# POLLEN SOURCES FORAGED BY DOMESTICATED STINGLESS BEE (*Heterotrigona itama*) REARED IN GELAM FORESTS OF TERENGGANU, MALAYSIA

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## ABSTRACT

Melittopalynological analysis is one of the principal tools used to reveal significant floral sources to bees. Gelam (*Melaleuca* spp.) forest provides habitat for bees and provision for high quality honey. However, the forests are declining due to anthropogenic activities which directly reduced the availability of botanical sources on which bees rely for survival. Hence, this study aimed to investigate the pollen sources preferred by domesticated stingless bees (*Heterotrigona itama*) reared in fragmented Gelam forests from two meliponiaries in Terengganu. The palynological analysis was carried out on pollen load foraged by the stingless bees. Thirty seven pollen types belonging to 13 plant families were collected from the pollen loads of 251 stingless bee foragers. Tiliagraphs with CONISS and rank abundance curves were used to illustrate the pollen grains preferred by *H. itama*. Results clearly showed that *Melastoma malabathricum* and *Cyperus aromaticus* were the most frequently visited and vital pollen sources for *H. itama* in the Gelam forests. Interestingly, Gelam (*Melaleuca cajuputi*) pollen is one of the floral sources, suggesting that Gelam is one of the preferred pollen and nectar sources for stingless bees. This study discovered a causal link between the fragmentation of Gelam forests and the low abundance of *Melaleuca cajuputi* pollen foraged by *H. itama*. Information on this dynamic is critically important for maintaining the health of bee colonies as well as for the conservation efforts of Gelam forests in Terengganu, Malaysia.

**Keywords**: Stingless bees, melittopalynology, honey, *Melaleuca*, Myrtaceae

## ABSTRAK

Analisis melittopalinologi adalah salah satu alat utama yang digunakan untuk mendedahkan sumber bunga yang penting kepada lebah. Hutan Gelam (*Melaleuca* spp.) menyediakan habitat kepada lebah dan madu berkualiti tinggi. Namun, hutan ini semakin berkurangan disebabkan oleh aktiviti antropogenik yang secara langsung mengurangkan ketersediaan sumber botani yang diperlukan oleh lebah untuk terus hidup. Oleh itu, matlamat kajian ini adalah untuk menyiasat sumber debunga yang digemari oleh lebah kelulut (*Heterotrigona itama*) yang diternak di dua ladang kelulut di hutan Gelam Terengganu. Analisis palinologi telah dijalankan ke atas butiran debunga yang dimakan oleh lebah kelulut. Tiga puluh tujuh jenis debunga dari 13 famili tumbuhan telah dikumpulkan daripada 251 lebah kelulut pencari makanan. Tiliagraf dengan CONISS dan lekuk pangkat kelimpahan digunakan untuk mengilustrasikan butiran debunga yang digemari oleh *H. itama.* Keputusan jelas menunjukkan bahawa *Melastoma malabathricum* dan *Cyperus aromaticus* merupakan sumber debunga paling kerap dilawati dan penting kepada *H. itama* di hutan Gelam. Menariknya, debunga Gelam (*Melaleuca cajuputi*) adalah salah satu sumber bunga yang direkodkan dan ini menunjukkan bahawa Gelam adalah salah satu sumber debunga dan nektar pilihan untuk lebah kelulut. Kajian ini menemui hubungan antara fragmentasi hutan Gelam dengan kelimpahan debunga *Melaleuca cajuputi* yang rendah yang diambil oleh *H. itama*. Maklumat dinamik ini amat penting untuk mengekalkan kesihatan koloni lebah serta untuk usaha pemuliharaan hutan Gelam di Terengganu.

**Kata kunci**: Kelulut, melittopalinologi, madu, *Melaleuca*, myrtaceae

## INTRODUCTION

Gelam forest or *Melaleuca* forest is commonly known as ‘Hutan Gelam’ by local Malaysian people, occurs in the beach ridges interspersed with the Swales (BRIS) ecosystem. This biome is diverse for many taxonomic groups mainly plants, birds, bats, fishes, insects and microbes (Ismail & Mohamed 2017; Salim & Mohamad 2011). Gelam forest covers 23,000 hectares in Peninsular Malaysia, and Terengganu is classified as the largest *Melaleuca* forest, with approximately 15,000 hectares (Omar et al. 2020). The forests are highly dominated by *Melaleuca cajuputi*, which is often called as ‘Kayu Putih’or Cajuput oil tree due to leaves from this tree capable of producing commercial essential oil and important for therapeutic purposes (Desdiani et al. 2022). Gelam forests are also critical for local communities livelihoods as the honey breeding areas, trees are utilised for timber, charcoal production, poles, and fencing posts (Giensen 2015; Salim et al. 2015).

Honey derived from *M. cajuputi* resources is categorised as Gelam honey. Gelam honey which is exclusively obtained from Gelam forest has long been known as one of the side incomes of local people and is claimed to have a unique and pleasant flavour or aroma compared with other honey harvested from other forests (Salim et al. 2015). *Melaleuca cajuputi* produces inflorescent flowers that attract many insect groups to forage on both pollen and nectar as their essential food resource in the forests (Quang Tan 2008). Thus, it shows that Gelam Forest is crucial for providing bee subsistence (Quang Tan 2004), but its role remains unclear due to a lack of information and limited study in Malaysia, especially on the floral structure that makes it easy for mandibulate insects like bees to get access to nectar. There is an urgent need to understand the importance of the Gelam forest for stingless bees (*Heterotrigona itama*) reared in these forests, which local beekeepers domesticate for honey production.

*Heterotrigona itama* is one of the popular domesticated bee species that exerts vast contributions to the meliponiculture sector in Malaysia through its products, ecological and economic values (Razak & Ishak 2017). They offer high-quality foods such as honey and pollen bread. Honey produced by stingless bees has been found to contain essential pharmacological, therapeutic, and nutritional characteristics important for human health (Fletcher et al. 2020). Moreover, *H. itama* fulfills a crucial ecological function by acting as a pollinator for agricultural plant species, contributing to Malaysia's economic growth (Azmi et al. 2017). This species is also considered a generalist visitor because they show a high floral constancy on particular flowers closer to their hives (Cholis et al. 2020), where they tend to forage on plant resources within a 500-meter distance (Benedick et al. 2021).

The bee often exhibits behaviour that involves carrying pollen grains during their foraging activity, which is an important key to indicate the floral sources is frequently visited. Melittopalynology is a subfield of palynology that focuses on pollen grains collected by bees and which flowers are most favoured by bees (Ghazi et al. 2018). This study is one of the key tools for understanding bee foraging ecology and the geographical origin of pollen sources (Ponnuchamy et al. 2014). It is also helpful in assessing the exact information on bee floral availability and beneficial in understanding the shrinkage of botanical sources in certain regions.

Degradation of the BRIS ecosystem contributes to habitat fragmentation. Silica mining, land clearing for agricultural or aquaculture purposes, and housing areas are the major threats facing the Gelam forests in Terengganu (Nafiz et al. 2020; Salim et al. 2014). Anthropogenic forces on Gelam forests intensively affect almost all of the biodiversity and contribute to the loss of botanical sources that bees rely on for survival. So far, very limited information has been reported on palynological studies of *H. itama* that are employed for revealing important plants to bees from the Gelam forest. Therefore, the main objective of this study was to investigate the pollen preferences and foraging pattern of *H. itama* reared in fragmented Gelam forests from two meliponiaries of Terengganu.

## MATERIALS AND METHODS

### Study Sites

The samples were collected from two meliponiaries in the vicinity of fragmented Gelam forest biomes. The meliponiaries represent several anthropogenic factors, including deforestation and rural-residential development areas. Both meliponiaries are located in Terengganu at Bukit Mawi, Kampung Kijing (5°12'46.9"N 103°11'42.0"E), and Bidong Valley Farm (5°31'01.2"N 102°57'48.4"E) (Figure 1). The weather of both sites is hot and humid from May to September, with temperatures ranging from 29°C to 42°C.

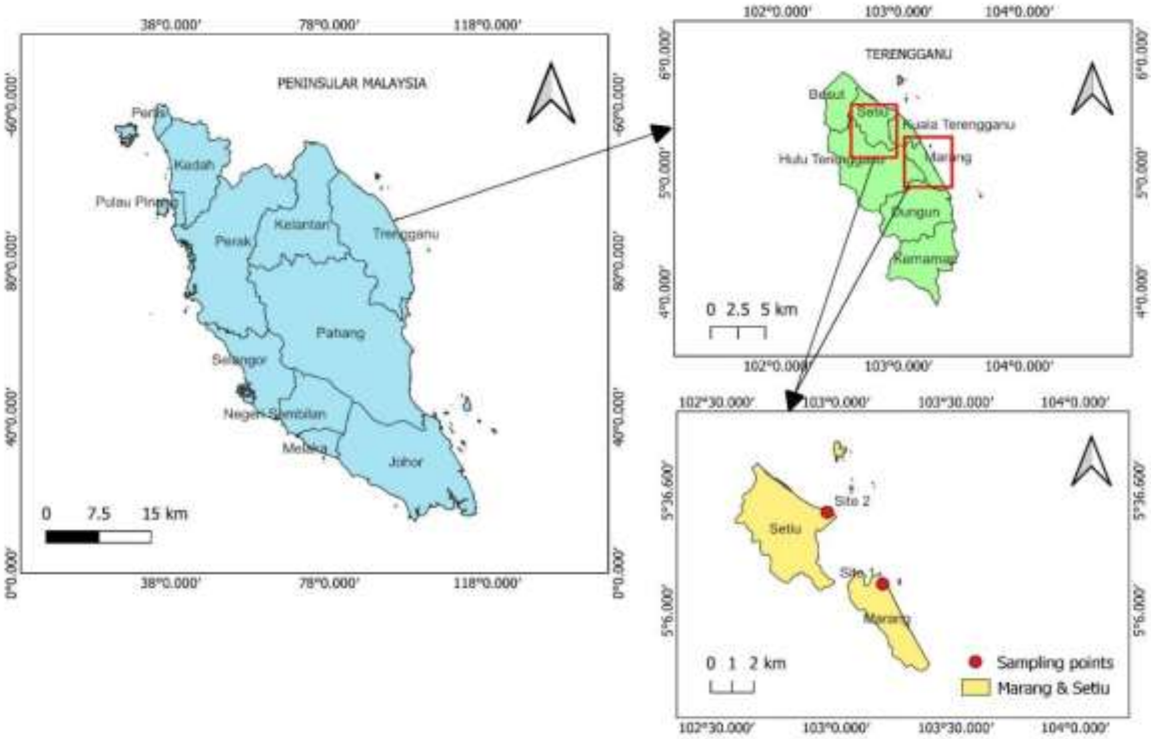


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Figure 1. Map of study sites at Bukit Mawi, Kampung Kijing, Marang (Site 1) and Bidong Valley Farm, Setiu (Site 2) in Terengganu

**Samples Collection**

A total of 10 colonies of stingless bee (*Heterotrigona itama*) were selected from each location (Marang = 5; Setiu = 5). Three individuals of *H. itama* foragers carrying pollen were collected from different colonies during each peak hour (0900am – 1000 am, 1000 am - 1100 am, 1100 am – 1200 pm) (Ghazi 2015; Mohamed 2021). The hive entrance was closed with a leaf, and incoming forager that returned to the hive was captured using microcentrifuge (1.5 ml) which contained 1 ml of 70% ethanol. This study was conducted during the period from May to September 2022.

**Palynological Analysis**

The pollen sediment in the centrifuge tube was stained with 10–20 ml of Safranin O following the method recommended by Wood et al. (1996) and Jayadi & Susandarini (2020). Then, pollen suspension was deposited onto glass slides covered with a coverslip for microscopy observation. The pollen grains were observed under a Leica DM750 microscope with 400x and 1000x magnifications. The pollen type images were captured using Leica Microsystem Software, the size (width and length) of pollen grains were measured in μm, and pollen morphological characteristics were recorded. The methodology for quantifying the pollen frequency was based on Tamic et al. (2011) and Cholis et al. (2020) with minor modifications. The identification of pollen grains was characterised by key publications (da Silva et al. 2020; El-Labban 2020; Kiew & Muid 1991). The characteristics of pollen grains were categorised using the palynological terminology for "pollen types" by Persano Oddo and Ricciardelli d'Albore (1989). All pollen load samples were analysed in the Laboratory of Apiculture, Universiti Malaysia Terengganu (UMT), Malaysia. The pollen type frequencies were classified according to Louveaux et al. (1978), which are Predominant pollen type (>45%), Secondary pollen type (16–45%), Important minor pollen type (3–15%), and Minor pollen type (<3%). The raw pollen loads data was organised using Microsoft Excel©2019.

**Statistical Analyses**

Pollen diagrams and cluster analyses were constructed using Tilia software (version 2.6.1) and Constrained Incremental Sum-of Squares Cluster Analysis (CONISS) (Grimm 1987). The heatmap was performed using GraphPad Prism version 9.0.0. The rank abundance curve was visualised in OriginPro® version 2022 software (OriginPro 2022). All analyses were used to reveal the preferences of pollen grains and understand the bee foraging patterns.

## RESULTS

**Pollen Loads Composition and Frequency Categories**

Palynology analysis of pollen loads revealed that *Heterotrigona itama* foraged on the heterogeneity of floral in the Gelam Forests. A total of 251 pair of corbicular pollen loads were collected from May to September 2022. Photomicrographs of pollen types collected by *H. itama* from Gelam forests in Marang and Setiu are shown in Figure 2. The pollen analysis conducted yielded 37 pollen types belonging to 13 plant families that spread across six life forms; trees, shrubs, herbs, creeper, climbers and sedge (Table 1). The most common families collected by *H. itama* were Myrtaceae (3) and Lamiaceae (3), followed by Fabaceae (2) and Asteraceae (2).

*Syzygium inacarnatum* was classified as secondary pollen type in May with 22.7%. In July, *Melastoma malabathricum* was categorised as the dominant pollen type with 45.3%, and *Cyperus aromaticus* was secondary pollen type with 21.5%. Meanwhile, in September *Cyperus aromaticus* and *Melastoma malabathricum* both pollen taxa were grouped into secondary pollen types with 22.5% and 19.1%, respectively. Unidentified pollen types were recorded in Table 1 at the minor frequency classification in all months. Nevertheless, the highest pollen frequency was Unidentified pollen 4, which ranged from 6.9% to 16.5%. Moreover, *Areca catechu, Cocos nucifera, Cucumis melo, Cyperus aromaticus, Melastoma malabathricum, Morus alba, Melaleuca cajuputi,* Unidentified pollen 4 and Unidentified pollen 12 were collected by *H. itama* from both sites during sampling periods.

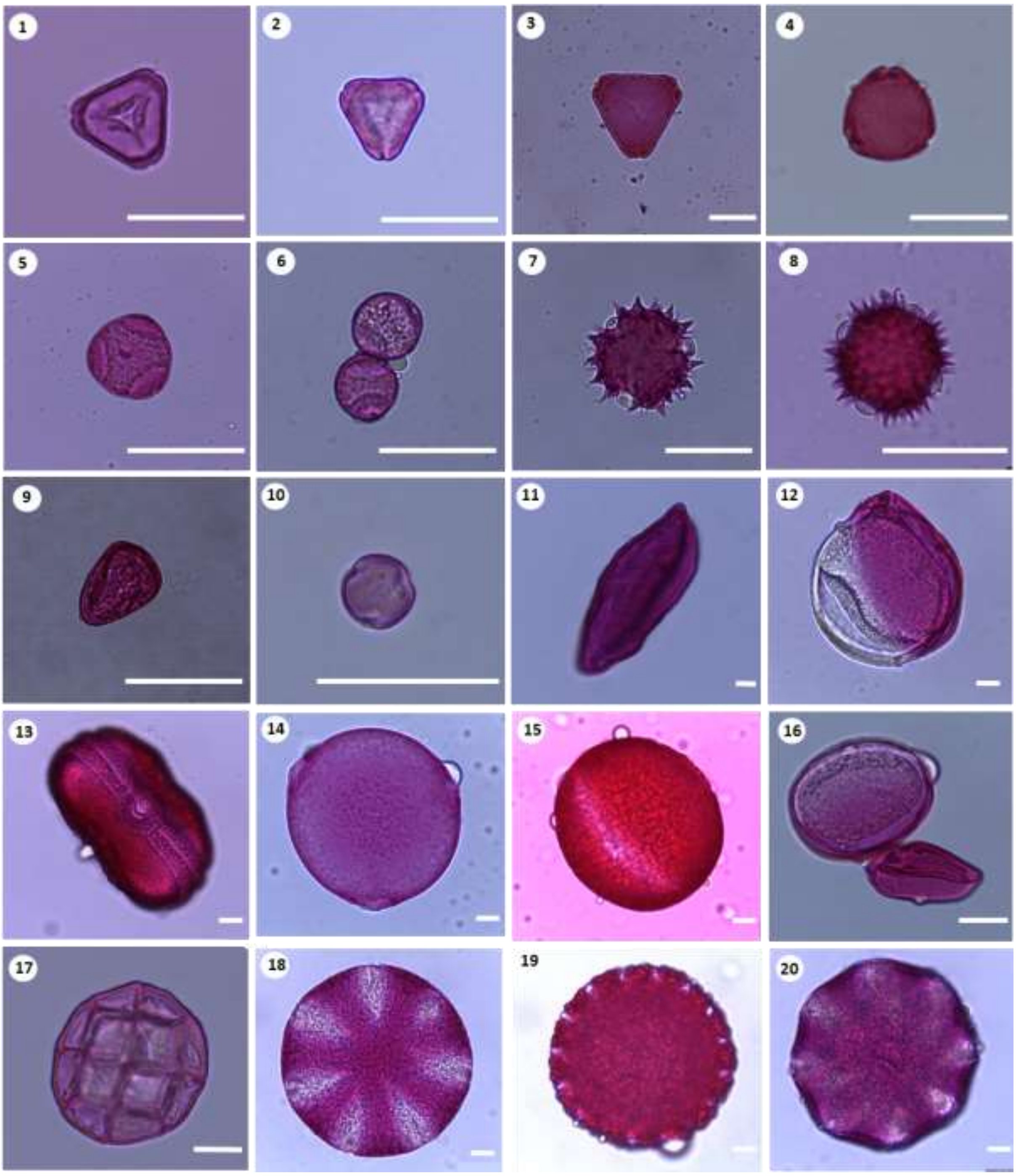

Figure 2. Photomicrographs of the pollen load of stingless bee foragers in a fragmented Melaleuca forests in Terengganu: 1. *Melaleuca cajuputi*, 2. *Syzygiumincarnatum*, 3. *Syzygium grande*, 4. *Melastoma malabathricum*, 5-6. *Morus alba*, 7. *Bidens pilosa*, 8. *Tridax procumbens*, 9. *Cyperus aromaticus*, 10. *Mangifera indica*, 11-12. *Cocos nucifera*, 13. *Asystasia gangetica*, 14. *Cucumis melo*, 15. *Antigonon leptopus*, 16. *Areca catechu*, 17. *Acacia mangium*, 18. Lamiaceae type 3, 19. Lamiaceae type 1, 20. Lamiaceae type 2. Scale bar :10 m

Table 1. Corbicular pollen loads analytical data of *Heterotrigona itama* from Marang and Setiu in Terengganu. Notes: D: Predominant pollen type >45%, S: Secondary pollen type 16-45%, I: Important minor pollen type 3-15% & M: Minor pollen type <3% (Louveaux et al. 1978)

| **Plant Families** | **Pollen Types** | **Vernacular Names** | **Habits** | **Pollen Frequencies Classes / Month** | | |
|---|---|---|---|---|---|---|
| | | | | **May** | **July** | **Sept** |
| Acanthaceae | *Asystasia gangetica* | Ara songsang | Herb | M | | |
| Anacardiaceae | *Mangifera indica* | Mangga | Tree | | I | I |
| Asteraceae | *Bidens pilosa* | Rumput juala | Herb | M | | M |
| | *Tridax precumbens* | Kancing baju | Herb | M | | |
| Arecaceae | *Areca catechu* | Pinang | Palm | M | M | M |
| | *Cocos nucifera* | Kelapa | Palm | M | M | M |
| Cucurbitaceae | *Cucumis melo* | Tembikai susu | Climber | M | M | M |
| Cyperaceae | *Cyperus aromaticus* | Rumput helikopter | Sedge | I | **S** | **S** |
| Fabaceae | *Acacia mangium* | Akasia | Tree | | I | |
| | *Mimosa pudica* | Semalu | Creeper | | | **S** |
| Lamiaceae | Lamiaceae type 1 | - | Herb | | | M |
| | Lamiaceae type 2 | - | Herb | | | M |
| | Lamiaceae type 3 | - | Herb | | | M |
| Melastomataceae | *Melastoma malabathricum* | Senduduk | Shrub | I | **D** | I |
| Moraceae | *Morus alba* | Mulberi | Shrub | I | I | M |
| Myrtaceae | *Melaleuca cajuputi* | Gelam | Tree | I | M | I |
| | *Syzygium incarnatum* | Kelat gelam | Tree | **S** | | |
| | *Syzygium grande* | Kelat jambu laut | Tree | M | | |
| Polygonaceae | *Antigonon leptopus* | Air mata pengantin | Climber | M | | |
| Rosaceae | Rosaceae type | - | Shrub | M | M | |
| Unidentified | Unidentified pollen 1 | | | M | | |
| | Unidentified pollen 2 | | | M | | |
| | Unidentified pollen 3 | | | M | | |
| | Unidentified pollen 4 | | | **S** | I | I |
| | Unidentified pollen 5 | | | M | | I |
| | Unidentified pollen 6 | | | M | | |
| | Unidentified pollen 7 | | | | M | |
| | Unidentified pollen 8 | | | | M | |
| | Unidentified pollen 9 | | | | | I |
| | Unidentified pollen 10 | | | | | M |
| | Unidentified pollen 11 | | | | | M |
| | Unidentified pollen 12 | | | M | M | M |
| | Unidentified pollen 13 | | | | M | |
| | Unidentified pollen 14 | | | | | M |
| | Unidentified pollen 15 | | | | | M |
| | Unidentified pollen 16 | | | | | M |
| | Unidentified pollen 17 | | | | | M |

**Pollen Abundance Data: Tiliagraphs and CONISS**

Pollen diagrams provide a framework for analysing groups of life forms and the foraging patterns of *H. itama*. Tiliagraph A revealed that 22 pollen types belonged to nine plant families from Marang. *Cyperus aromaticus* pollen was the highest frequency pollen percentage, as it was found in all hives, ranging from 23.1% to 63.8% (Figure 3A). Tiliagraph B represented 27 pollen types from 13 botanical families. *Melastoma malabathricum* pollen (10.4% – 73.8%) was the most abundant pollen observed in Gelam forests in Setiu (Figure 3B).

Based on the cluster analysis (CONISS), some groups formed in Tiliagraph A and B. Tiliagraph A shows Hive 3 and Hive 4 as subgroups, indicating both groups were highly similar in foraging preference of pollen (Figure 3A). Tiliagraph B demonstrated Hive 1 was excluded from the mixed group formed by the rest of the hives (Figure 3B). Hives 2, 3, 4, and 5 are closely related to each other, forming a subgroup. CONISS revealed that Hives 4 and 5, as well as Hives 2 and 3 shared a high degree of similarity.

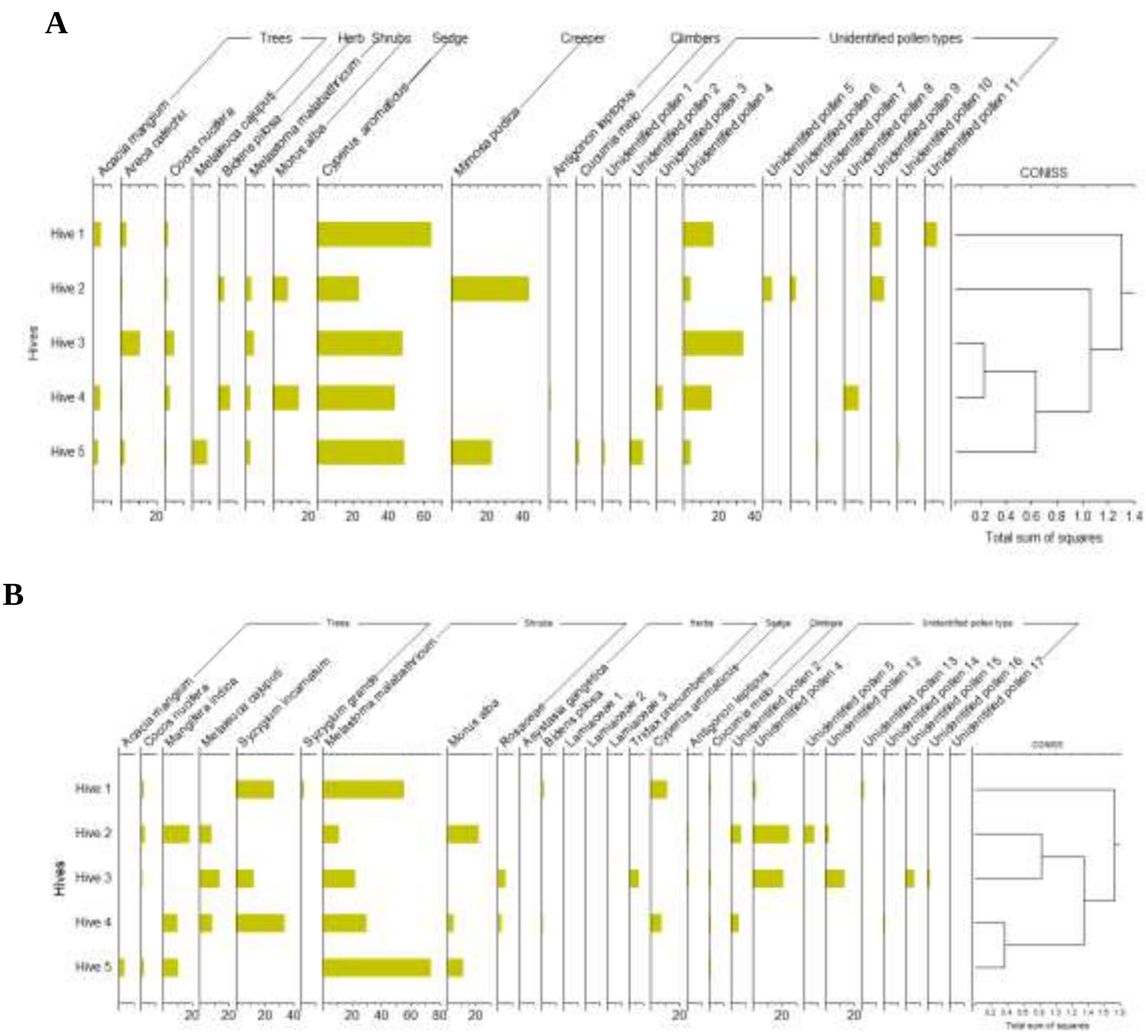


Figure 3. Pollen diagrams illustrating the percentage of pollen types collected by *H. itama* from Marang and Setiu. The *x*-axis (horizontal) shows the percent of total pollen for each type. Above *x* axis demonstrated different pollen types displayed side by side. Meanwhile, *y*-axis (vertical) displayed hives of stingless bees. Hives are plotted in a sequence (Hives 1-5) characterised by Cluster analysis (CONISS), shown at the right of the graph. Study sites: A= Marang, B=Setiu

**Pollen Abundance Data: Heatmap of Pollen Types**

The heatmap depicted the normalised total number of pollen grains in percentage (Figure 4). A total of 144,601 pollen grains were quantified with 54,389, 45,870, and 44,342 grains in May, July, and September, respectively. The highest percentage of pollen grains observed in this study were *Cyperus aromaticus* (23%) followed by *Melastoma malabathricum* (19%), and Unidentified Pollen type 4 (12%). The remaining pollen types recorded less than 10%. Pollen derived from *Syzygium incarnatum* showed high percentage values in May, with 23%. Meanwhile, the pollen loads of *H. itama* found a pollen predominance of *M. malabathricum* (45%) in July, and *Cyperus aromaticus* stood out (33%) in September.

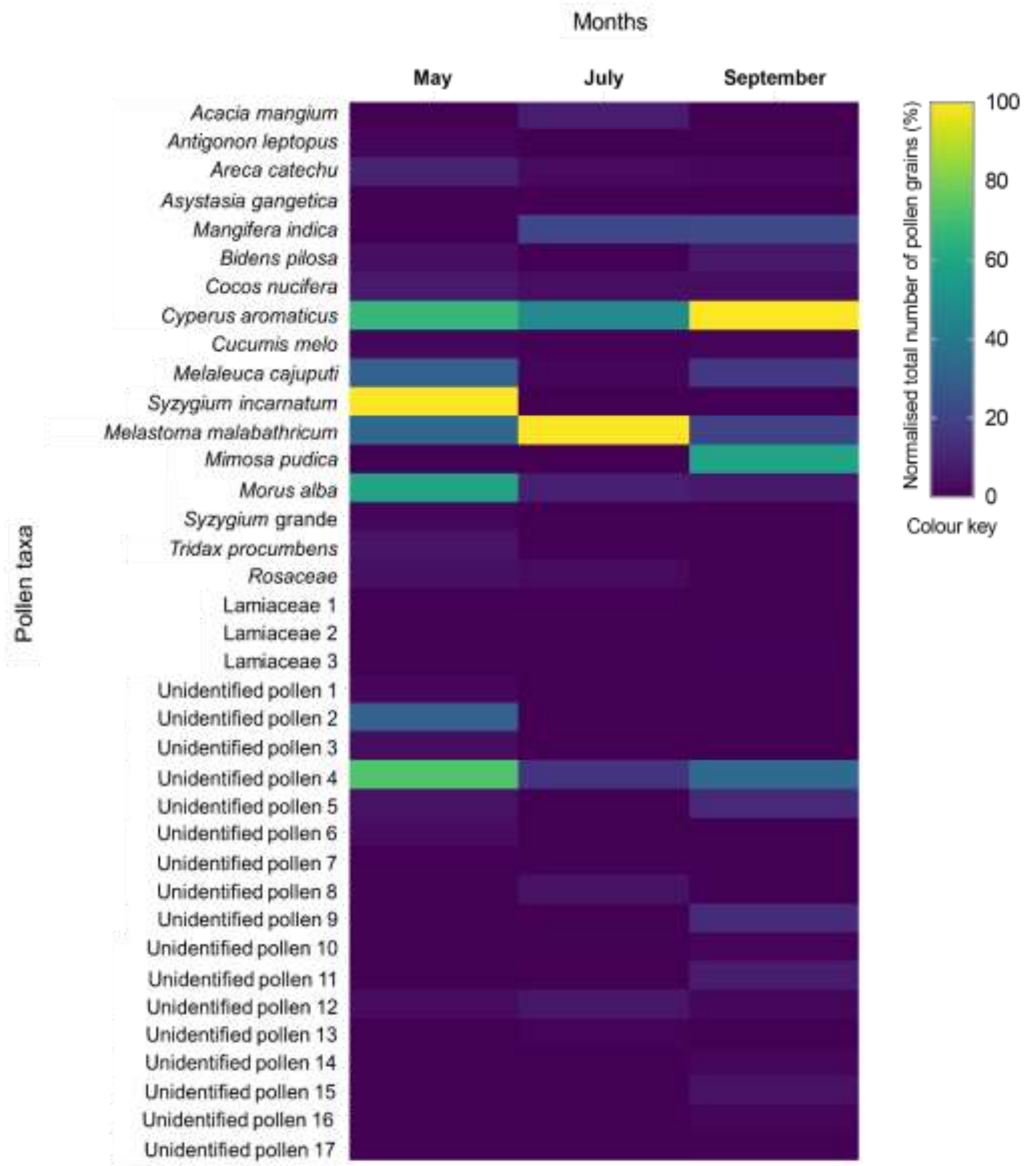


Figure 4. Heatmap represents the normalised total number of pollen grains of stingless bee pollen loads based on 3-month sampling periods. The heatmap shows the pollen foraging patterns of *Heterotrigona itama* in different months. The yellow colour represents stingless bee's dominant pollen grains, while dark purple represents the least pollen collected.

**Stingless bee Abundance Data: Rank Abundance Curve (RAC)**
Thirty seven pollen taxa were collected by stingless bees (N = 251) from Gelam forests. The RAC showed the foraging preferences of *H. itama* from the study areas. The curve for Marang and Setiu were significantly steeper, indicating that *H. itama* specialised in specific pollen sources for each site (Figure 6). *Cyperus aromaticus* and *Melastoma malabathricum* were highly collected by *H. itama* in Marang and Setiu, respectively (N = 55, N = 43). Meanwhile, *Areca catechu*, *Cocos nucifera*, and *Syzygium incarnatum* were also the species with the most pollen gathered. Moreover, *Acacia mangium*, *Mangifera indica*, *Melaleuca cajuputi*, *Mimosa pudica*, and other species are among the 14 pollen types represented by the intermediate pollen type collected by this bee at both sites.

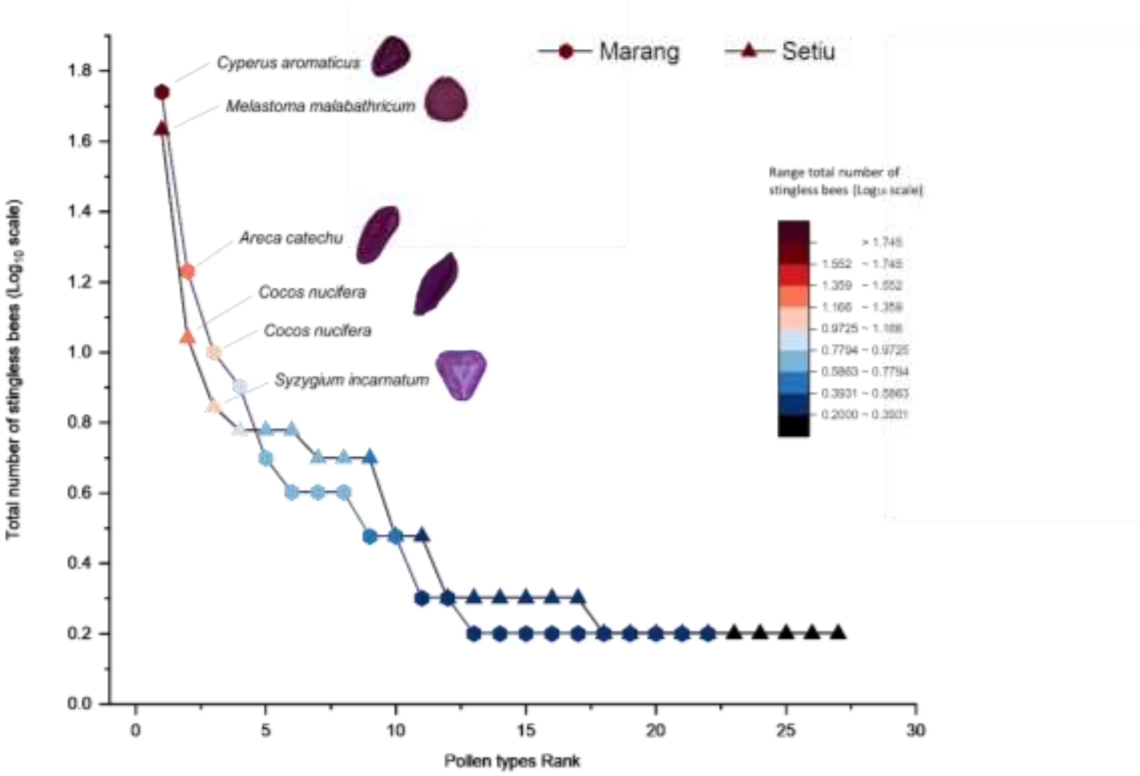


Figure 5. Rank abundance curve (RAC) showed the main pollen types collected by *Heterotrigona itama* (N = 251) reared in Gelam forests from Marang and Setiu. The y axis represents stingless bee abundance in log10 scale and the x-axis ranks each pollen type in order most to least abundant. The curve shows the pollen type preferences of *H. itama* over sampling periods. The graph pointed out the most pollen grains collected by the stingless bee.

**Stingless bee Abundance Data: Tiliagraph**
A tiliagraph shows the abundance of *H. itama* foraging on pollen sources from various lifeforms, including trees, shrubs, herbs, sedge, creeper, and climbers (Figure 6). The pollen diagram reveals the seasonality of the foraging pattern of *H. itama* between months on various plant types from Gelam forests. Overall, the pollen of trees was highly obtained by *H. itama* (N = 62), followed by sedge (N = 61) and shrubs (N = 56) (Figure 6). The monthly *H. itama* abundantly foraged on pollen from trees, shrubs, and sedges. In May, stingless bees showed a high preference for collecting pollen from trees species (N = 33). In July, shrubs became the

most prevalent pollen taxa foraged on (N = 32). Sedge was mainly gathered in September (N = 27), but the present study discovered sedge contained a single species which is *Cyperus aromaticus* collected by *H. itama.*

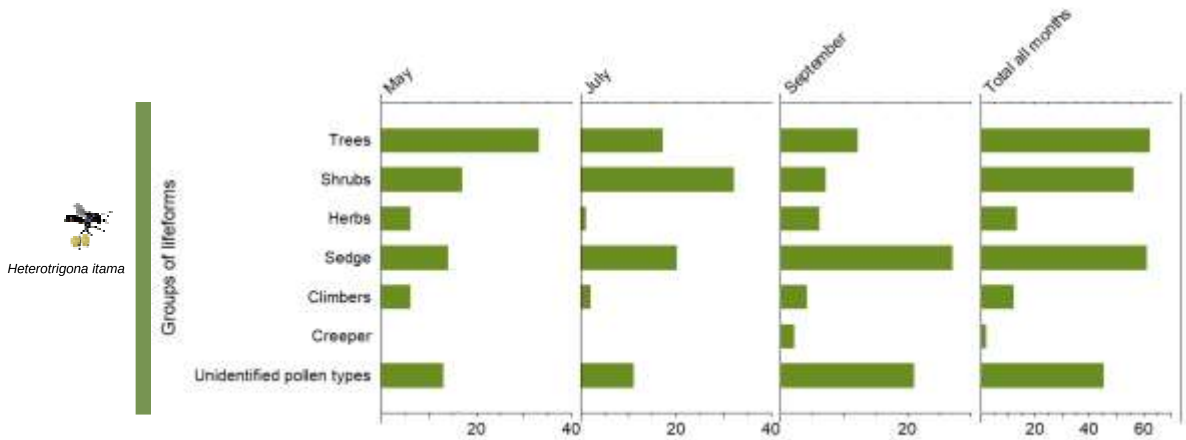


Figure 6. Tiliagraph demonstrating abundance of stingless bees collected pollen taxa of trees, shrubs herbs, sedge, climber and creeper during May to September

## DISCUSSION

The Gelam forest biome offers a heterogeneity of floral resources to domesticated stingless bees (*Heterotrigona itama),* which is crucial for high-quality honey production and commercialisation in Terengganu. The botanical sources collected by this *H. itama* reflected variations of bee flora found in Gelam forests that are significant to bees. This study also revealed the foraging behaviour and patterns of *H. itama* gathering pollen sources from distinct plant species. The present study discovered *H. itama* showed polylectic behaviour which involved foraging on numerous flowering plant species for pollen sources over the study period. Moreover, *H. itama* has detectably foraged on six life forms, including trees (*Melaleuca cajuputi*), shrubs (*Melastoma malabathricum*), herbs (*Asystasia gangetica*), sedge (*Cyperus aromaticus*), climbers (*Antigonon leptopus*), and creeper (*Mimosa pudica*).

In particular, the findings of this study discovered that *H. itama* has extensively used numerous plant families including trees (Anacardiaceae, Arecaceae, Fabaceae and Myrtaceae), shrubs (Melastomataceae and Moraceae) and sedge (Cyperaceae) as documented in previous studies of *H. itama* and other species of stingless bees (Azmi et al. 2015; Fahimee et al. 2021; Loeis & Basukriadi 2021; Majid et al. 2020; Zaki & Razak 2018). Another interesting note, the result indicates that *H. itama* abundantly collect pollen from trees. This suggests that the Gelam forests are important resources for *H. itama* including *M. cajuputi*, *Syzygium incarnatum* and *Syzygium grande,* which are abundantly found in the BRIS ecosystem (Salim et al. 2011; Shahrudin et al. 2016). The remaining trees are crop species cultivated around Meliponiculture localities such as *Areca catechu*, *Cocos nucifera* and *Mangifera indica.* However, this study discovered that the cultivated plants offer good resources to the stingless bee that may also contribute to honey production. The foraging preferences and patterns of *H. itama* that were recorded in the present study suggest more studies emphasis on pollen sources, which uncover novel and astonishing elements regarding *H. itama* that are strongly preferred for trees, shrubs and sedges from Gelam forests. This finding shows that forests are critical important bee-flora for domesticated stingless bees.

Stingless bee colonies utilised various pollen grains (22 pollen types) in Marang. *Cyperus aromaticus* was intensively used by *H. itama* in Marang followed by Palm species including *Areca catechu* and *Cocos nucifera*. *Cyperus aromaticus* locally known as 'rumput helikopter' and abundantly found in Malaysia. The weed is an invasive sedge that flourishes in Asia and many other countries, where it is fast-growing and aggressively dominates particular areas. In Malaysia, this sedge can be found in a diverse range of environments, including wasteland, roadside, railways, grassland, and forests (Chadha et al. 2021). The results of this study confirmed that *H. itama* foraged highly on *Cyperus aromaticus* for pollen sources. This could be due to *Cyperus aromaticus* ecologically massive production of flowers and seeds, which allows bees to forage for food sources (Chadha et al. 2021). Some studies have supported *Cyperus* sp. being recognised as an essential pollen resource for bees during floral scarcity (dearth periods), which plays an important role in providing sufficient food resources for maintaining colonies' health and honey production (Kumari & Kumar 2017). Pollen of Cyperaceae also a crucial polleniferous floral collected by bees species (honey bees and stingless bees) as has been discovered by Yano et al. (2013) and Loeis & Basukriadi (2021).

Twenty seven pollen types were collected by stingless bee colonies in Setiu. Pollen of *Melastoma malabathricum*, *Cocos nucifera,* and *Syzygium incarnatum* were highly foraged by *H. itama*, which indicates that the stingless bees preferred these floral from Setiu. *Melastoma malabathricum* is often called 'Senduduk' by Malaysian. The plant is a common shrub omnipresent in lowland and mountain forests, roadside, cleared land and wasteland (Joffry et al. 2012). The attractiveness of flower traits and flowers year-round makes *Melastoma malabathricum* beneficial for various insect groups, including hoverflies (Syrphidae), bees (Apidae and Megachilidae), wasps (Vespidae), and butterflies (Papilionidae), which are often foraged on *Melastoma* flowers (Huang et al. 2021). Therefore, the result corroborates that *H. itama* shows highly preference for pollen grains of *Melastoma malabathricum* in Setiu. Similar findings by Zaki & Razak (2018) also found evidence that *H. itama* foraged pollen grains from this plant in Terengganu. The other species of stingless bees (*Melipona rufvenrtris* and *Melipona quadrifasciata anthidioides*) showed similar preferences on *Melastoma* flowers, indicating dominant pollen throughout the study periods with pollen frequency ranging from 55–99% (Barth et al. 2020). The pollen spectrum of the family Melastomataceae was recorded as having the highest pollen selectivity and richness by a stingless bee (*Melipona capixaba*) and other Meliponine (Luz et al. 2011; Ocaña-Cabrera et al. 2022). Based on these points of view, this study can infer that *Melastoma* flowers are an ecologically important plant for several species of stingless bees and many groups of insects.

Despite being regarded as an opportunistic stingless bee species, *H. itama* has shown a predilection for particular botanical taxa from Gelam forests. The present investigation discovered adaptive behaviour and preferences of *H. itama* on one single flower species for each study site. This behaviour is commonly known as flower constancy. In this study, most of the corbicular pollen loads of stingless bees originated from *Cyperus aromaticus* in Marang, meanwhile, *Melastoma malabathricum* in Setiu, reflects the high degree of flower constancy behaviour in *H. itama* exploiting food resources for both floral species. The flower constancy behaviour of bees is often influenced by several aspects such as the distance of hives between flowers, food sources availability, reward quantity, colour, shape and odour of flowers (Grüter & Ratnieks 2011).

The colour of the flower is one of the most vital floral traits in terms of luring bees to forage on pollen and nectar. The central part of the flower (the anther) serves as a floral guide, which allows bees to allocate resources (de Ibarra et al. 2015; Lunau et al. 1995; 2017). The findings of this study confirmed that both the anthers and pollen grains of *Cyperus aromaticus* and *Melastoma malabathricum* display a yellow colour. The yellowish colouration in the anther and pollen might be one of the main reasons a large number of *H. itama* foraged on pollen sources from these flowers. In this scenario, *H. itama* shows a solid attraction for both flowers, and this preference may be due to their innate response to the yellow UV-absorbing zones of anthers and pollen grains, which plays an integral part as a neural signaling mechanism to attract bees for resources recognition (Lunau et al. 2017). However, the pollen size also offers a reasonable explanation for the high frequencies of pollen loads and the pollen abundance in this study. The study found that the smallest pollen grains sizes, such as *Cyperus aromaticus*, *Morus alba*, *Melastoma malabathricum*, *Mangifera indica*, *Mimosa pudica*, *Melaleuca cajuputi and Syzygium incarantum,* ranged from 10 μm to 31 μm. All these pollen grains were recorded among the highest frequencies, which suggests that the components that contribute to high frequency and pollen abundance are small in pollen size.

Nevertheless, *Melaleuca cajuputi* is a plant species native to the Malay Peninsula and can be found abundantly in Terengganu. *Melaleuca* flower is attractive with a whitish colour and fragrant scent, which offers a vast number of pollen and nectar resources to a diverse group of insects including Hymenoptera (bees and wasps), Diptera (syrphid flies), Lepidoptera (butterflies), Hemiptera (true bugs), Coleoptera (beetles), and Thysanoptera (thrips) (Baskorowati et al. 2010; Quang Tan 2002; 2008). Interestingly, the present study highlighted the evidence that *H. itama* foraged on pollen sources of *M. cajuputi* from the study areas. Therefore, it may be deduced that *M. cajuputi* is ecologically important for stingless bees, as it offers food resources for the bees' diet and assist in sustaining healthy bee colonies (Razak 2021). Previous study also reported that the *Melaleuca* pollen also had been collected by *H. itama* in Terengganu (Zaki & Razak 2018). The botanical sources of *Melaleuca* also play a vital role for wild honey bees in the honey production of Terengganu (Ibrahim et al. 2012).

On the contrary, the findings of this study showed Tiliagraphs and RAC analysis revealed that the *H. itama* collect relatively low amounts of *Melaleuca* pollens and the least recorded number of stingless bees foraged on this pollen grain. This could be linked to the anthropogenic impacts (deforestation and rural housing development areas) that occur within both locations, which have directly impacted the floral supplies of *M. cajuputi* to domesticated bees around Gelam forests. Furthermore, pollen load composition was illustrated as Tiliagraph supported by CONISS. The cluster analysis generates a hierarchical clustering of the pollen collected by *H. itama* from each hive. This component is one of the beneficial analyses applied to melittopalynological studies. The analysis enables tracing the particular pollen types and identical pollen collected by bees for each hive. This analysis is significant in uncovering the foraging preferences and patterns of *H. itama* in Gelam forests. Thus, the findings of this study indicate that ten colonies of *H. itama* showed distinctly different foraging patterns in each hive. However, the result showed these colonies strongly preferred *Cyperus aromaticus* and *Melastoma malabathricum* during the study period.

## CONCLUSION

Floral heterogeneity found in the Gelam forest provides subsistence for domesticated stingless bees (*H. itama*) for survival. Melittopalynology is a crucial tool for disentangling the importance of pollen sources for *H. itama* in Gelam forests. Findings of this study highlighted the importance of floral sources from *the Melaleuca* forest, which can be helpful in the context of conservation forests. The presence of 37 pollen types from distinct life forms reflects that the Gelam forest serves a broad spectrum of vegetation that are significant to *H. itama* for their foraging activities. *Cyperus aromaticus* and *Melastoma malabathricum* were discovered to be vital pollen sources for stingless bees. The study shed light on some botanical species that were also ecologically important for domesticated bees during the study period, such as *Areca catechu, Cocos nucifera, Mangifera indica, Melaleuca cajuputi* and *Syzygium incarnatum*. Understanding the botanical preference of stingless bees based on the pollen analysis can improve future meliponiculture strategies. The findings of this study are helpful to the meliponiculture industry as well as the government sector, as there is a need to enhance management practices in order to conserve the plant biodiversity found in the Gelam forests. Therefore, the Gelam forest should be prioritised for forest conservation efforts and future recommendation studies.

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**Conflict of Interest**
The authors declare that they have no conflict of interest.

**Ethics Declarations**
No ethical issue required for this research.

**Data Availability Statement**
Not applicable

**Authors' Contributions**
WAA, NZM, CMO and DMA conceived this research and designed experiments; MIIM, WAA, and NZM participated in the design and interpretation of the data; MIIM, SK and MFLF performed experiments and analysis; MIIM,  and WAA wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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