Pollen Ultrastructure from *Heterotrigona Itama* Foragers at the Indo-Malayan Meliponine Repository Sekayu, Terengganu, Malaysia

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Abstract

Stingless bees play an important role as pollinators of plants. *Heterotrigona itama* is considered as important pollinator's bee and the most popular species in meliponiculture for high value honey. As a generalist stingless bee, they collected many types of pollen. Thirteen (13) types of pollens collected by *H.itama* have been identified in Indo-Malayan Meliponine Repository Sekayu, Terengganu. They are *Antigonon leptopus* (Polygonaceae), *Biden pilosa* (Asteraceae), *Cocos nucifera* (Arecaceae), *Capsicum annuum* (Solanaceae), *Mimosa pudica* (Fabaceae), *Acacia auriculiformis (*Fabaceae), *Amaranthus spinosus (*Amaranthaceae), *Averrhoea carambola* (Oxalidaceae), *Cosmos sulphureus* (Asteraceae), *Hymenocallis littoralis* (Amaryllidaceae), *Sphagneticola tribolata* (Asteraceae); *Solanum melongena* (Solanaceae) *and Andrographis paniculata* (Acanthaceae). These pollens were loaded mainly on corbiculae, which is called as "selective pollen acquisition" and also adhered abundantly on other body regions, which is called as "incidental pollen acquisition". These pollens were found adhered on antenna, compound eye, mandible, tongue, other legs, and abdomen; and also spread on the surface of both wings and on thorax.Those pollens on corbiculae or the selective pollens may not serve a reproductive role for the plant as they almost invariably moistened with nectar. While the incidental pollens are considered important and play an effective role in plant pollination as the chances of losing individual grains to get into contact with stigmas and perform pollination during floral visits are very likely. All thirteen (13) type of pollens were occured as both "selective pollen acquisition" and as "incidental pollen acquisition".

Keywords: *H. itama*, incidental pollen, selective pollen, pollinator, stingless bee

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Introduction

Stingless bees (Hymenoptera, Apidae, Meliponini) are the largest group of eusocial bees on Earth. They are a group of comparatively small bee species and play an important ecological role in the pollination process of plant life, particularly wild flowers in most tropical countries [1,2]. Stingless bees are economically important pollinators in Peninsular Malaysia's dipterocarp forests and agriculture [3,4,5]. More than 500 species have been recorded and found mostly in tropical areas [2, 6]. Malaysia hosts a great number and diversity of honey bees and stingless bee species. There are about 35 species of Indo-Malayan stingless bees which have been inventoried in Malaysia [4]. *Heterotrigona itama* is one of four common species of stingless bees (*Heterotrigona itama , Geniotrigona thoracica, Lepidotrigona terminata, Tetragonula laevisep*) in southeast Asia [3,5,7] and the most popular species in meliponiculture [8,9,10] for their honey, propolis and beebread.

The floral sources used by bees can be identified by analyzing pollen grains obtained from their bodies [10,11,12], feces, brood cells, or storage pots in the nests [13]. Each individual flower species has a unique morphology pollen grain which can be studied to determine flower constancy of stingless bee. Stingless bees collect pollen and nectar flowers to nourish their colony and this simultaneously transfer pollen between flowers. Generally, bees use their proboscis to scrape off pollen grains from the anther, with subsequent scraping motions by the foreleg to remove pollen from the mouthparts and head to the hind legs [14,15]. Then the pollens will be moistened with nectar and saliva, and packed on pollen basket (corbiculae) in the form of pasty pellets called pollen loads [10,16]. Generally the collected pollens is believed may fertilize ovules in which stingless bees lose inadvertently on floral stigmata as they go about collecting nectar, pollen, or other material. This behaviour of stingless bees answers the question of how the stingless bees function as good pollinators. This is because the transported pollen loads were moistened with nectar and saliva and this reduce the chances of losing individual grains to contact stigmas and perform pollination during floral visits.

This study is aiming to identify pollens collected by stingless bees at the Indo-Malayan Meliponine Repository Sekayu, Terengganu, Malaysia and the adherence of those pollens on their body other on the corbiculae. *H. itama* as the common stingless bees in Malaysia is the subject used to this study, using Field Emission Scanning Electron Microscope (FESEM) to view pollens at the nature state. Data on the pollens plant species that *H. itama* collect and the pollens adhered on various parts of the body provides a base for studies on the interactions between the bees and their preferred plants of which are related to pollination.

Materials and Methods

Study Area

Collections of *H. itama* were sampled from the apiary center known as Indo-Malayan Meliponine Repository Sekayu, Terengganu, Malaysia. This center is located about 15km from Kuala Berang and 52km south of Kuala Terengganu with geographical coordinates at N04° 58' 0" and E102° 58' 0'. (The internet map location at 4.9652926, 102.9571952). The area covers approximately 93 hectares of secondary forest which also includes a fruit orchard. There are many species of flowering plants, a large numbers of trees, climbers, shrubs, herbs, ephiphytes and saprophytes. The climate is hot and humid.

Samples Collection - Stingless bee foragers with pollen after visiting flowers & Pollens from references flower.

To assess pollen loads content, the returning foragers of *H.itama* with pollens were captured from several colonies by intercepting these bees before they entered the hive. They were captured gently and carefully by either using pooter methods or directly trap individual bees into clean vials. They were kept in properly clean and labelled vials. Pollens from reference flowers which were seen visited by stingless bees, were collected and kept in clean labelled containers. Both types of sample were taken to laboratory for ultrastructural analysis as soon as possible to avoid contamination and the bees become very stiff.

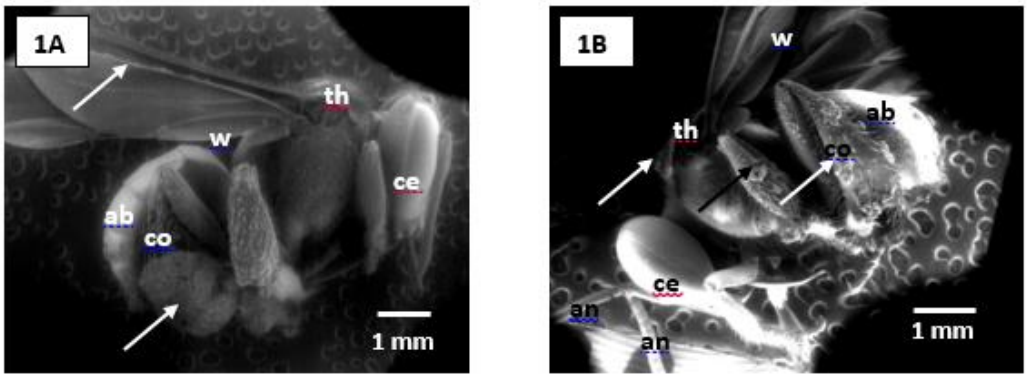
Samples Preparation and Microscopy

Stingless bees with pollens were carefully and gently placed on top of SEM stub which was layered with carbon conductive adhesive tape. They were positioned with the pollen basket facing up using toothpick and stereomicroscope. Pollens from reference flowers were picked up using toothpicks. They were placed onto SEM stub which was layered with carbon conductive adhesive tape.

Analysis of the pollen collected by the bees was performed using controlled low-vacuum mode FESEM Quanta FEG 650 at University of Malaya at various applicable magnification. SEM stub were placed on the SEM stage inside SEM chamber. They were viewed under controlled low-vacuum mode FESEM. The accelerating voltage was 1.0 – 5 KV and the spotsize was 2.5 – 5.00. The working distance of the specimen was 10nm. Image was recorded to be analysed.

Results and Discussion

H. itama were observed to collect thirteen (13) types of pollens as shown in Table 1. The "selective pollen acquisition" was observed as pollen load on pollen basket as shown on Figure 1A and 1B. The "incidental pollen acquisition" was observed to spread abundantly on other parts of body such as antenna, compound eyes, mandibles and tongue, other legs and abdomen; and loose pollen were also found on both wings and thorax respectably as on Table 1 and Figures 2A, 2B, 2C, 2D, 2E and 2 F.

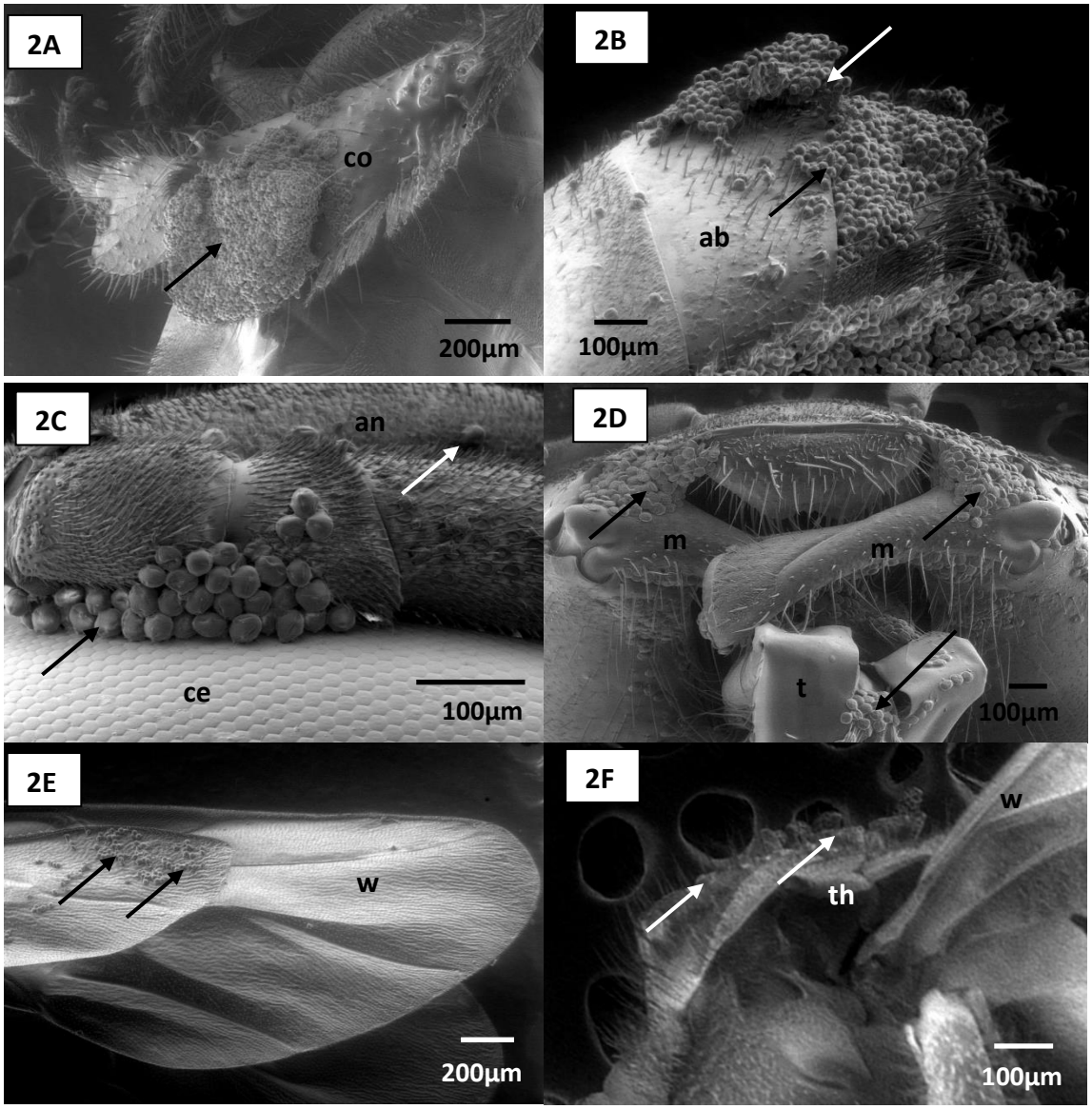


Figures 1A and 1B: Scanning Electron Microscope (Ultrastructure) Image of *H.itama* with pollens (arrows) on their corbiculae (pollen basket) (co) and other area. ab, abdomen; an, antenna; ce, compound eye; co, corbiculae; th, thorax; w, wing. (Note: Pollen load on pollenbasket on Figures 1B were scraped off).

Table 1: List of pollens collected and pollen acquisition on the body parts of *H.itama*

	Pollens	Antenna	Compound Eye	Mandible	Tongue	Other Legs	Pollen-basket	Thorax	Abdomen	Wings
1.	*Amaranthus spinosus* (Amaranthaceae)	++	+	++++	+++	+++	+++++	+	+++	+++
2.	*Antigonon leptopus* (Polygonaceae)	++	++	++++	++++	+++	+++++	+	+++	++
3.	*Acacia auriculiformis* (Fabaceae)	++	++	++++	+++	+++	+++++	++	+++	++
4.	*Andrographis paniculata* (Acanthaceae)	+	+	++	++	++	+++++	+	++	++
5.	*Averrhoa carambola* (Oxalidaceae)	++	+	++++	++	+++	+++++	+	++	++
6.	*Biden pilosa* (Asteraceae)	++	+	++++	++	+++	+++++	+	++	++
7.	*Capsicum annuum* (Solanaceae)	++	+	+++	+++	+++	+++++	+	++	++
8.	*Cocos nucifera* (Areacaceae)	++	+	+++	+++	+++	+++++	+	+++	++
9.	*Cosmos sulphureus* (Asteraceae)	++	+	+++	+++	+++	+++++	+	+++	++
10.	*Mimosa pudica* (Fabaceae)	++	+	+++	+++	+++	+++++	+	+++	++
11.	*Hymenocallis littoralis* (Amaryllidaceae)	+-	-	+++	+++	++++	+++++	++	+++	+
12.	*Sphagneticola tribolata* (Asteraceae)	+-	-	+++	+++	+++	+++++	+	++	++
13	*Solanum melongena* (Solanaceae)	+	+	+++	+++	+++	+++++	+	++	++

Scale : + the density of pollen
Note : +- very small number of pollen (less than five)



Figures 2A B, C, D. E, and F are the patterns of pollens (arrows) collection/acquisition pattern on the body of *H.itama*. 2A is corbiculae, 2B is abdomen (metasoma), 2C is antenna, 2D is mandibles and tongue, 2E are wings and 2F is side view of thorax. Figure 2A shows the ball of pollen on the corbiculae surface which is "selective pollen acquisition" where the pollen collected is on the corbiculae. Figures 2B, C, D, E and F are "incidental pollen acquisition" which is accidentally picked up on the body of the bee. an, antenna; ce, compound eye; co, corbiculae; m, mandible; t, tongue; th, thorax; w, wing. (Pollens on figures are A - *Amaranthus spinosus*, B, C and E - *Antigonon leptopus*, D - *Acacia auriculiformis*, F - *Hymenocallis littoralis.*

Study of pollen identification is important as it can be used to revealed the botanical diversity to bees. Morphology of pollens has been analysed in detail under the scanning electron micoscope. The used of Field Emission Scanning Electron Microscope (FESEM) revealed the unique morphology of pollen on it nature state. The ultrastructural morphology

of pollen in nature state is like a fingerprint which can be used to identify plant taxonomy by using combination of the size, shape and the surface pattern of pollen [12,17].

In this study, results showed that *H.itama* collected almost thirteen (13) types of pollens (Table 1). They were *Amaranthus spinosus (*Amaranthaceae), *Antigonon leptopus* (Polygonaceae), *Acacia auriculiformis (*Fabaceae), *Andrographis paniculata* (Acanthaceae), *Averrhoea carambola* (Oxalidaceae), *Biden pilosa* (Oxalidaceae), *Capsicum annuum* (Solanaceae), *Cocos nucifera* (Arecaceae), *Cosmos sulphureus* (Asteraceae), *Mimosa pudica* (Fabaceae), *Hymenocallis littoralis* (Amaryllidaceae), *Sphagneticola tribolata* (Asteraceae); *Solanum melongena* (Solanaceae). All the identified plant species are common plant species grow within and nearby areas of Sekayu. Majority of identified plant species were common with other studies [7,9,10,11]. The plants species that always reported among meliponini keeper in many stingless bees' areas are *A. leptopus*, *C. nucifera*, *C. annuum*, *M. pudica*, *A. auriculiformis* and *C. Sulphureus* [1,7,9,10,11]. Pollen constancy and specialization in stingless bees as collected on corbiculae is believed not for efficient pollination but rather as food for their colony [18]. However, the broad spectrum of pollen acquisition by the *H .itama* suggested that this species is a generalist forager as they visit multiple types of flowers to obtain resources. In Malaysia *H. itama* is also reported by many researchers as playing a vital role as an effective pollinator of many wild plant's [11,18] and agricultural crops [3,9].

All the thirteen identified pollens were observed loaded mainly on corbiculae as it is the spesific structure to collect pollens [7,19]. These corbiculae pollens is called as "selective pollen acquisition" [20]. The pollens were also adhered abundantly on other parts of body such as antenna, compound eye, mandible, tongue, other legs, and abdomen; and small number of pollens also adhered on the surface corner of both wings and thorax. [20] considered the other region pollens as as "incidental pollen acquisition". In this study, the mandible, tongue and abdomen were found with more pollen compared to other regions. These areas are the regions which easily exposed to pollens anter compared to other body region when the bee landed on flowers. However, in this study pollens of *H. littoralis* and *S. tribolata* were hardly attached to antenna and compound eyes as both species considered have bigger size of pollens.

The "selective pollen acquisition"on corbiculae occurred as a huge packed ball shaped of pollens. Normally bee moistens the forelegs with its protruding tongue and brushes the pollen that has collected on its head, body and forward appendages to the hind legs. The pollens were then transferred to the pollen comb on the hind legs and then combed, pressed, compacted, and transferred to the corbicula on the outside surface of the tibia of the hind legs as on Figures 1A and 1B. Such pollen is almost invariably moistened with nectar. This corbiculae pollen is mainly for the purpose of food for bee larvae (brood). The moistening process during collection was suspected to impair pollen viability and may not serve a reproductive role for the plant. [20,21] previously suggested moistened pollens were not condusive for plant pollination.

The "incidental pollen acquisition" pollens are those which adhered randomly and abundantly on other body regions such as antenna, compound eye, mandible, tongue, other legs, abdomen, both wings and thorax (Figures 2A, 2B, 2C, 2D, 2E and 2F). Such pollen is often picked up as a by-products when bees visit flowers for nectar for their own consumption while out of the nest or other purposes during foraging or scouting bouts [22] This result is in agreement with [13], where they reported that while collecting pollen and/or nectar from flowers, bees usually stay with pollen grains adhered to various parts of the body besides

those pollen stored into the corbiculae. This incidental pollen collection is believed to contribute effectively in plant pollination. The incidental pollen acquisition presumably occurred when stingless bee make contact with pollen sources, in which pollens were incidentally picked up on the body of the bees as they encountered a variety of flower morphologies of many plant species. The incidental pollens can also be often picked up as a by-product when bees visit flowers for nectar for their own consumption while out of the nest or other purposes during foraging [22]. As a result, on subsequent visits, the bees may unwittingly perform pollination [23]. Among the body regions area which were adhered with pollens; the mandible, tongue, other legs and abdomen were found with more pollens compared to other regions. However, the tongue is a moistened area which may impairs pollen viability.

Conclusion

H.itama is considered as a "generalist forager" where they collected almost thirteen (13) types of pollens in Indo-Malayan Meliponine Repository Sekayu, Terengganu, Malaysia. They were *A. spinosus, A. leptopus, A. auriculiformis, A.paniculata, A. carambola, B. pilosa, C. annuum, C. nucifera, C. sulphureus, M. pudica, H. littoralis, S. tribolata, and S. melongena*. These identified plant species are common plant species grow within the areas of Sekayu. All the thirteen (13) types of pollens on *H itama* were occured as both "selective pollen acquisition" and "incidental pollen acquisition". The "selective pollen" where the pollens were mainly loaded on corbiculae; and those pollens on tongue may not serve a reproductive role for the plant as they were moistened and suspected to impair pollen viability [20,21]. The "incidental pollen" that play effective role in plant pollination are those easily to expose on pollens anter when it landed on flowers. They are adhered randomly on antenna, compound eye, mandible, other legs, abdomen, both wings and thorax.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors report no conflicts of interest in this work.

Compliance with Ethical Standards

For this type of study formal consent is not required.

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