Application of Scanning Electron Microscope in Palynology Study of Floral Resources By Indo-Malayan Stingless Bees Genus Tetragonula

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Abstract

Palynology or study of pollen (pollen analysis) allows the identification of floral sources of stingless bees. The palynology (pollen analysis) was carried out on pollens grains foraged by the Indo-Malayan stingless bees. The objective of this study is to identifiy the flower preferred by the *Tetragonula* in their surrounding habitat. The pollens collected by eight species of *Tetragonula* and their referrences flower were investigated using scanning electron microscope (SEM) with some modification on the SEM parameters to suit the samples. Pollen's unique morphology is like fingerprint which can provide a basis for the identification of plant species. About twenty-three (23) pollens have been observed and identified. The predominant pollens were *Antigonon leptopus* (Polygonaceae) and *Elaeis guineensis* (Arecaceae) with each collected by about four (4) species of stingless bees; followed by Averrhoa carambola (Oxalidaceae), *Capsicum annuum* (Solanaceae), *Citrus microcarpa* (Rutaceae), *Cocos nucifera* (Arecaceae) and *Sphagneticola tribolata* (Asteraceae) with each collected by about three (3) species of stingless bees. *Tetragonula fuscobalteata* was found as the most generalist foragers which had collected almost twelve (12) type of pollens, followed by *Tetragonula laeviceps* with nine (9) species of pollens. It can be concluded the identification of pollens and stingless bee-collected pollens based on morphological ultrastructural detail using SEM can provide the valuable and specific information of pollens and plant-insect interaction.

Keywords: pollen, *Tetragonula*, Scanning Electron Microscope

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Introduction

Stingless bees (Hymenoptera, Apidae, and Meliponini) are the largest group of eusocial bees which play an important ecological role in the pollination of many flowering plants in subtropical and tropical regions [1]. The stingless bees species are classified into five genera: Melipona, Trigona, Meliponula, Dectylurina and Lestrimelitta [2] .

Tetragonula is small size group of stingless bees with their length 2.5 – 4.5mm [2]. Many of them are similar to one another and often difficult to recognize, differing in minute aspects of coloration, size, and setation [3] . Malaysia is home to diverse species of stingless bees which 35 species of stingless bees has been identified in Peninsular Malaysia. Most of the research of stingless bee-pollens in Malaysia was focussed on common apiculture stingless bees which are *Heterotrigona itama* and *Geniotrigona thoracica* ([4, 5, 6]. Research by Malaysian Agricultural Research and Development Institute (MARDI) listed that *Tetragonula fuscobalteata*, and *Tetragonula laeviceps* are among the common species available in Peninsular Malaysia beside *Heterotrigona itama, Geniotrigona thoracica, Lepidotrigona terminata,* and *Tetragonilla atripes* [7].

Meliponine bees and flowering plants have been considered as mutualistic relation. Stingless bees need flowering plants for nectar and pollen as source of food and flowering plants need the bees for pollination which is the key aspect in the sexual reproduction plants. Stingless bees may have preferences in selecting flowers to pollinate. Palynology or study of pollen is crucial in understanding the crops and plants that are foraged by bees as their food source because bees have species-specific preferences of pollens. Most of studies on identification of pollen grain related to the stingless bees are generally done by stereomicroscope and light microscope [4, 8] . However, examination of pollens grain (from honey) using scanning electron microscope (SEM) highlighted that it could able to differentiate and recognize the ultrastructure pollens better than by light microscope, and using of light microscope is not sufficient to determine the kind of honey flow [6, 9] .

The aim of this study was to determine the composition and identify the pollens and flower contancy foraged by of eight (8) species by subgenus *Tetragonula* using scanning electron microscope (SEM). The species are *Tetragonula* (*Tetragonilla*) *collina, Tetragonula minor, T.fuscobalteata, T.laeviceps, T.melanocephala, T.minangkabau, T.sirindhornae* and *T.testaceitarsis*. It is in need to study potential impact of smallest sizes stingless bees especially in agricultural ecosystem. The results obtained will also hopefully to provide useful knowledge on species diversity of *Tetragonula* and their pollen food sources.

Materials and methods

Method of low-vacuum mode of SEM to study pollen in situ was a modification from [8, 9].

Samples Collection - Stingless bees with pollen on pollenbasket (corbiculae)

Six (6) species of stingless bees were sampled from Indo-Malayan Meliponine Repository Sekayu, Terengganu and two (2) from Borneo. The arriving foragers that had pollen loads were captured gently and carefully by pooter methods. Samples were collected by sucking using a tube into a small bottle or vial. Each sample were colected by individual tube (in order to prevent pollen contamination) and kept in properly labelled vial.

Samples Collection – Pollens from referrences flower

Pollens from references flower were collected which were seen visited by stingless bees. Those flowers were kept in labelled container and brought back to laboratory for examination. These are reference pollens to be compared with pollen from the pollen basket of stingless bee foragers.

Samples mounting

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 references flowers were picked up using toothpick. They were placed onto SEM stub which was layered with carbon conductive adhesive tape.

Microscopy and Imaging System

SEM stub were placed on the SEM stage inside SEM chamber. They were viewed under controlled low-vacuum mode FESEM Quanta FEG 650 at University of Malaya. 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

The stingless bees Genus *Tetragonula* in Sekayu and Borneo related with their preferences flower is summarized as on Table 1. *Tetragonula* (*Tetragonilla*) *collina* collected three (3) type of pollens, *T. fuscobalteata* twelve (12), *T. laeviceps* nine (9), *T. minor*, *T. melanocephala* and *T. minangkabau* collected five (5), *T.sirindhornae* (4) and *T. testaceitarsis* two (2).

Twenty-three (23) identified species and family of pollens collected by studied *Tetragonula* has been identified and also listed inTable 1. Seven (7) predominant pollens collected by *Tetragonula* observed using SEM which were *Antigonon leptopus* (Polygonaceae), *Elaeis guineensis* (Arecaceae), *Averrhoa carambola* (Oxalidaceae), *Capsicum annuum* (Solanaceae), *Citrus microcarpa* (Rutaceae). *Cocos nucifera* (Arecaceae) and *Sphagneticola tribolata* (Asteraceae) related with the flowers are shown on Figure 1.

Table 1: Family and species of Pollen sources by 8 species of Indo-Malayan Tetragonula

Family	Plant species	Indo-Malayan Stingless Bee : Tetragonula								
		Tetragonula (Tetragonilla) ...	*Tetragonula minor*	*T.fuscobalteata*	*T.laeviceps*	*T.melanocephala*	*T.minangkabau*	*T.sirindhornae*	*T.testaceitarsis*	Total number of pollen ...
Arecaceae	*Cocos nucifera*	■	■			■				3
	Elaeis guineensis			■	■	■	■			4
	Wodyetia bifurcata				■	■				2
Asteraceae	*Biden pilosa*				■	■				2
	Cosmos sulphureus						■			1
	Helianthus annus				■			■		2
	Sphagneticola tribolata				■	■		■		3
Apocynaceae	*Catharanthus roseus*			■						1
Cleomaceae	*Cleome rutidosperma*			■						1
Euphorbiaceae	*Manihot esculenta*				■					1
Fabaceae	*Acacia auriculiformis*			■	■					2
	Mimosa pudica		■				■			2
Lythraceae	*Lagerstroemia indica*			■				■		2
Mutingiaceae	*Muntingia calabura*			■						1
Oxalidaceae	*Averrhoa bilimbi*								■	1
	Averrhoa carambola		■	■			■			3
Passifloraceae	*Turnera subulata*				■					1
Polygonaceae	*Antigonon leptopus*			■	■		■		■	4
Rubiceae	*Ixora coccinea*			■						1
Rutaceae	*Citrus microcarpa*		■	■				■		3
Sapindaceae	*Nephelium mutabile*	■								1
Solanaceae	*Capsicum annuum*	■	■	■						3
	Solanum melongena			■						1
Total number of species on each bees		3	5	12	9	5	5	4	2	

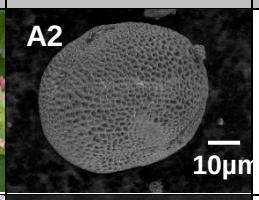
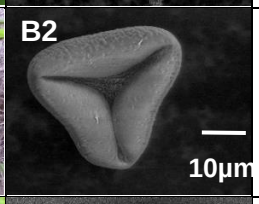
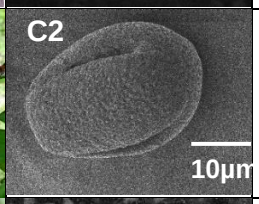
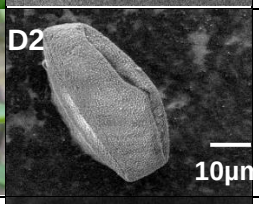
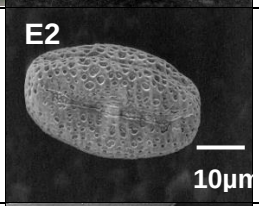
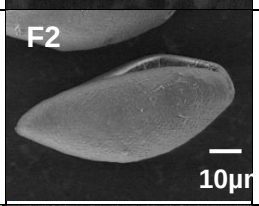
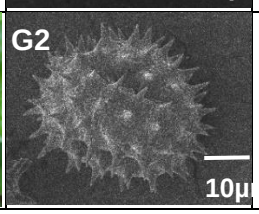
Flowers	Pollens	Species
		*Antigonon leptopus* Family: Polygonaceae
		*Elaeis guineensis* Family: Arecaceae
		*Averrhoa carambola* Family: Oxalidaceae
		*Capsicum annuum* Family: Solanaceae
		*Citrus microcarpa* Family: Rutaceae
		*Cocos nucifera* Family: Arecaceae
		*Sphagneticola tribolata* Family: Asteraceae

Figure 1: The predominant pollens collected by *Tetragonula* observed using SEM. (A) *Antigonon leptopus* (Polygonaceae); B) *Elaeis guineensis* (Arecaceae); (C) *Averrhoa carambola* (Oxalidaceae), (D) *Capsicum annuum* (Solanaceae); (E) *Citrus microcarpa* (Rutaceae); F) *Cocos nucifera* (Arecaceae) and (G) *Sphagneticola tribolata* (Asteraceae). (1) Flower and (2) Pollens for the respective plant.

Discussion

The detail and unique ultrastructure morphology of pollens under scanning electron micoscope is like fingerprint, which can be used to identify plants genus by using combination of the size, shape and the surface pattern of pollen [10, 11]. The studied pollens has been observed and identified based on their unique and distinctive ultrastructural morphological characteristic. All the pollens were found from those plants near their hives. Most of this plants were small type flower plants. These type of flowers has attractant of nectar and pollen [1].

Stingless bees are generalist foragers in floral resource and visit flowers of more plant species. *Tetragonula fuscobalteata* was found as the most generalist foragers which had collected almost twelve (12) type of pollens, followed by *Tetragonula laeviceps* with nine (9) species of pollens. *T. fuscobalteata* has been reported as the highest pollen carrier among all nature stingless bee in Thung Salaeng Luang National Park, lower northern Thailand [12]. Among the other *Tetragonula, T. laeviceps* are common *Tetragonula* studied. [13] reported *T. laevisep* and *T.collina* as the main pollens collector among ten other species of stingless bees collected in Nam Nao National Park, Thailand. Moreover, [5] reported the pollens constancy by *T. laeviceps* showed it's constancy more on the flowers of grass in Poaceae family (76.49%), followed by two Rutaceae sp.

The pollens collected by *Tetragonula* showed *Antigonon leptopus* (Polygonaceae) and *Elaeis guineensis* (Arecaceae) are the most dominant pollen. It was collected by *T.fuscobalteata, T.laeviceps, T.minangkabau* and *T.testaceitarsis*. Many literature reported the *A.leptopus* as the common pollens and attracts many Indo-Malayan stingless bee [4]. The flowers are visited by a myriad of pollinators (bees, flies, hummingbirds, butterflies), facilitating sexual reproduction outside of its natural range [14]. *Elaeis guineensis* is a monoecious plant, the species depends on effective pollination mainly by insects and to a lesser extent by wind [15]. In this study pollens of *E. guineensis* was collected by *T.fuscobalteata*, *T.laeviceps*, *T.melanocephala* and *T.minangkabau.*

Conclusions

Pollen from the different flowers has specific shape, size and unique morphological detail out the ultrastructure. The pollens ultrastructural characteristic viewed by SEM can be used in identification in detail of pollens related to stingless bees. There are about twenty-three (23) unique pollens related to Indo-Malayan *Tetragonula* stingless bees have been identified in Indo-Malayan Meliponine Repository Sekayu, Terengganu and Borneo. The pollen of *Antigonon leptopus* (Polygonaceae) is well-known pollen plant visited by many stingless bees. *Tetragonula* could be considered as potential pollinator of oil palm plant.

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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 have no disclosures to declare.

Compliance with ethical standards
The work is compliant with ethical standards.

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