Morphological, Nest Architecture and Colony Characteristics of Stingless Bees (Hymenoptera; Apidae; Meliponini) from Tasik Kenyir, Terengganu

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Abstract The objectives of this study were to investigate the morphological characteristics, nest architecture and colony characteristics of two common stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica* from Taman Tropika Kenyir, Tasik Kenyir, Hulu Terengganu. Variations were found in terms of the colours, length and width of the body parts. All the measurements of the morphological characters in *G. thoracica* were significantly greater than *H. itama* ($p < 0.05$). The average body size of *H. itama* was 4.7 ± 1.55 mm whilst *G. thoracica* was 7.44 ± 2.05 mm (N = 50). *Heterotrigona itama* are black in colour with grey wings while on the contrary, *G. thoracica* are brown in colour with dark brown wings and white tips at the apex of the wings. Both species have corbiculated hind legs fringed with sensilla, ten flagellum antennae segments, six abdomen segments and three ocelli. Three nest cavities of both species were also measured and observed to investigate the nest architecture between the two species. The nest measurements showed that *G. thoracica* nest was significantly larger compared to *H. itama* ($p < 0.05$). Both of the nests also have different unique characteristics from each other in terms of the colour, shape and smell. Both of the colonies shared similar behaviours especially on their foraging, dumping and nesting activities. Nevertheless, the defense mechanism between the two species was totally different from each other where *H. itama* colonies were observed to be more aggressive compared to *G. thoracica* colonies.

Keywords Stingless bee · Morphological characteristics · Nest architecture · Colony behaviour · Tasik Kenyir

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Introduction

Stingless bees (Hymenoptera: Apidae: Meliponini) are closely related to the common honey bees and are widely distributed in tropical and subtropical regions (Salim et al. 2012). There are about 700 species have been recorded worldwide and stingless bees are the smallest of the honey producing bees (Heard 1999). They are highly social insects that living in permanent colonies, nesting in old walls, logs, crevices and such other concealed places. The sting of stingless bee is greatly reduced without an effective tip. Hence, the defense behaviour is by chasing the intruders and enemies by biting, becoming entangled in the intruder's hairs and getting into the nose, ears and the eyes (Danaraddi 2007).

Malaysia is home to diverse species of stingless bees which consists of ~33 species (Mohd Norowi et al. 2008). Stingless bees are native to Malaysia, thus they are resistant to the diseases and parasites of European honey bees, *Apis mellifera* (Delfinado et al. 1989). Besides, stingless bees are considered domesticated species because they are being reared for their products such as honey, propolis, bee bread (which is the pollen and other ingredients stored in the brood cells) and bee wax.

Stingless bees are known to be important pollinators in tropical rainforest (Eltz et al. 2003) and also good candidates for providing pollination services in agricultural ecosystem such as starfruits, mango, durian, watermelon, guava and coconut (Slaa et al. 2006). The value of insect pollination service of these crops was estimated more than USD 19 million (Heard 1999). At least four species of stingless bees, *H. itama, G. thoracica, Tetragonilla atripes* and *Tetrigona peninsularis* can be potentially domesticated for pollination service in Malaysia agricultural ecosystem. Their small size allows them to have access to many kinds of flowers whose openings are too narrow to permit penetration by other bees and they are common visitors to flowering plants in the tropics (Heard 1999). Other than pollination, stingless bees also play important roles in health care through the production of medicinal hive products such as propolis, beebread, and also honey which is believed to have higher nutrients content compared to *Apis mellifera* (Kwapong et al. 2010).

To date, there is limited collection of voucher specimens for taxonomic purposes on the stingless bees from Tasik Kenyir, Terengganu. Descriptions of most of the known species of stingless bee from Peninsular Malaysia and Borneo, including collections from earlier work carried out in mid 1950s can be found in Schwarz (1939) and Eltz et al. (2003). Therefore, it is important to stress that the taxonomic work on Tasik Kenyir stingless bees is in exhaustive and particular attention must be given, now that the taxa are considered for its economic and health properties. Even though considerable studies have been conducted on the use of stingless bees for commercial pollination purposes, however to date, very little attention has been made to study the colony and morphological characteristics, and the nest architecture of Malaysian stingless bees. Outcome of this study is hoped would give an insight on their biology, ecology and behaviour which are absolutely essential for the sustainability of meliponiniculture industry in Malaysia.

Morphological Characteristics

The most common stingless bee species in Tasik Kenyir, *H. itama* and *G. thoracica* were collected from Taman Tropika Kenyir (TTK), Pulau Tekak Besar, Tasik Kenyir, Terengganu (5°02′ N, 102°44′E). Samplings of the stingless bees were carried out from August until October 2013 (N = 50). Twenty five individuals of each species (*H. itama* and *G.thoracica*) were selected for the morphological characteristics study. The external morphological characters between the species such as the length and width of the head, thorax, abdomen, fore wing, hind wing, and leg parts were measured using the digital vernier callipers (mm). Images and measurement for each morphological character were captured using Dino-Eye microscope camera. Morphological characteristics such as the colour of the wing and body were also observed.

In this study, the leg part of the stingless bees is divided into coxa, trochanter, femur, tibia, and tarsus. The front leg of both species *G. thoracica* and *H. itama* have broader tibia which is subclavate compared to the tibia on the hind leg (Fig. 1). The front and hind legs of *G. thoracica* are almost similar with *H. itama* except that the legs of *G. thoracica* (7.04 ± 0.09 mm) are longer than *H. itama* (4.25 ± 0.05 mm). Both of the species leg parts are covered with sensillum, a sense organ in insects, typically consisting of a receptor organ in the integument connected to sensory neurons. Stingless bees are depending on the sensilla for choosing profitable food source, resins, water sources, and also for nestmate recognition (de Brito Sanchez 2011). Another unique feature on the hind leg part is the pollen basket (corbiculum). The pollen basket is formed by the outer and inner rows of long, curved hairs. The pollen basket is used to collect and also to carry pollens. Pollen grains, which are collected between the dense hairs on the body of the worker, are removed and compressed into pellet form; the pellet is placed into the pollen basket which is located

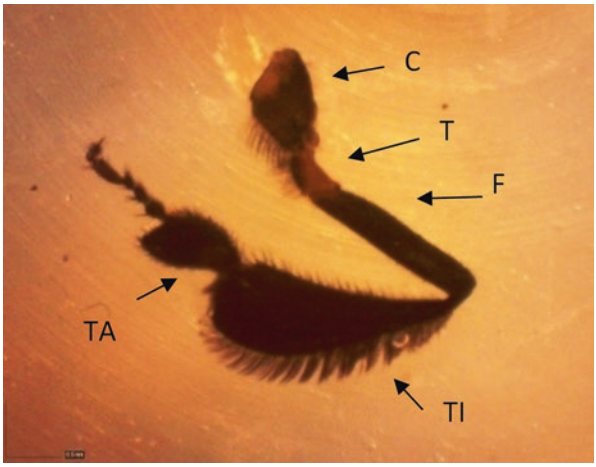

Fig. 1 The front leg of *G. thoracica* which are divided into coxa, trochanter, femur, tibia and tarsus at 71.5× magnifications. *C* Coxa, *T* Trochanter, *F* Femur, *TI* Tibia, *TA* Tarsus

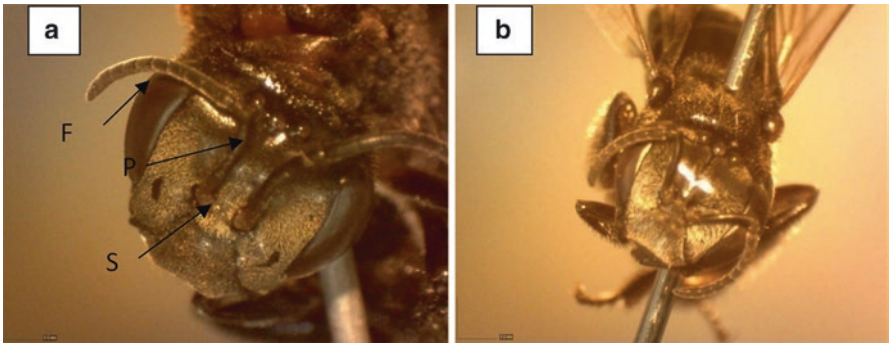

Fig. 2 The antennae part of *G. thoracica* (**a**) which are divided into scape, pedicel, and flagellum and *H. itama* (**b**) geniculate antenna with ten flagellum segments at 71.5× magnifications. *S* Scape, *P* Pedicel, *F* Flagellum

on the outer face of each hind leg and once the baskets are fully loaded, the worker returns to the hive to store the pollen for later use (Arita et al. 1989).

The antennae are paired segmented appendages located on the head usually between or below the compound eyes. The antennae arise from the antennae sclerite in front of the anterior margin of the compound eye at the frontal sutures. The three basic segments of the stingless bee antenna are the scape (base), the pedicel (stem), and finally the flagellum (Fig. 2a). The antennae of *G. thoracica* and *H. itama* shared similar shape and number of flagellum segments (ten segments). The antennae of both species are geniculate in which they are hinged or bent like an elbow (Fig. 2b). Apart from that, similarly to the leg part, gustatory sensilla are also abundant on the antennae which act as a sensory organ (de Brito Sanchez 2011).

Geniotrigona thoracica and *H. itama* have two pairs of wings which are the fore wings and hind wings that are attached on the mesothorax and metathorax respectively (Fig. 3a, b). The forewings are relatively larger and broader compared to the hind wing. The fore wing is leathery and it is used to protect the hind wing whilst the hind wing is thinner and more papery. The wings of *G. thoracica* are different from the *H. itama* in terms of colour and size. *Geniotrigona thoracica* wing is light brown in colour with white tips in the end of it while on the contrary, *H. itama*'swing is dark grey in colour. The wing size of *G. thoracica* is also larger (7.34 ± 0.25 mm) than *H. itama* (4.24 ± 1.03 mm). However, the hind wings of both species have a unique characteristic for wings adaptation and modification which is called hamuli. Hamuli are the tiny hooks that hold the front and the hind wings together and there are eight tiny hooks altogether attached on the hind wings of both species (Fig. 3c). Besides that, the wings also have tiny sensory hairs attached on the surface.

Abdomen is the third functional region or tagma of the bee's body; the abdomen is located just behind the thorax (Fig. 4a, b). Thorax is the second (middle) tagma of

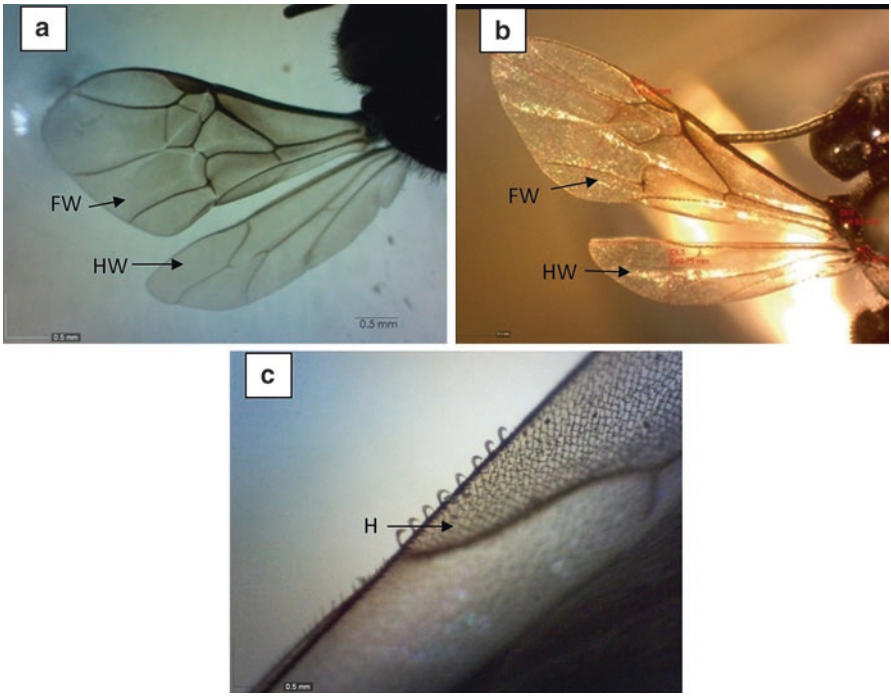

Fig. 3 The forewing and hind wing of *H. itama* (**a**) and *G. thoracica* (**b**) at 71.5× magnifications. The hamuli (**c**) on the hind wing which attach the forewing and the hind wing together at 82.9× magnifications. *FW* Forewing, *HW* Hind wing, *H* Hamuli

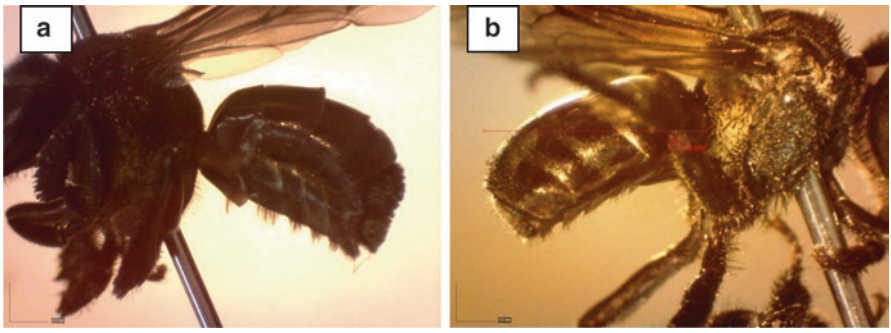

Fig. 4 The abdomen of *G. thoracica* (**a**) and *H. itama* (**b**) at 71.5× magnifications

an insect's body. This region is almost exclusively adapted for locomotion where it contains three pairs of walking legs and two pairs of wings. Structurally, the thorax is composed of three body segments which are the prothorax, mesothorax, and metathorax.

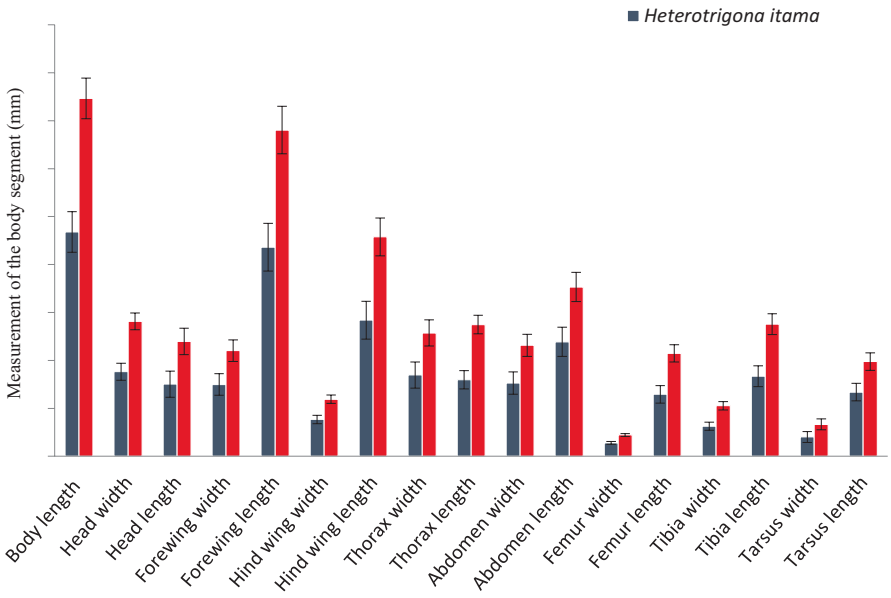

Fig. 5 Mean and standard deviation of *H. itama* and *G. thoracica* body segments' measurements (N = 50)

The junction between the thorax and abdomen is petiolate giving the appearance of a "wasp-waist" (Meyer 2005). Each segment of the abdomen consists of a dorsal sclerite, the tergum and a ventral sclerite, the sternum, joined to one another laterally by a pleural membrane. The thorax and abdomen of *G. thoracica* is brownish in colour compared to *H. itama* which has black body. However, the thorax and abdomen surface of both species are covered with sensilla. Uniquely, the sensilla are longer, more plumose and rough on the lower abdomen area compared to the upper part of the abdomen where the sensilla are much shorter and softer.

Figure 5 shows the measurements for 25 individuals of *H. itama* and *G. thoracica* that were collected from Taman Tropika Kenyir. The length and width of the body, legs, thorax, abdomen, head and wings of *G. thoracica* are relatively larger than *H. itama.* The t-test analysis revealed that all of these morphological measurements are significantly different between the two species ($p < 0.05$). This is supported by Smith (2012), where he reported that *G. thoracica* is larger in size compared to *H. itama* and probably the largest species in Asia.

Nest Architecture and Colony Characteristics

Three nests' cavities of each species were opened and observed to compare the nest architecture between the two species. The length and width of the nests were taken using the electronic vernier calipers. Other than that, shape, colour, scent of nests

and location of the brood cells, entrance tube, honey and pollen pots were also measured and described. The behaviours of the stingless bees were observed without influencing the bees for about 72 h at the same time from 0800 to 1800 h. The behaviours observed include of (i) foraging activity (the act of searching for food and provisions); (ii) defense mechanism (observed by opening and disturbing their nest by inserting small stick in the entrance); (iii) territory and guarding; and (iv) nesting and dumping activities.

Generally, the length and width of entrance tube, honey pots, pollen pots and brood cells of of *G. thoracica* nest is larger than *H. itama* (Table 1). The nests of stingless bees are usually consist of an external tube, internal tunnel, resin dump, waste dumps, and food pots for storing pollen and honey. Brood cells, honey and pollen pots are arranged in separate clusters. Brood cells and food pots are made of cerumen which is a mixture of wax and resin. The cells of combs will be in contact with one another, sometimes connected by small pillars or connectives of soft cerumen (Danaraddi 2007).

The characteristics of the nests between the two species are also quite different from each other (Table 2). The colour and surface texture of the entrance tube of *G. thoracica* is dark brown in colour and has a very coarse and sticky surface compared

Table 1 The mean and standard deviation of the nest characteristics from both species *H. itama* and *G. thoracica* recorded from Tasik Kenyir

No	Nest parameters	Mean ± standard error mean (mm)	
		Heterotrigona itama	*Geniotrigona thoracica*
1	Entrance tube length*	114.817 ± 50.378	41.591 ± 2.364
2	Entrance tube width*	15.807 ± 3.566	28.060 ± 1.797
3	Honey/pollen pots length	20.364 ± 2.511	25.920 ± 4.576
4	Honey/pollen pots width*	14.023 ± 0.740	21.517 ± 2.541
5	Brood cells length	4.663 ± 0.133	5.753 ± 0.901
6	Brood cells width	3.303 ± 0.133	4.010 ± 0.424

* = $p < 0.05$

Table 2 The characteristics of the nest entrance tube, honey/pollen pots, and also the brood cells of *H. itama* and *G. thoracica*

No	Nest parameters	*Heterotrigona itama*	*Geniotrigona thoracica*
1	Entrance tube shape	Cylindrical/tube-like	Cylindrical/tube-like
2	Entrance tube colour	Light brown	Dark brown
3	Entrance tube surface	Very soft and brittle	Very coarse and sticky
4	Honey/pollen pots shape	Oval/capsule-like	Oval/capsule-like
5	Honey/pollen pots colour	Dark brown	Dark brown
6	Brood cells shape	Round/bead- like shape	Round/bead-like shape
7	Brood cells colour	Yellow	Yellow
8	Other characteristics	Have no sweet scent	Have strong sweet scent

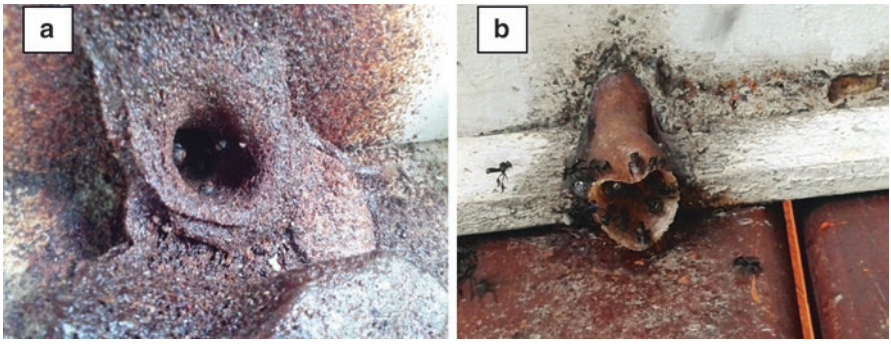

Fig. 6 The nest entrance tube of *G. thoracica* (**a**) which is larger and coarser than *H. itama* (**b**)

to *H. itama* which is more smooth, brittle, and lighter in colour (Fig. 6a, b). The nest of *G. thoracica* also has a very strong sweet smell (vanilla-like smell) which is obtained from the propolis and resins. Nevertheless, although the nest of *H. itama* also has propolis attached to it, the nest is somehow do not smell sweet at all. Despite the differences, they also shared some similarities especially in terms of the shape and colour of the brood cells and honey or pollen pots as well as the entrance tube shape (Fig. 7a, b).

The colony characteristics and behaviour of each species such as defense mechanisms, nesting and dumping activities, and their foraging behaviour were observed. In this study, the defense mechanism between the two species is very different from each other. The guard bees of *G. thoracica* were very timid and passive. They were withdrawing when a small stick was inserted in the entrance. They also did not crawl in hairs or clothes and they did not bite at all even when the nest was opened. They stayed calm for the whole day from early morning to late evening. On the contrary, *H. itama* were very active and the guard bees were usually guarding in the opening. They deposited a lot of sticky resin droplets around the inner and outer sides of the opening when a small stick was inserted. Numerous landed on hairs and skin and they performed disturbing biting when the nest was opened. As for the nesting and dumping activities, the workers from both species carried waste pellets (small leaves/foreign materials) outside of the nest. As a result, a pile of waste dump was found in front of the nest entrance. Some workers of *G. thoracica* were found cooling their nest by flapping their wings vigorously which was to improve their nest ventilation.

Nests ‘breathe’ in the sense that tidal gas exchange occurs frequently, although the entrance tube is the only connection to outside (Roubik 2006). Circulation is accomplished by workers that fan their wings while facing outward toward the entrance. This behavior is called ‘nest-cooling’ where the stingless bees cool down their nest by wing fanning. There was no ‘nest-cooling’ activity found within

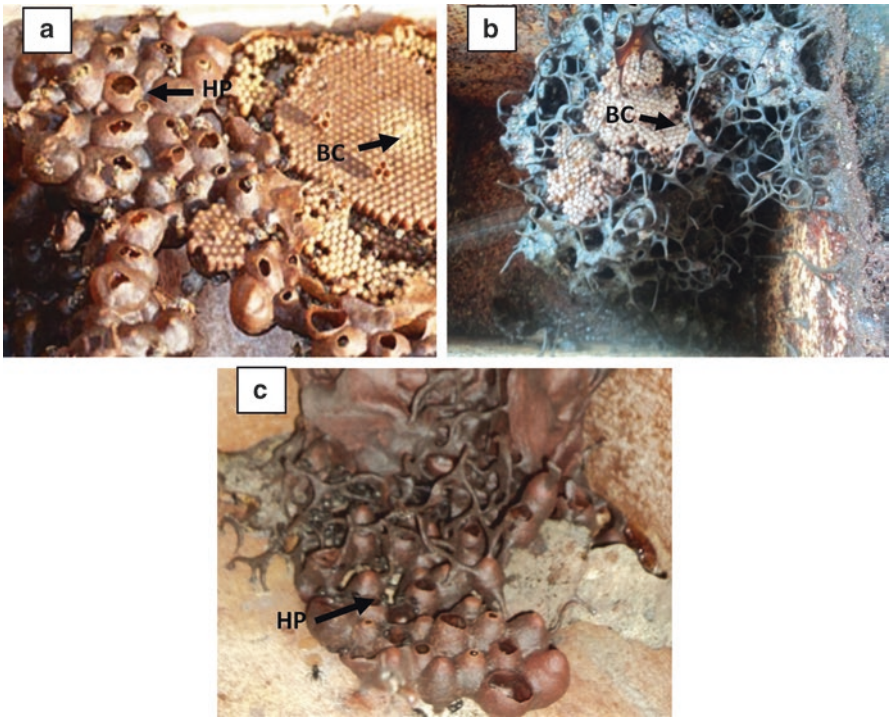

Fig. 7 The nest brood cells and honey pots of *G. thoracica* (**a**) and also the brood cells (**b**) and honey pots (**c**) of *H. itama*. *HP* Honey pots, *BC* Brood cell

H. itama colonies probably because their nest are still small. Both species were actively nesting in the morning and decrease gradually in time in which they were more relaxed in the evening. Both species were very active during morning and early afternoon (0800–1200) in which they were actively foraging. They were seen carrying pollens and propolis inside and outside of the nest. They also could be seen perching on flowers during that time range (Fig. 8). However, both species were a lot calmer in the late afternoon and evening (1300–1800) in which the number of workers carrying pollens and propolis were declining. In the late evening, very few bees were spotted foraging and most of them were resting inside their nest.

The stingless bees communicate locations of forage sources by secreting chemical scents (pheromones) and through the use of the sun's direction (Kwapong et al. 2010). The bees were not foraging during the late evening where the sun is down. Workers begin foraging activities as early as dawn and end by dusk depending upon weather conditions and availability of flower.

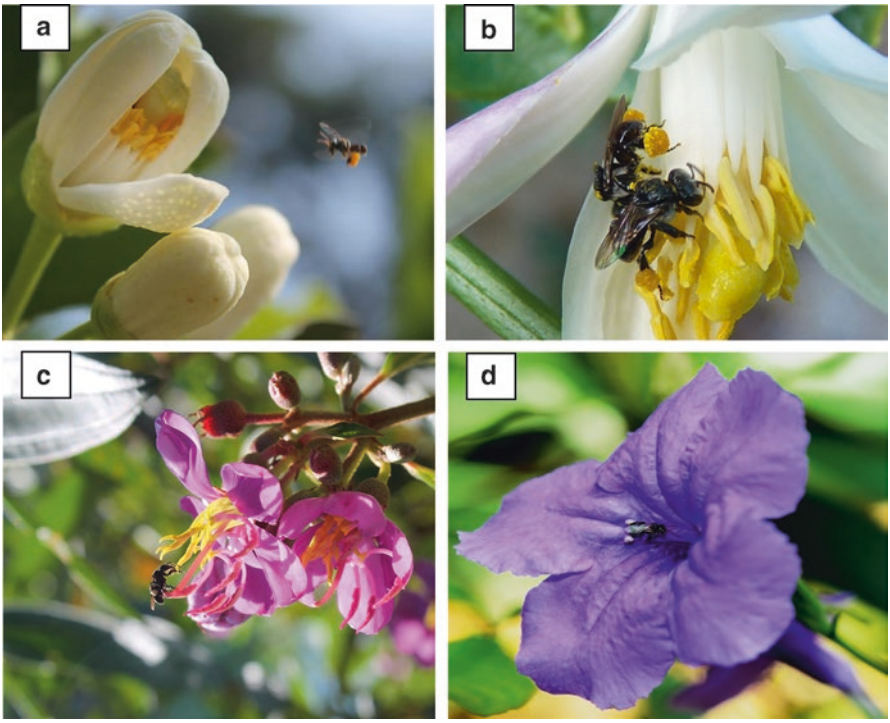

Fig. 8 *G. thoracica* (**a**) and *H.itama* (**b**–**d**) were spotted perching on flowers carrying along pollens on their legs in the morning and early afternoon

Conclusion

Heterotrigona itama and *G. thoracica* can be distinguished by the size and also the colour of their body. In general, *H. itama* are black in colour with grey wings and also known as the black jet species whilst *G. thoracica* has brown body and dark brown wings with white tips in the end. *Heterotrigona itama* is relatively larger in size compared to *G. thoracica*. Both species has corbiculated hind legs fringed with sensilla. They also have ten flagellum segments of antennae, six abdomen segments and three ocelli. The nests of *G. thoracica* are larger in size compared to *H. itama*. The nests of both species have different unique characteristics from each other in terms of colour, shape, nest surface, and also smell. Nevertheless, they also share two similarities in terms of the brood cells shape (bead-like shape) and colour (yellow) and also the honey and pollen pots which are capsule-like and dark brown in colour. As for the colony characteristics, both of the colonies were similar in terms on their foraging behaviour, dumping and nesting activities. However, it seemed that the defense mechanisms between the two species were very different from each other in which *H. itama* colonies were observed to be more aggressive when

compared to *G. thoracica* colonies. For the best utilization of the stingless bees, research on their diversity, biology, ecology and behaviour is absolutely essential. Therefore, through this study, we hope to establish a baseline of the indigenous stingless bees in promoting Tasik Kenyir as a potential area of sustainable apiculture industry particularly in Terengganu state.

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References

Arita, H. L., Sakai, W. S., & Moniz, J. J. (1989). Pollen types collected by honey bees at three localities on the Island Hawaii. Part I: Flora. *Source Identification, 29*, 125–132.

Danaraddi, C. S. (2007). Studies on stingless bee, *Trigona iridipennis* Smith with special reference to foraging behaviour and melissopalynology at Dharwad, Karnataka. (M.Sc. dissertation). University of Agricultural Sciences, Dharwad, India.

de Brito Sanchez, M. G. (2011). Taste perception in honey bees. *Chemical Senses, 36*(8), 675–692.

Delfinado, B. M., Baker, E. W., & Phoon, A. C. G. (1989). Mites (Acari) associated with bees (Apidae) in Asia, with description of a new species. *Annual Bee Journal, 129*(9), 609–613.

Eltz, T., Brühl, C. A., Imiyabir, Z., & Linsenmair, K. E. (2003). Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland dipterocarp forests in Sabah, Malaysia, with implications for forest management. *Forest Ecology and Management, 172*, 301–313.

Heard, T. A. (1999). The role of stingless bees in crop pollination. *Annual Review of Entomology, 44*, 183–206.

Kwapong, P., Aidoo, K., Combey, R., & Karikari, A. (2010). *Stingless bees: Importance, management, and utilisation. A training manual for stingless beekeeping*. Ghana: Unimax Macmillan Ltd.

Meyer, J. R. (2005). External anatomy: The abdomen, general entomology. Retrieve from https://projects.ncsu.edu/cals/course/ent425/library/tutorials/external_anatomy/abdomen.html.

Mohd Norowi, H., Sajap, A. S., Rosliza, J., Mohd Fahimie, J., & Suri, R. (2008). *Conservation and sustainable utilization of stingless bees for pollination services in agricultural ecosystems in Malaysia* (p. 5). Kuala Lumpur: Department of Agriculture.

Roubik, D. W. (2006). Stingless bee nesting biology. *Apidologie, 37*(2), 124–143.

Salim, H. M. W., Ahmad, D. D., Harrison, R. D., Fletcher, C., Kassim, A. R., & Potts, M. D. (2012). Stingless bee (Hymenoptera: Apidae: Meliponini) diversity in dipterocarp forest reserves in peninsular Malaysia. *The Raffles Bulletin of Zoology, 60*(1), 213–219.

Schwarz, H. F. (1939). The Indo-Malayan species of *Trigona*. *Bulletin of the American Museum of Natural History, 76*, 83–141.

Slaa, E. J., Chaves, L. A. S., Malagodi-Braga, K. S., & Hofstede, F. E. (2006). Stingless bees in applied pollination: Practice and perspectives. *Apidologie, 37*(2), 293–315.

Smith, D. R. (2012). *Key to workers of Indo-Malayan stingless bees* [Powerpoint slides]. Retrieve from https://ses.library.edu.usa/bitstream/2123/111123/4/Poster122.pdf.