the ecological balance within the ecosystem.

Their ecological importance, the foraging behavior and resource utilization patterns of *H. itama* within coastal forests remain relatively unexplored. While melissopalynological studies in Malaysia are emerging, they still lack comprehensive coverage across different regions and bee species. Previous research by Rosdi et al. (2016) in northern Malaysia, Selvaraju et al. (2019) on the West Coast of Malaysia, and Majid et al. (2020) in Southern Malaysia provides valuable insights but remains geographically limited. Additionally, research by Azmi et al. (2015) in Besut, Terengganu, on the melissopalynology of *Lepidotrigona terminata* expands our understanding of different stingless bee species. Furthermore, the study melissopalynology of *H. itama* by Chelong et al. (2020) conducted in the Pattani coastal forest of Thailand, illustrates regional variations in pollen foraging and plant preferences. However, there is need for more localized research in Malaysia to build a comprehensive understanding of the ecological roles of stingless bees, emphasizing the importance of expanding both geographical and species-specific studies. Few melittopalynological studies have been conducted on the east coast of Peninsular Malaysia. For example, a previous study shows that

stingless bees visit various plants such as *Flacourtia jangomas*, *Lithocarpus* spp., *Mimusops elengi*, *Capsicum* spp., *Citrus hystrix* and *Murraya paniculata* (Ghazi et al. 2018). Additionally, Azmi et al. (2015) reported that in Besut, Terengganu, stingless bees are attracted to *Murraya paniculata*, *Citrus hystrix*, *Calophyllum inophyllum*, *Ixora coccinea*, *Bougainvillea glabra*, *Mimosa pudica*, *Asystasia gangetica* and *Suregada multiflora*.

The current trend in research focuses solely on the composition of honey, dependent on the types and origin of bees' foods, which are flower nectar and plant honeydew (Sujanto et al. 2022). Thus, understanding the foraging activities of *H. itama* is essential for comprehending their ecological role and conserving the coastal forest ecosystems especially in Terengganu. Thus, this study aims to investigate the palynology of *H. itama* at the coastal forest of Terengganu. The study utilized a combination of field observations and analysis of collected pollen samples to achieve its objectives. The findings of this study will not only contribute to the understanding of the ecological role of *H. itama* bees but also provide crucial information for the conservation and management of the coastal forests of Terengganu.

MATERIALS AND METHODS

Sampling Location

The study was conducted at the Taman Rimba Ilmu Tanah Beris (TRIBE), Universiti Sultan Zainal Abidin (UniSZA), Besut Campus in the Terengganu state, on the east coast of Peninsular Malaysia. The TRIBE area is approximately 100 acres and is situated within the UniSZA coastal forest reserve. The sampling site was the UniSZA stingless bee apiary located at coordinates '5.760371 N, 102.634221 E'. TRIBE contains a large number of trees, climbers, lianas, and shrubs. The dominant mangrove plants, including both exclusive and non-exclusive species found at TRIBE, are *M. cajuputi* and *Acacia auriculiformis*. In addition to these, some of the dominant vegetation found in the study area includes *Asystasia gangetica*, *Olea brachiata*, *Antigonon leptopus*, *Synsepalum dulcificum*, *Passiflora edulis*, *Citrus* spp., and *Syzygium* spp.

Stingless Bee Hive Selection

In total, 50 stingless bee colonies were kept in TRIBE for honey production. However, only six mature colonies of stingless bees, at least 1 year old, that were in great condition, free of any disease, and had an active colony, were selected for this study. The age of the colonies has been identified and confirmed by the structure of the nest and the queen. Normally the 1-year-old hive developed 9 layers of eggs. The selected colonies were maintained and acclimatized in the field for approximately six months under natural climatic conditions before the experiment. The distance between selected hives is approximately three meters between each hive. The hives were built topping from wooden box of 40 cm x 30cm x 5 cm.

Sampling of Pollen Grains

Samplings of pollen grains from the bodies of *H. itama* foragers were conducted once per month for 12 months (March 2016 until February 2017). However, the data for January 2017 is unavailable due to heavy flooding at the location of the study, which caused the access road to be closed. Pollen grains were collected using a modified method from Marques-souza et al. (1996). The samplings were done on hot sunny days, between 0900 and 1100 hours. The time of sampling is related to the optimal foraging conditions and the biology of flowering plants. Most of the flowers at TRIBE open and release their pollen during the early morning hours, which means there is an abundance of fresh, newly opened flowers available for stingless bees to forage during this time.

To capture the stingless bee foragers with pollen loads, the entrance of the hives was temporarily closed with a net. Twenty arriving workers from each hive that had pollen loads were caught using forceps. After removing the pollen loads with a blunt needle, the stingless bee workers were released. The pollen clusters from each *H. itama* individual were preserved in vials containing 70% ethanol and the vials were properly labeled.

Pollen Slides Preparation

One microliter of the preserved pollen grains in each vial was transferred to a slide using a micropipette (10 μ l, Eppendorf, Germany) and covered with a slide cover. Each vial had four replicates of slides to ensure accurate data. The slides were observed under a 1300 light microscope (Moticam, United States) with 40X magnification. The pollen images were captured and the length and width of the pollen were measured. The pollen grains collected from the bodies of stingless bee foragers were identified by comparing them to the reference slides and the reference book (Hesse et al. 2009; Jaapar et al. 2015; Kiew & Muid 2019; Zaki et al. 2018). Based on the obtained images, a qualitative analysis of pollen types based on shape, size, and surface structure, as well as their classification into family, genus, or species, was performed,

Data Analysis

Paleontological Statistics Software Package for Education and Data Analysis (PAST) version 4.13 (Hammer et al. 2001) was used for all of the statistical analyses. One-way Analysis of Variance (ANOVA) was used to examine the data to determine whether there was a significant difference at a 95% confidence level ($P=0.05$). The stingless beehives were defined as an explanatory variable and significant differences between mean values were investigated using Least Significant Difference (LSD). The frequencies and occurrences of pollen grains were adopted from Luz and Barth (2012). The following frequencies to estimate the pollen grains comprised of dominant pollen = >45%; accessory pollen = 15% to 45%; isolated pollen = 3% to 15% and occasional pollen = <3%. Two-way Cluster Analysis was used to sort the type of pollen grains into groups in order to classify the similarity of pollen grains collected by *H. itama* at each hive and month. Detrended correspondence analysis (DCA) was used to illustrate the pollen assemblage ordination based on different months and hives.

RESULTS AND DISCUSSION

Types of Pollen

Overall, palynological analysis of 1283 pollen baskets from *H. itama* at TRIBE revealed that 43 plant species belonging to 20 families were visited by the stingless bee. However, out of 43 species plant species visited by *H. itama* during the period of March 2016 until February 2017, only 21 plant species belonging to 20 families of flowering plants were successfully identified which include of mangrove species, ornamental trees, underutilized fruits, agriculture fruits and others. There were 22 morphotype of pollens cannot be identified.

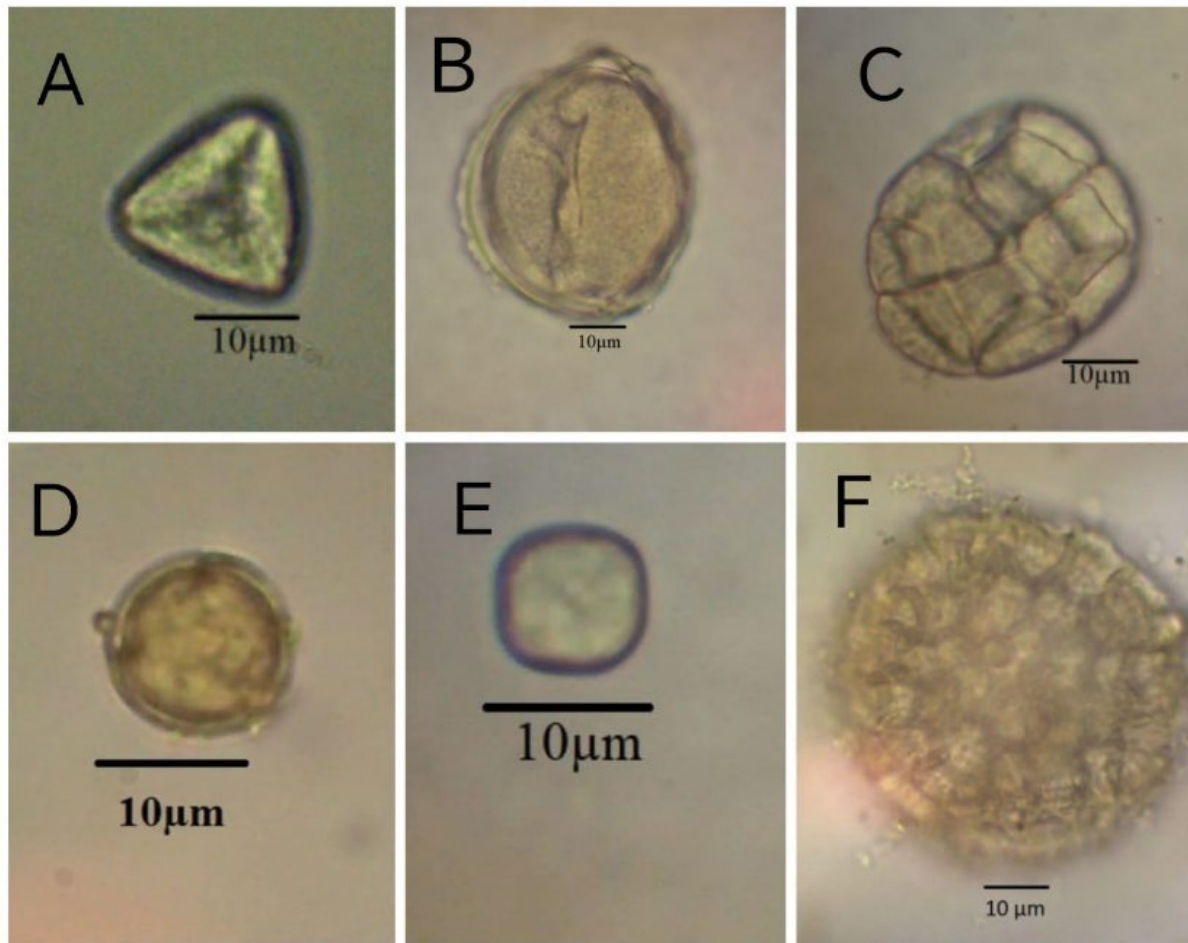


Figure 1. The main pollen types identified during the study (A) *Melaleuca cajuputi*, (B) *Cocos nucifera*, (C) *Acacia auriculiformis*, (D) *Muntingia calabura*, (E) *Mimosa pudica* and (F) *Passiflora edulis*

The total number of pollen grain types collected by *H. itama* varied significantly between months (Figure 2). The highest number of pollen grain types was collected in May, with a total of 20 types, while the lowest number was observed in October, with only 11 types. There was a significant difference in the total number of pollen grain types collected by *H. itama* in different months ($F_{10, 55} = 3.69, P < 0.05$).

Regarding the hive-specific data, Hive 3 had the highest number of pollen grain types collected, totaling 35 types, followed by Hive 6 with 32 types, and Hive 2 with 30 types. Hive 5 had the lowest number of types, with only 25. There was no significant difference in the total number of pollen grain types collected by the different hives of *H. itama* ($F_{5, 1278} = 0.089, P > 0.05$). The total number of pollen grain types collected by *H. itama* for each hive was fairly similar, ranging from 25 to 35 types of pollen grains.

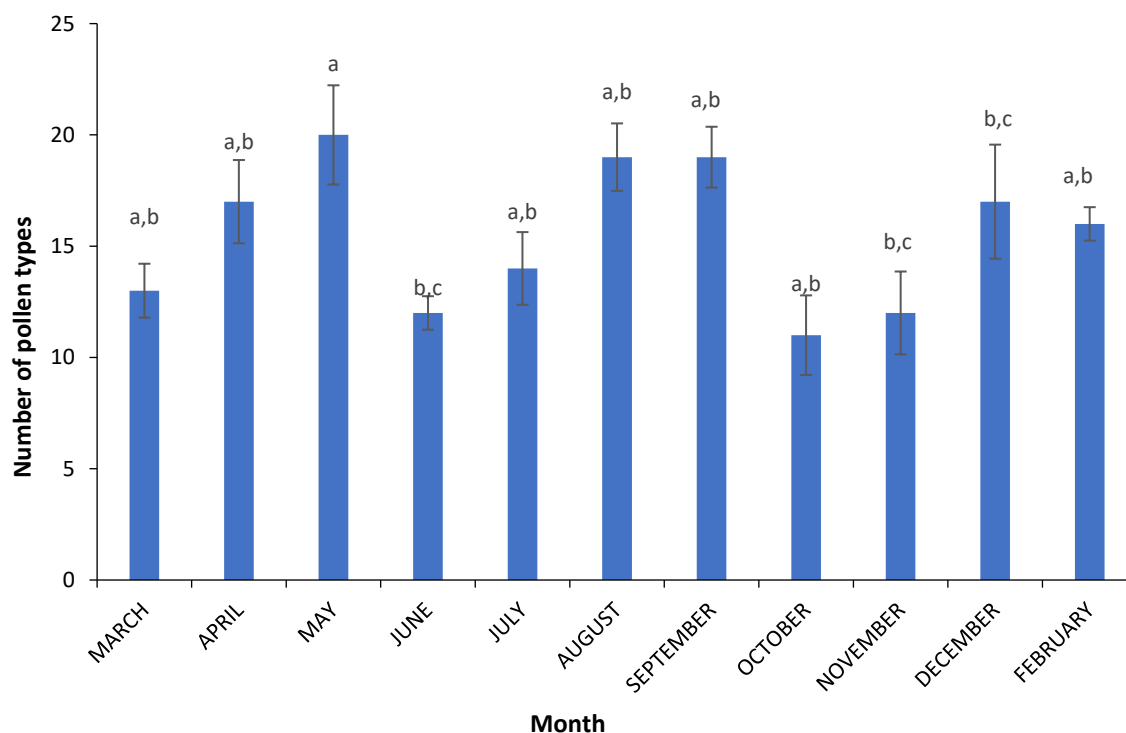


Figure 2. Total types of pollen grains collected by *H. itama* at TRIBE. Different letters indicate significant difference between the total types of pollen collected for each month. Error bars as standard deviation

Temporal Composition of Pollen Grains Collected by *Heterotriona itama*

The main pollen type found in Mac 2016 was unidentified A (48%) followed by *Cassia biflora* (13%). *Lindernia crustacea* (Linderniaceae, 11 %) constituted the third highest type of pollen collected by *H. itama*. However, the types of pollen grains were considered as accessory pollen grains (Table 1). The lowest were *P. edulis* (Passifloraceae; 1%), *Cocos nucifera* (Palmea; 1%), *Citrus* spp. and (Rutaceae; 1%). All of them were categorized as occasional pollen grains. During April, the most prevalent pollen collected by *H. itama* was *M. cajuputi* from the Myrtaceae family, accounting for 39% of the pollen grains, also classified as accessory pollen. The least collected pollen was *Cleome rutidosperma* (Capparaceae, 1%), and *A. gangetica* (Acanthaceae; 1%) Pollen in April was grouped as isolated and occasional pollen.

May exhibited the highest diversity of pollen types among all months, with 20 different species identified. *Passiflora edulis* (Passifloraceae; 25%) was the most abundant, followed by *Bidens pilosa* (Asteraceae; 12%). Meanwhile, the lowest pollen grains included *C. nucifera* (Palmea; 1%), *Acacia* spp (Fabaceae, 1%), *Muntingia* and *calabura* (Tiliaceae; 1%). In June, the most prevalent pollen was identified as Unidentified G (53%), categorized as dominant pollen grains, followed by *C. rutidosperma* (Capparaceae, 19%), classified as accessory pollen. *Passiflora edulis* (Passifloraceae; 1%) was the least collected.

In July *Orthosiphon aristatus* (Lamiaceae; 13%) was observed to have the the highest pollen collected, trailed by *S. dulcificum* (Sapotaceae; 11%). The lowest pollen collected was from *C. nucifera* (Asteraceae; 1.67%). In August 2016, *H. itama* collected 19 types of pollen grains, though only 12 could be identified. Majority of the pollen was considered occasional, except for *L. crustacea* and *M. cajuputi*, which were classified as accessory pollen. *Lindernia*

crustacea (Linderniaceae, 47%) was the most abundant, followed by *M. cajuputi* (Myrtaceae: 9%), whereas *B. Pilosa* (Asteraceae; 1%), *Mimosa pudica* (Leguminosea; 1%), and one pollen from Lamiacea (1%).

September exhibited a dominant presence of *P. edulis* (Passifloraceae; 20%), followed by *Acacia* spp. (Fabaceae, 12%) and *O. aristatus* (Lamiaceae; 10%). Only *P. edulis* was considered accessory pollen, with the rest categorized as isolated and occasional pollen. In October, Unidentified G (59%) stood as the most prevalent pollen type, classified as dominant pollen. *Passiflora edulis* (Passifloraceae; 15%) and *M. cajuputi* (Myrtaceae: 9%) followed. The least collected included *M. pudica* (Leguminosea; 1%).

November witnessed *Acacia* spp. (Fabaceae, 33%) as the most abundant, categorized as accessory pollen grains. The lowest collected pollen grains were categorized as occasional pollen, including *O. aristatus* (Lamiaceae; 10%) and *B. pilosa* (Asteraceae; 1%). In December, *Melastoma malabathricum* (Melastomataceae; 59%) was the dominant pollen, classified as dominant pollen grains. Six types of pollen grains were considered occasional, including *Bidens Pilosa* (Asteraceae; 2%), *P. edulis* (Passifloraceae; 2%), *Acacia* spp (Fabaceae, 1%), *Muntingia calabura* (Tiliaceae; 1%), *Commelina diffusa* (Commelinaceae; 1%), *Citrus* sp. (Rutaceae; 1%), and *B. pilosa* (Asteraceae; 1%). Lastly, in February, Unidentified H (33%) represented the highest pollen type collected, categorized as accessory pollen grains. *Commelina diffusa* (Commelinaceae; 1%), *Citrus* sp. (Rutaceae; 1%), *Capsicum* sp. (Solanaceae; 1%), and four unidentified types were the least collected, all considered occasional pollen grains.

Based on to 2-way cluster analysis, it is clear that *H. itama* collected similar pollen grains in different months. For example, in April until June 2016 *H. itama* collected the pollen grains of *C. rutidosperma* while *M. pudica* was collected in July 2016 until February 2017 (Figure 3). In addition, *Acacia* spp. and *P. edulis* was collected by *H. itama* in every month. The most interesting outcome from this analysis was that there were certain types of pollen were carried at certain month. For example, pollen in family Lamiacea was only found collected in March and *Mimusops elengi* in June.

Table 1. Frequencies and occurrences of pollen types collected by *H. itama* at TRIBE from Mac 2016 to February 2017

Scientific Names	Mac	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Feb
Mangrove species											
<i>Acacia</i> spp.	I*	I*	O*	O*	I*	I*	I*	I*	A*	O*	I*
<i>Melaleuca cajuputi</i>		A*	O*			I*	I*	A*	I*		I*
Ornamental plants											
<i>Antigonun leptopus</i>					I*						
<i>Mimusops elengi</i>							I*				
Shrubs											
<i>Asystasia gangetica</i>	I*	O*		I*			I*	I*			I*
<i>Melastoma malabathricum</i>										I*	
<i>Cosmos caudatus</i>			O*								
<i>Mimosa pudica</i>						O*	*	I*	I*	*	I*
<i>Commelina diffusa</i>	*	O*								I*	I*
<i>Lindernia crustacea</i>	I*	I*	I*			D*	I*				

Vegetables											
<i>Cleome rutidosperma</i>	*	O*	O*	A*							
<i>Capsicum sp.</i>					A*						I*
Underutilized fruits											
<i>Passiflora edulis</i>	O*	I*	A*	O*	O*	I*	I*	I*	I*	I*	I*
<i>Muntingia calabura</i>	*	I*	O*	*	*	I*	I*	*	*	I*	*
Agriculture Fruits											
<i>Cocos nucifera</i>	O*	I*	O*	O*	O*	*	O*	I*	*	*	*
<i>Citrus sp.</i>	O*	I*	I*	*	O*	*	O*	*	*	I*	I*
<i>Averrhoa bilimbi</i>	A*	I*	I*				I*	*	I*	I*	*
Others											
<i>Bidens Pilosa</i>	I*	*	I*	I*	I*	O*	O*	*	I*	I*	A*
<i>Orthosiphon aristatus</i>	*	I*	I*	I*	A*	I*	I*	*	*	I*	*
<i>Synsepalum dulcificum</i>	O*	I*	I*	I*	A*				I*		

Note: (D = dominant pollen (>45%); A = accessory pollen (15%-45%); I = isolated pollen (3%-15%); O = occasional pollen (<3%). * Indicate that the trees are flowering.

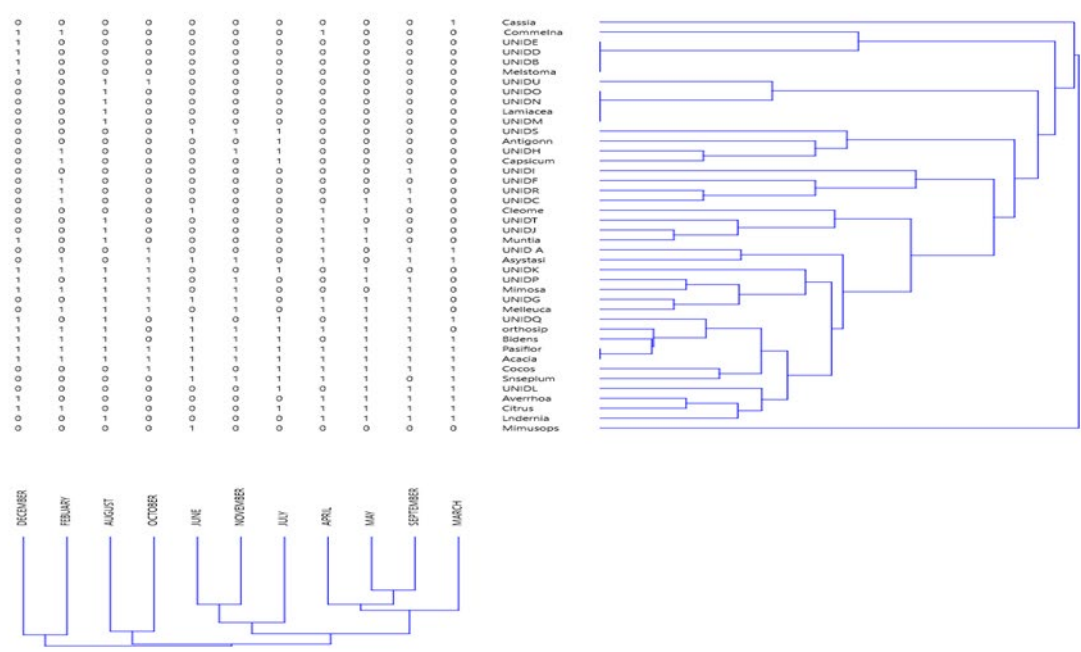


Figure 3. Detrended Correspondence Analysis (DCA) between months and types of pollen grains collected by *H. itama*

Figure 3 shows the Detrended Correspondence Analysis (DCA) between months and types of pollen grains collected by *H. itama*. From the DCA graph, all the months were grouped together, while some of the pollen grains were clearly separated into another group. These included *Averrhoa bilimbi* and *Commelina diffusa*.

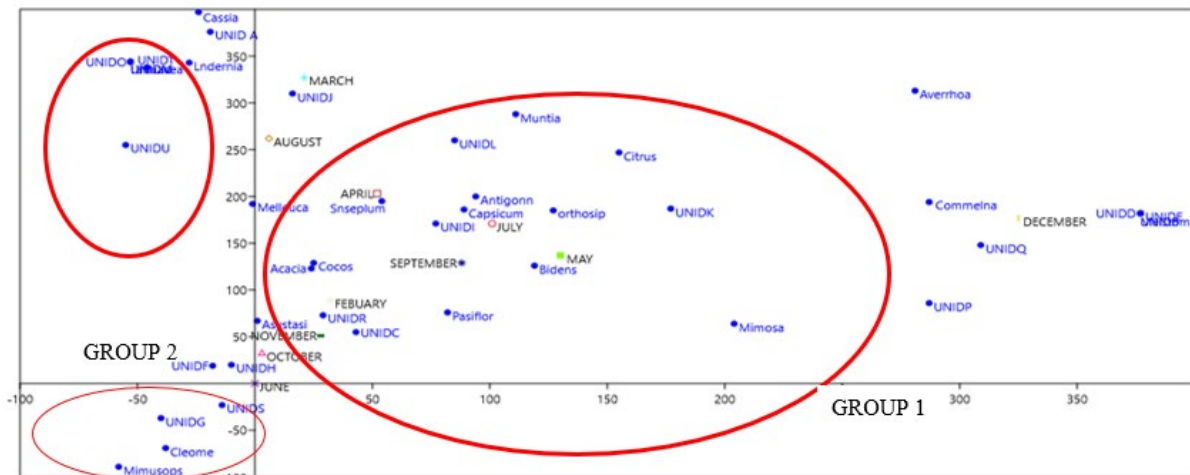


Figure 4. Detrended Correspondence Analysis (DCA) between months with types of pollen grains

Palynological analysis of pollen loads revealed that *H. itama* collected a high diversity of pollen types, which belong to native plant species, ruderal species, as well as introduced and cultivated species in the study area. Based on a study done by Ghazi et al. (2018), 59 types of pollens were collected by *H. itama* in Taman Tropika Kenyir, showing that the foragers collect a variety of pollen types. Moreover, *Lepidopera terminata* collected 11 different types of pollen, as reported in a study by Azmi et al. (2015). Additionally, according to Zaki and Razak (2018), *H. itama* collected 37 types of pollen in the rubber estate of Tepoh, Terengganu. The presence of a variety of pollen types fulfills the nutritional requirements of the bee, leading to potential improvements in the foraging behavior of the stingless bee. Pollen resources play a crucial role in the colony's brood rearing and the development of worker bees (Di Pasquale et al. 2016). Research by Barroso-Arévalo et al. (2019) found that colonies with higher brood cells had significantly lower pollen stores, suggesting the importance of pollen storage in maintaining the health of broods within the colony. Additionally, pollen contains lipids that possess antimicrobial properties, capable of inhibiting the growth of spores and bee pathogens (Manning 2015).

There was a significant difference in the total abundance of pollen grains collected by *H. itama* from March 2016 to February 2017 in TRIBE. In May, the pollen grains collected were significantly higher compared to other months. However, the total types of pollen grains collected by *H. itama* in June were the lowest. This pattern suggests that May might be the peak of the flowering season for many plant species in the study area. Mamat et al. (2023) found that May was highest pollen collected by stingless bee in Gelam forest Terengganu. The pollen types gathered by *H. itama* appear to be influenced by the varying flowering periods of different plant species. This variation in pollen types is likely due to the different flowering patterns and densities of these plants. The behavior of stingless bee is affected by general flowering events occurring in lowland flowering mixed dipterocarp forests, which are influenced by changes in available floral resources (Ghazi 2015). Moreover, *H. itama* have a particular tree or plant they favor for collecting pollen each month. This bee species exhibits specific preferences for certain types of flowers or food sources, which can vary with the changing seasons or environmental conditions. As an example, in April, *M. cajuputi* (39%) was the most dominant, while in August, *L. crustacea* (Linderniaceae, 47%) and November was *Acacia* spp. (33%).

There are several characteristics that might influence stingless bee to be attracted to certain flowers. The first characteristic is the colour of the flower. Some researchers found that bright colours of flower, for example blue was attractive which may have a positive influence on the abundance and frequency of the flower visitors (Dyer et al. 2021). However, in this study, it has been found that the *H. itama* collected pollen grains mostly from the white and creamy white flowers. For examples, the flowers that were visited by stingless bees were *Capsicum* sp., *Citrus* sp., *M. elengi*, *M. calabura*, *S. dulcificum* *P. edulis* and *C. nucifera*. This finding was supported by previous studies which were done by Ghazi et al. (2018) at Taman Tropica Kenyir found that stingless bees collected pollen of *Capsicum* sp., *Citrus* sp., *Coffea* sp., *Erioglossum rubiginosum*, *Eugenia michelii*, *Flacourtia jangomas* *Mikania* sp., *M. elengi*, *M. calabura*, *Phaleria atmacrocarpa*, *S. dulcificum* and *Syzygium* sp. According to Kiew and Muid (1991), 66% of flowers that were visited by bees in Malaysia were either white, creamy white and yellow in colour. In this study, *P. edulis* was the highest pollen grains collected by *H. itama*, followed by *Acacia* spp. and *M. cajuputi*. This result suggested that stingless bees are also attracted to white and yellow flowers. This finding is also supported by Obregon and Parra (2014), whereby the obtained result of 52.2% *Melipona eburnea* prefers flower colour of white and creamy flowers. Interestingly, *H. itama* in the current study area also forages of flowers that are purple in colour such as *M. pudica*, and *L. crustacea*.

The size of flowers is another significant factor that influences the preference of pollen types for *H. itama*. During foraging, *H. itama* mostly favors small-sized flowers. This preference may be attributed to the hypothesis that larger bees are more efficient at foraging, as they can meet the physiological demands required during the process (Cassano & Naug 2022). Stingless bees' preference for small flowers could be due to the easy access they provide to floral rewards, such as honey and pollen, without facing competition from other pollinators like butterflies and birds. The predominant flowers, such as *Acacia* spp. and *M. cajuputi*, were indeed very small, measuring around 0.5-2.5 cm. This size match between the visitor (*H. itama*) and the flowers may make *H. itama* an effective pollinator for these specific flowers. This observation aligns with the findings of Azmi et al. (2015), who revealed that stingless bees generally prefer small flowers when selecting crops or flowers for foraging purposes. Thus, the preference of *H. itama* for small-sized flowers may be attributed to their ease of access to floral rewards and their ability to efficiently collect pollen and nectar from such flowers. In this study, most of the flowers such as *A. gangetica*, *Averrhoa bilimbi*, *Averrhoa carambola*, *Capsicum* sp., *Citrus* sp., *Cosmos caudatus*, *M. cajuputi*, *M. calabura* and *S. dulcificum* possess the same characteristic of small flowers. In accordance to its body size, *H. itama* can carry pollen that is small in size (Cholis et al. 2020).

Other than color and size, previous studies have also shown that the shape and odor of flowers play important roles in facilitating the recognition of rewarding resources. According to Wang et al. (2019), flower scent is an important factor in attracting pollinators, and the chemical profile of the scent may represent a pollination syndrome. *Mimusops elengi*, *Citrus* sp., and *M. cajuputi* have a fresh, mild, and pleasant odor, which may explain why these flowers were preferred by *H. itama* in this study. Bobadoye et al. (2023) have also found that food-marking odors play a vital role in the recruitment of social insects by assisting foragers and recruited nestmates in orienting themselves. Earlier, Reichle et al. (2010), who reported stingless bee, *Scaptotrigona pectoralis* could learn a series of odor mixtures, including linalool, phenylacetaldehyde, geraniol, and eugenol, from the nest atmosphere.

This finding also indicates that *H. itama* is a flexible pollinator, capable of collecting pollen from small flowers that may not be pollinated by larger insects or birds, as well as from

larger flowers. *Heterotrigona itama* is able to collect pollen grains of various sizes, ranging from small to large. In a study reported by Eltz (2001) in Sabah, stingless bees were observed collecting pollen of various sizes, including 16 μ m (*Rhizophora* sp.), 31 μ m (*Elaeis guineensis*), 35 μ m (*Passiflora* sp.), 62 μ m (*Jasminium* sp.), 166 μ m (*Hibiscus* sp.), and others. This flexibility in collecting different pollen sizes is advantageous for *H. itama*, allowing them to survive in areas with diverse types of pollen grains. Furthermore, this ability indirectly strengthens the claim that *H. itama* is an effective pollinator in tropical rainforests.

Based on the two-way cluster analysis, the dominant pollen types collected were *Acacia* spp. and *P. edulis* because these two pollen types were the most abundant in the vicinity of the bee's hive. *Passiflora edulis* flowers all year round, and *H. itama* collects its pollen every month. This finding is similar to Putra et al. (2023), who discovered that *T. laevicep* is one of the pollinator agents of *P. edulis* in Indonesia. The researchers observed that *T. laevicep* actively collected pollen from the flowers of *P. edulis* during their study.

Acacia spp. was identified as the primary source of pollen, mainly due to its secretion of highly concentrated extrafloral nectars that act as attractive floral rewards. This leads to a greater diversity of pollinators being drawn to *Acacia* spp. compared to other flowers that only offer pollen rewards. The dominance of *Acacia* spp. in supplying pollen is crucial as it significantly contributes to honey production by providing abundant nectar sources (Begum et al. 2021; Mondal et al. 2023). Throughout the sampling period, *Acacia* spp. was widely available, making it the preferred choice for *H. itama*. Studies have indicated that bees prioritize plants that occur in high densities at a given location, and *Acacia* spp. was indeed the most commonly collected plant type during sampling periods. This preference can be attributed to *Acacia* spp. offering a rewarding product, which discourages bees from seeking out alternative plant species when their preferred food source is abundantly available (Keller et al. 2005).

CONCLUSION

Findings of this study has provided valuable insights into stingless bee feeding preferences, resource availability, and potential plant-pollinator interactions in the coastal forest ecosystem. This information can aid in the identification of key plant species in the coastal forest that are dependent on *H. itama* for pollination, thereby facilitating conservation efforts aimed at protecting these plants and their associated ecosystems. Future research in the field of palynology and stingless bees could focus on investigating the influence of different habitat types on the pollen spectrum carried by *H. itama* would provide a better understanding of their foraging preferences and behavior. This could be achieved by conducting similar studies in different forest types, such as the secondary forest or peat swamp forest in Terengganu. Furthermore, investigating the potential impacts of human activities, such as deforestation or habitat fragmentation, on the foraging behavior and pollen spectrum of *H. itama* would be relevant for conservation efforts. This might involve comparing the pollen spectra between bees in protected areas and those in areas influenced by anthropogenic activities. Overall, further research in palynology and stingless bees in the coastal forest of Terengganu will not only contribute to our understanding of the ecological role of *H. itama* but also aid in the formulation of effective conservation strategies to protect their habitat and preserve the plant diversity in the region.

ACKNOWLEDGEMENTS

We would like to extend our sincere gratitude to the farm staff of the Faculty of Bioresources and Food Industry UniSZA for their exceptional technical support throughout this research project. Their expertise, dedication, and unwavering assistance were crucial in overcoming technical challenges and ensuring the successful completion of this study.

AUTHORS DECLARATIONS

Funding Statement

Not applicable

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

Not applicable

Authors' Contributions

RoZIAH Ghazi (RG): Conducting research, analysis the data and writing the draft manuscript; Abd Jamil Zakaria (ABZ): Conceptual and designing the experiment; Wahizatul Afzan Azmi (WAA): Conceptual and designing the experiment; Fahimee Jaapar (FJ): Data analysis, reviewing and editing the manuscript; Norhayati Ngah (NN): Conceptualization, reviewing and editing the manuscript.

REFERENCES

- Azmi, W.A., Zulqurnain, N.S. & Ghazi, R. 2015. Melissopalynology and foraging activity of stingless bees *Lepidotrigona terminata* (Hymenoptera: Apidae) from an apiary in Besut, Terengganu. *Journal of Sustainability Science and Management* 10(1): 27-35.
- Barroso-Arévalo, S., Vicente-Rubiano, M., Ruiz, J.A., Bentabol, A. & Sánchez-Vizcaíno, J.M. 2019. Does pollen diversity influence honey bee colony health? *Spanish Journal of Agricultural Research* 17(3): e0504.
- Begum, H.A., Iqbal, J. & Aziz, A. 2021. Characterization of pollen profile of *Apis mellifera* L. in arid region of Pakistan. *Saudi Journal of Biological Sciences* 28(5): 2964-2974.
- Bobadoye, B.O., Alamu, O.T., George-Onaho, J.A., Ete, J.A., Ayandokun, A.E., Agboola, I.S., Adeoye, O.T. & Adekola, P.J. 2023. Recruitment behaviour in an African meliponine bee species (Hymenoptera, Apidae: Meliponini): Understanding glandular origin and pheromonal components. *Tanzania Journal of Science* 49(1): 207-217.
- Bueno, F.G., Kendall, L., Alves, D.A., Tamara, M.L., Heard, T., Latty, T. & Gloag, R. 2023. Stingless bee floral visitation in the global tropics and subtropics. *Global Ecology and Conservation* 43: 1-13.
- Cassano, J. & Naug, D. 2022. Metabolic rate shapes differences in foraging efficiency among honeybee foragers. *Behavioral Ecology* 33(6): 1188-1195.
- Chelong, I., Adulyasas, L. & Adair, A. 2020. Melissopalynology and foraging plants of stingless bees, *Heterotrigona itama* (Hymenoptera: Apidae) from Pattani, Thailand. *International Journal of Advanced Research in Engineering and Technology* 11(9): 252-259.
- Cholis, M.N., Alpionita, R., Prawasti, T.S. & Atmowidi, T. 2020. Pollen load and flower constancy of stingless bees *Tetragonula laeviceps* (Smith) and *Heterotrigona itama* (Cockerell) (Apidae: Meliponinae). *Advances in Biological Sciences Research* 8: 285–289.
- Di Pasquale, G., Alaux, C., Le Conte, Y., Odoux, J.F., Pioz, M., Vaissière, B.E., Belzunces, L.P. & Decourtye, A. 2016. Variations in the availability of pollen resources affect honey bee health. *PloS One* 11(9): e0162818.
- Dyer, A.G., Jentsch, A., Burd, M., Garcia, J.E., Giejsztowt, J., Camargo, M.G., Tjørve, E., Tjørve, K.M., White, P. & Shrestha, M. 2021. Fragmentary blue: Resolving the rarity paradox in flower colors. *Frontiers in Plant Science* 11:618203.
- Eltz, T. 2001. Ecology of stingless bees (Apidae, Meliponini) in lowland dipterocarp forests in Sabah, Malaysia, and an evaluation of logging impact on populations and communities. PHD Thesis. Universität Würzburg, Germany.
- Forster, C.Y., Mourmourakis, F., Hochuli, D.F., White, T.E., Latty, T. & Gloag, R. 2023. Flower choice by the stingless bee *Tetragonula carbonaria* is not influenced by colour similarity to a higher reward flower in the same patch. *Apidologie* 54(2) :16.

- Friess, D.A., Yando, E.S., Alemu, J.B., Wong, L.W., Soto, S.D. & Bhatia, N. 2020. Ecosystem services and disservices of mangrove forests and salt marshes. In: Hawkins, S.J. et al. (eds). *Oceanography and Marine Biology*, pp. 107-141. London: Taylor & Francis.
- Ghazi, R. 2015. Melissopalynology and foraging activity of the stingless bee, *Heterotrigona itama* (Hymenoptera: Apidae) at Taman Tropika Kenyir, *Terengganu*. Master Thesis. University Malaysia Terengganu.
- Ghazi, R., Zulqurnain, N.S. & Azmi, W.A. 2018. Melittopalynological studies of stingless bees from the East Coast of Peninsular Malaysia. In: Vit, P., Pedro, S. & Roubik, D. (eds.). *Pot-Pollen in Stingless Bee Melittology*, pp. 77-88. Switzerland: Springer.
- Hamdan, O., Tariq M.H. & Ismail, P. 2020. *Status of Mangrove in Malaysia*. Kuala Lumpur: FRIM Special Publication.
- Hammer, Ø., & Harper, D. A. 2001. Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 4-9.
- Hesse, M., Halbritter, H., Zetter, R., Weber, M. Bunchuer, R., Frosch-Radivo, A. & Ulrich, S. 2009. *Pollen Terminology*. Austria: Springer Wien New York.
- Jaapar, M.F., Jajuli, R. & Radzali, M. 2015. *Lebah Kelulut Malaysia: Biologi dan Penternakan*. Serdang: Penerbit Institut Penyelidikan dan Kemajuan Asas Tani (MARDI).
- Keller, I., Fluri, P. & Imdorf, A. 2005. Pollen nutrition and colony development in honey bees—Part II. *Bee World* 86(2): 27-34.
- Kiew, R. & Muid, M. 1991. *Beekeeping in Malaysia: Pollen Atlas*. Kuala Lumpur, Malaysia: United Selangor Press Sdn. Bhd.
- Lob, S., Bahri, S. & Razak, A. 2017. Composition and identification of pollen collected by stingless bee (*Heterotrigona itama*) in forested and costal area of Terengganu, Malaysia. *Malaysian Applied Biology* 46(3): 227–232.
- Luz, C.F.P.D. & Barth, O.M. 2012. Pollen analysis of honey and beebread derived from Brazilian mangroves. *Brazilian Journal of Botany* 35: 79-85.
- Majid, M., Ellulu, M.S. & Abu Bakar, M.F. 2020. Melissopalynological study, phenolic compounds, and antioxidant properties of *Heterotrigona itama* honey from Johor, Malaysia. *Scientifica* 2020(1): 2529592.
- Mamat, M.I.I., Khamis, S., Fuad, F.L. Mohamed, N.Z., Omar, C.M., Abdullah D.M. & Azmi W.A. 2023. Pollen sources foraged by domesticated stingless bee (*Heterotrigona itama*) reared in Gelam Forests of Terengganu, Malaysia. *Serangga* 28(2): 120-138.
- Manning, R. 2015. Fatty acids in pollen: a review of their importance for honey bees. *Bee World* 82(2): 60-75.

- Marques-souza, A.C., Moura, C.D.O. & Nelson, B.W. 1996. Pollen collected by *Trigona williana* (Hymenoptera: Apidae) in Central Amazonia. *Review Biology Tropical* 44(2): 567–573.
- Mondal, R., Das, N., Layek, U., De, S.K. & Karmakar, P. 2023. Pollen sources of Asian honeybee (*Apis cerana* Fabricius) in Paschim Medinipur district of West Bengal, India. *Grana* 27: 1-13.
- Obregon, D. & Nates-Parra, G. 2014. Floral Preference of *Melipona eburnea* Friese (Hymenoptera: Apidae) in a Colombian Andean Region. *Neotropical entomology* 43(1): 53-60.
- Pesiu, E., Lee, G.E., Salam, M.R., Salim, J.M., Lau, K.H., Yong, J.W.H. & Abdullah, M.T. 2022. Species composition, diversity, and biomass estimation in coastal and marine protected areas of Terengganu, Peninsular Malaysia. *Agronomy* 12(10): 2380.
- Putra, R.E., Wibisana, G., Kinasih, I., Raffiudin, R., Soesilohadi, R.C.H. & Purnobasuki, H. 2023. Pollination biology of yellow passion fruit (*Passiflora edulis* forma *flavicarpa*) at typical Indonesian small-scale farming. *Biodiversitas* 24: 2179-2188.
- Reichle, C., Jarau, S., Aguilar, I. & Ayasse, M. 2010. Recruits of the stingless bee *Scaptotrigona pectoralis* learn food odors from the nest atmosphere. *Naturwissenschaften* 97(5): 519–24.
- Rosdi, I.N., Selvaraju, K., Vikram, P., Thevan, K. & Arifullah, M. 2016. Melissopalynological analysis of forest honey from north Malaysia. *Journal of Tropical Resources and Sustainable Science* 4(2): 128-132.
- Roubik, D.W., Heard, T.A. & Kwapong, P. 2018. Stingless bee colonies and pollination. *The pollination of cultivated plants: A compendium for practitioners* 2: 39-64.
- Selvaraju, K., Vikram, P., Soon, J.M., Krishnan, K.T. & Mohammed, A. 2019. Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. *Journal of Food Science and Technology* 56: 2508-2521.
- Sujanto, I.S.R., Ramly, N.S., Tang, J.Y.H., Abd Ghani, A., Alias, N., Mohamed, S. & Ngah, N. 2022. Influence of spatial variation on the physicochemical properties and mineral content of stingless bee honey (*Heterotrigona itama*) in Terengganu, Malaysia. *Asian Journal of Agriculture & Biology* 2022(4): 1-10.
- Wang T.N., Clifford, M.R., Martínez-Gómez, J., Johnson, J.C, Riffell, J.A. & Di Stilio V.S. 2019. Scent matters: Differential contribution of scent to insect response in flowers with insect vs. wind pollination traits. *Annals of Botany* 123: 289–301.
- Zaki, N.N.M. & Razak, S.B.A. 2018. Pollen profile by stingless bee (*Heterotrigona itama*) reared in rubber smallholding environment at Tepoh, Terengganu. *Malaysian Journal of Microscopy* 14(1): 38-54.