Malays. Appl. Biol. (2017) 46(3): 227–232

COMPOSITION AND IDENTIFICATION OF POLLEN COLLECTED BY STINGLESS BEE (*Heterotrigona itama*) IN FORESTED AND COASTAL AREA OF TERENGGANU, MALAYSIA

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Accepted 7 September 2017, Published online 4 October 2017

ABSTRACT

The aim of this study was to determine the composition and identify the pollen foraged by *Heterotrigona itama* in coastal and forested area over a period of nine months. The sampling has been conducted started from June 2016 until February 2017. Pollen composition obtained from the pollen basket of *H. itama* in coastal area and forested area of Terengganu was observed and recorded using optical microscope. Up until February, *H. itama* in coastal area had collected 14 different type of pollen, however only 10 types of pollen have been successfully identified. The identified pollen were *Antigonan leptopus, Amaranthus tricolor, Hibiscus rosa-sinensis, Cucumis melo, Ixora coccinea, Tridax procumbens, Biden pilosa, Turnera subulata*, *Ixora javanica* and *Portula grandiflora* which was the dominant pollen that has the highest percentage. Meanwhile *H. itama* in forested area has collected 15 different type of pollen with only 7 types of pollen have been successfully identified. The identified pollen were dominated by *Asystasia gangetica* followed by *Biden pilosa, Antigonan leptopus, Reullia brittonia*, *Amaranthus tricolor, Hibiscus sabdariffa* and *Portulaca grandiflora.* This observation could be useful for plants and *H. itama* conservation purposes and also in planning a suitable beescape for Meliponiculture in Malaysia.

Key words: Stingless bee, *Heterotrigona itama*, pollen distribution, palynology

INTRODUCTION

Both stingless bee and honeybee are known as eusocial insect. Apinae and meliponinae is highly beneficial in agriculture industry since bees are one of the main pollinator, which can help in enhancing the plant productions (Anguilet *et al.,* 2015). Previously, only honeybees such as *Apis cerana* and *Apis mellifera* become a highlight in honey and bee pollen production. However, these few years stingless bee had become a phenomenon in honey production in worldwide including in Malaysia. Stingless bee or known as '*kelulut*' by Malaysian native has high potential to generate passive income for the beekeepers.

Stingless bee keeping or meliponiculture is now trending in Malaysia. Nowadays, there are many beekeepers among Malaysian who had started to keep stingless bee in a large scale. The main product of meliponiculture is honey while other by-product such as beebread and propolis are also high cash value. Product from meliponiculture has been used as one of the ingredient to skincare and healthcare products.

There are about 64 genera and 500 species of stingless bee recorded in the world (Heard, 1988; Ruttner, 1988). It have been recorded that Malaysia has around 32 different species of stingless bee

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(Schwarz, 1939; Norowi, 2010; Salim *et al.,* 2012). Stingless bee belongs to family Apidae (De Camargo & Menezas Pedro, 1992), which recently the tribe has been renamed to meliponini (Michener, 2000) that have few different characteristics if compared to honey bee. Major differences between honey bee and stingless bee is that, the size of honeybee is bigger compared to stingless bee. Aside of that, honeybee and stingless bee can differ in terms of their color, wing's color and hive. As being recorded by Sommeijer *et al.* (2003), the smallest species of stingless bee is 3mm and has a very small nest that can fits into a cigarette packet meanwhile the largest can be as big as honey bee (*Apis mellifera*).

Bees are well known of their capability of carrying microscopic particles and most pollen are microscopic (Wodehouse, 1959), thus bees being one of the main pollinator in ecosystem. Pollen is the fine powder like material that produced by flowering plant for plant reproduction and is gathered by the worker bees (Abouda *et al.,* 2011). Stingless bees are one of an important pollinator that mainly found at several continents, mainly on tropical and subtropical regions (Macharia *et al.,* 2007). Bees are able to contribute to plant diversity as well as improving the quantity and quality of fruits and vegetable production (Gallai *et al.,* 2009). Like honeybee, stingless bees also collect pollen and contribute to the flower pollination and helping in fruit production (Heard, 1988).

The analysis of pollen is known as palynology where this method is to identify the bee flora (Barth, 2013). Riding & Kyffin-Hughes, (2004) stated that palynology is the study regarding microscopic fossil, it was originally known as 'pollen analysis' and encompassed the study of quaternary pollen grains and plant spores. Therefore, this study aimed to gather the information on the pollen composition and identify the pollen collected by the stingless bee in forest and coastal area in Terengganu.

MATERIALS AND METHODS

The forager of *Heterotrigama itama* in coastal area was represented by the samples collected at Universiti Malaysia Terengganu (UMT), meanwhile for *H. itama* foragers in forest area was collected from Taman Pertanian Sekayu, Terengganu. The sampling was carried out once a month for the period of nine months. Five colonies involves during sampling. Five returning foraging stingless bees with pollen load were collected for pollen identification. Sampling was conducted between 8 a.m to 11 a.m during the sampling period. Flowers around the sampling sites (coastal and forest) were also collected as the pollen references. The entrance of *H. itama* was briefly closed using a piece of white paper or a leaf to prevent returning foragers from entering the hive. The foragers with pollen load were suck in using a pooter and transferred into an Eppendorf tube and brought to the laboratory at UMT. The pollen load were removed from the corbicula of the bees and transferred into a vials containing distilled water. The pollen was diluted in the vials and a drop of pollen suspension was deposited into a microscopy slide and a cover slip was gently pressed onto the solution. The prepared slides were observe under 40× and 100× magnification using optical microscope Leica DM750.

RESULTS AND DISCUSSION

Palynology is a study of pollen which an analysis regarding pollen gathered by the stingless bee which very important in apiculture industry. Stingless bee including honey, propolis and the beebread are highly potential to be commercialized as honeybee products (Azmi *et al.,* 2015). Stingless bee is not only the important pollinators in open field and greenhouse crops (Azmi *et al*., 2017), but also one of an important pollinator in tropical rainforest (Macharia *et al.,* 2007). Bees will collect pollen grains from plants groups, which are specific to each species. The pollen collection of *H. itama* in coastal area showed that *Portula grandiflora* is the most dominant pollen sample (Figure 1). Meanwhile, *Antigonan leptopus, Amaranthus tricolor, Hibiscus rosa-sinensis, Cucumis melo, Ixora coccinea, Tridax procumbens, Biden pilosa, Turnera subulata* and *Ixora javanica* were the other pollen types that have been collected by *H. itama* in coastal area. The morphology of the identified pollen are presented in Figure 2.

Unknown pollen A, B, C and D (Fig. 3) are the pollen types that still not being identified. The analysis of pollen was expressed as the most dominant pollen in the sample either in honey, beebread or pollen obtained from the pollen basket of the stingless bee (Louveaux *et al.,* 1978). *Portula grandiflora* is a perennial plant that takes longer period to produce their first flowers. However, once this plant start flowering, it can be last throughout the year. In addition *Portula grandiflora* is drought and heat tolerant plants that require very little maintenance. The availability of this plant as *H. itama* food source throughout the year made this plant becoming *H. itama*'s favourites in coastal area.

Within nine months of pollen collection, *H. itama* has collected pollen from 15 plant species in the forest area. It has been documented that the stingless bee including *H. itama* plays a vital role as effective pollinator of many wild plant species (Slaa *et al.,* 2006). Of the 15 types of pollen

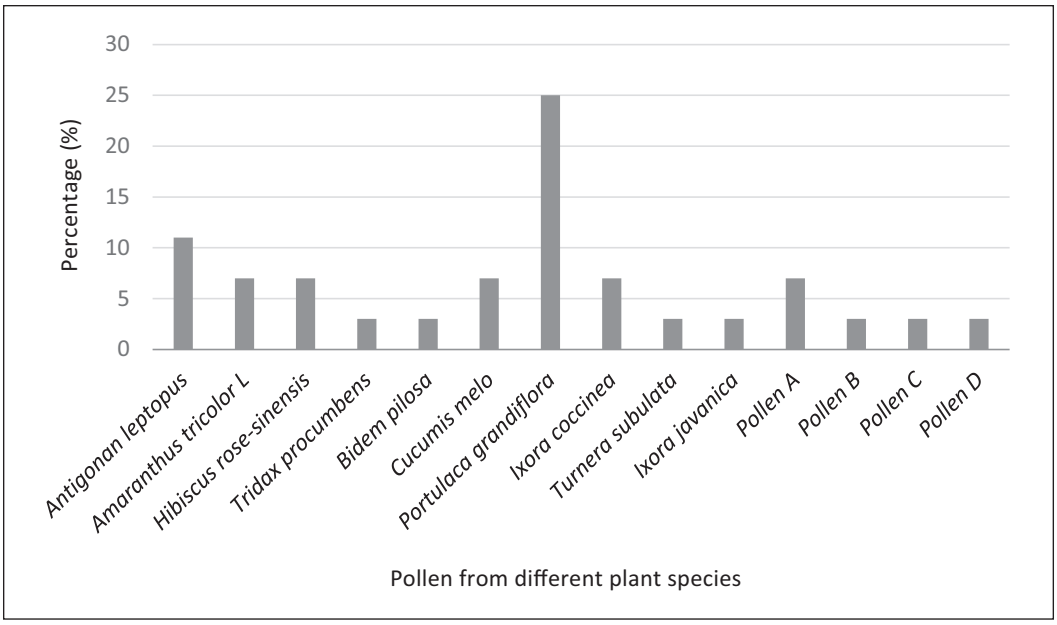

Fig. 1. Percentage of pollen based on plant species foraged by *Heterotrigona itama* in coastal area sampled from June 2016 until February 2017.

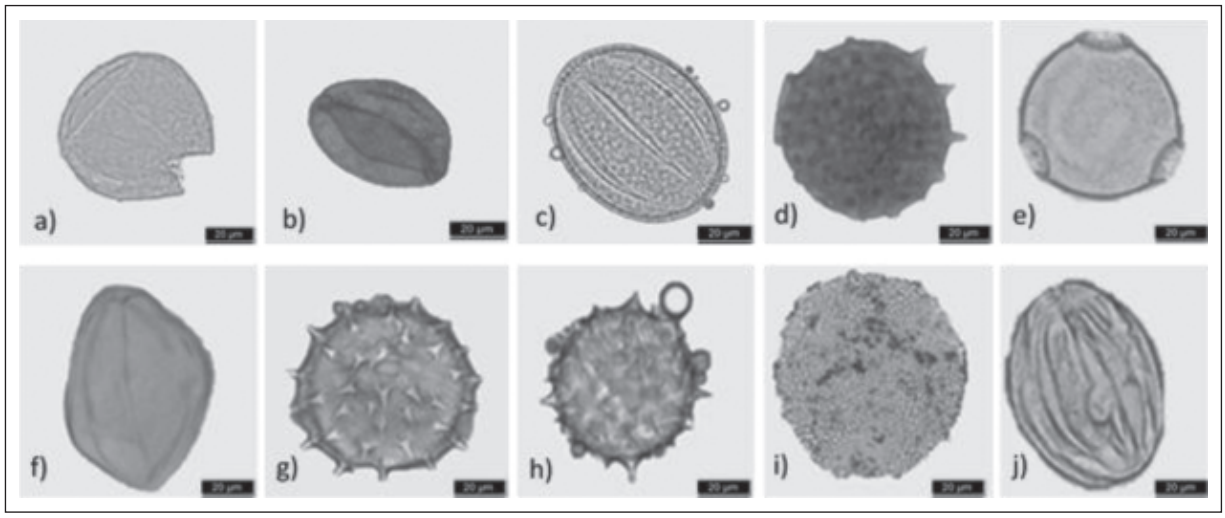

Fig. 2. Light micrograph of pollen foraged by *Heterotrigona itama* from different plant species sampled in coastal area from June 2016 until February 2017; (a) *Portula grandiflora,* (b) *Antigonan leptopus,* (c) *Amaranthus tricolor L.,* (d) *Hibiscus rosa-sinensis,* (e) *Cucumis melo,* (f) *Ixora coccinea,* (g) *Tridax procumbens,* (h) *Biden pilosa,* (i) *Turnera subulata,* and (j) *Ixora javanica.*

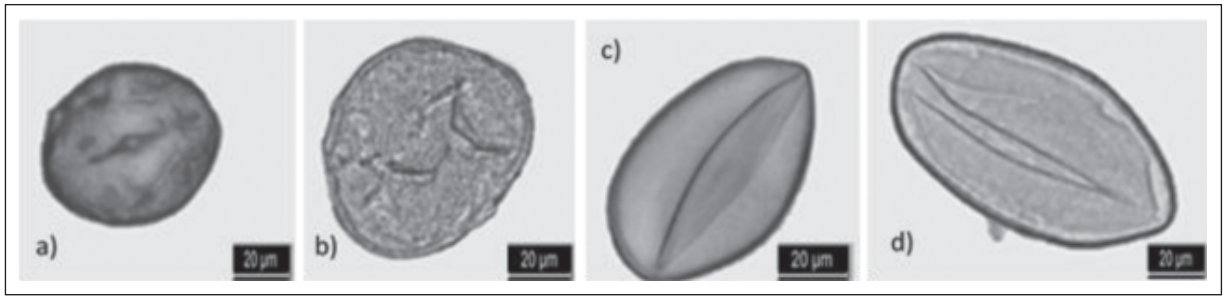

Fig. 3. Light micrograph of pollen foraged by *Heterotrigona itama* from unknown plant species sampled in coastal area from June 2016 until February 2017; a) Pollen A, b) Pollen B, c) Pollen C, and d) Pollen D.

collected, only seven pollens have been identified while another eight type of pollen still unknown. Figure 4 showed that the highest pollen distribution was from *Asytasia gangetica* (18%) followed by *Biden pilosa* and *Antigonan leptopus* with 14% each. *Reullia brittonia* has 7% of pollen distribution, meanwhile *Amaranthus tricolor, Hibiscus sabdariffa* and *Portulaca grandiflora* all has 3% of pollen distribution. The morphology of the identified pollen is presented in Figure 5.

Asytasia gangetica is categorized as shrub plant and commonly found growing as weed anywhere in Malaysia. *Asytasia gangetica* easy to breed and can stand in any weather condition. It is tolerant of many soil types, but optimal growth occurs in well drained, but moist soils that are rich in compost. However, there were also several pollen types that has not been identified in this study (Fig. 6).

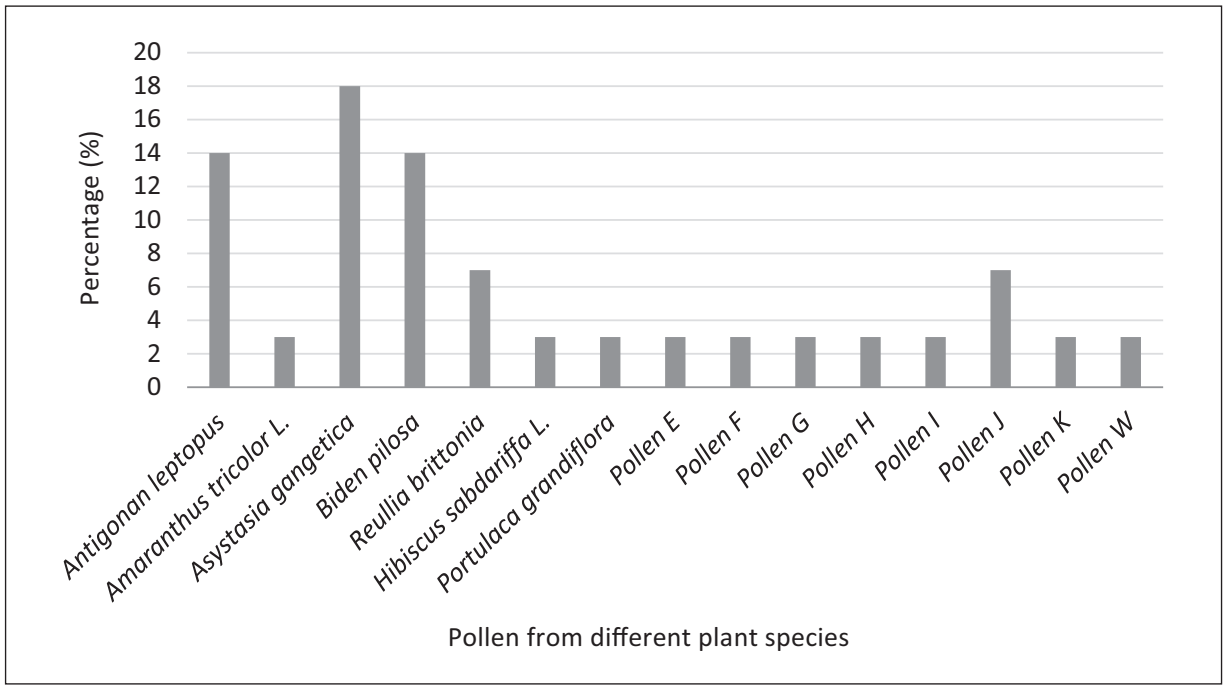

Fig. 4. Percentage of pollen based on plant species foraged by *Heterotrigona itama* in forest area sampled from June 2016 until February 2017.

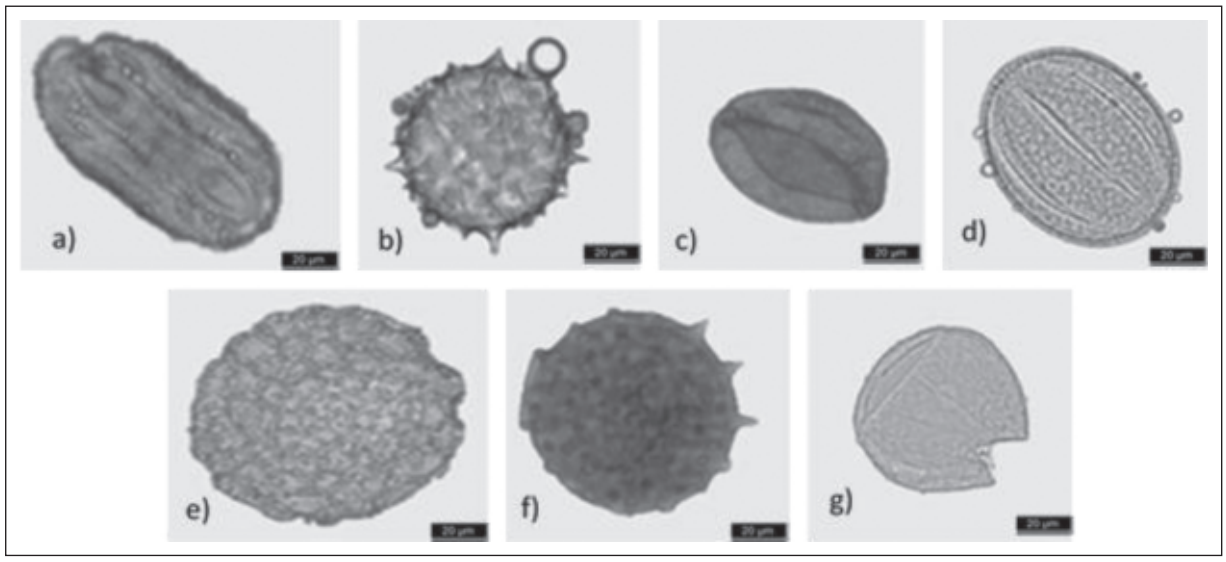

Fig. 5. Light micrograph of pollen foraged by *Heterotrigona itama* from different plant species sampled in forest area from June 2016 until February 2017; (a) *Asytasia gangetica,* (b) *Biden pilosa,* (c) *Antigonan leptopus,* (d) *Amaranthus tricolor,* (e) *Reullia brittonia,* (f) *Hibiscus sabdariffa,* and (g) *Portulaca grandiflora.*

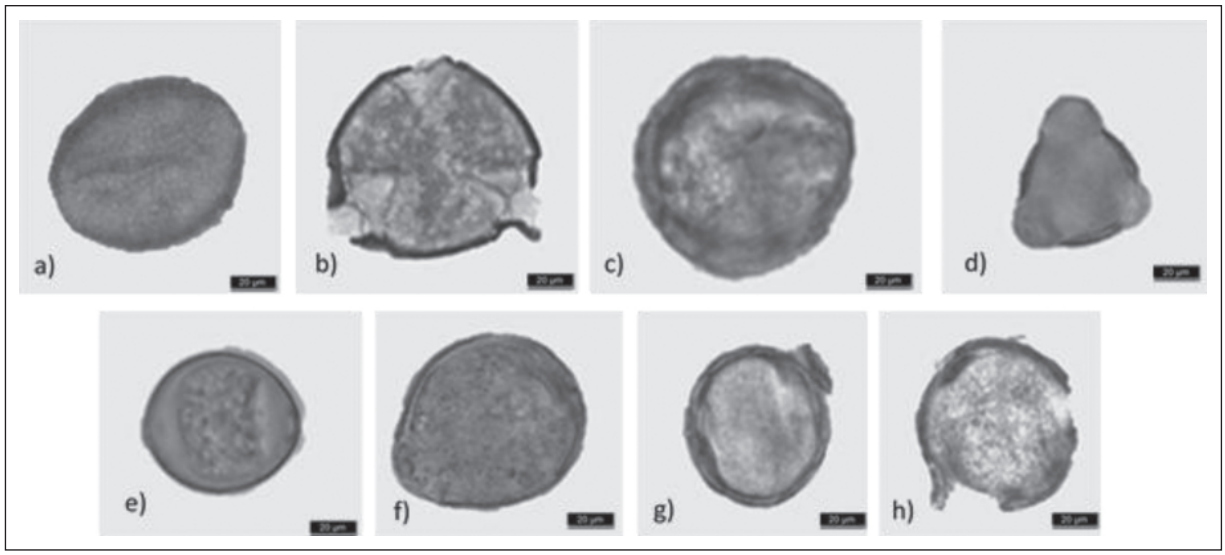

Fig. 6. Light micrograph of pollen foraged by *Heterotrigona itama* from unknown plant species sampled in forest area from June 2016 until February 2017; (a) Pollen E, (b) Pollen F, (c) Pollen G, (d) Pollen H, (e) Pollen I, (f) Pollen J, (g) Pollen K, and (h) Pollen W.

CONCLUSION

This experiment has gathered the pollen identity and composition in coastal and forest rearing area. The pollen composition foraged by *Heterotrigona itama* in coastal and forested area is dependant on the floral availability and the climate change. *Portulaca grandiflora* and *Asytasia gangetica* have shown the highest composition due to their availability throughout the year. The information obtained from this study will help to construct the pollen calendar, which could contribute in planning the suitable floral source for meliponiculture in Malaysia.

ACKNOWLEDGEMENTS

The authors wish to thank Terengganu Agriculture Department and Sekayu Agriculture Park for giving their full support during the collection of pollen samples. The technical assistance from Histocenter (M) Sdn. Bhd personnel in getting great microscopic image of pollens using LEICA Stereo and optical microscope is also appreciated. The research is supported by Universiti Malaysia Terengganu and Ministry of High Education through Niche Research Grant Scheme (NRGS Vot 53131).

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