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Distribution and Morphometrical Variations of Stingless Bees (*Apidae: Meliponini*) In Urban and Forest Areas of Penang Island, Malaysia

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Abstract

Stingless bees from four different places in Penang, northern peninsular Malaysia including Universiti Sains Malaysia campus(USM), Botanical Garden, Balik Pulau and Teluk Bahang were sampled randomly and identified. A total of six species of stingless bee were successfully recorded; *Heterotrigona itama*, *Lepidotrigona terminata*, *Tetrigona apicalis*, *Tetragonula iridipennis*, *Tetragonula laeviceps*, and *Tetragonula pagdeni*. *Heterotrigona itama* is the most abundance species in both urban and forest area. Their hive can be found in all study areas. The results showed that Balik Pulau (forest) had the most diverse stingless bee species (5 species) compared to other selected sites. Morphometrical studies comprising fifteen characters of *H.itama* were measured. There were significant difference for *H. itama* body length and appendages between samples collected from urban and forest areas. *H.itama* in forest areas have larger body size compared to those in the urban area. Overall, stingless bees in urban areas was less diverse compared to forest area.

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1. Introduction

Pollination is an important ecosystem service that effects the growth of flowers and fruits thus provide food resources for human and animals. Bees and many other animals are important pollinators which involved in this ecosystem services [1]. Pollination by bees plays a very important role to ensure the quality and quantity of seeds and fruits bear by the trees [2].

Stingless bees are different from honey bees but they play important roles to continue the pollination services in ecosystem. As an eusocial insects, they live together in cavities with a queen, workers and drones. Stingless bees are tropical insects that active all year round although in cold weather they are less active, with some species presenting diapause. Different species have different type of preferred habitats and climatic conditions [3]. About 800 species of stingless bees in entire tropical regions of the world were recorded, consisting of many ecologically habitats as dry savannah or cerrado to humid rainforest [4]. Their greatest abundance and diversity is in the Amazonian rain forest. Stingless bees do not sting but if their nests being threaten they will bite the enemy as a defence mechanism. Furthermore, some species have mandibular secretions that cause painful blisters [3]. Although stingless bees are lacking of sting, they may have very huge colonies. Huge colony size is necessary for bees' nest protection. Usually stingless bees love to hover around the entrance of the nest and are able to catch intruders in flight [5].

Stingless bees visit flowers of forest trees and crops as well as shrubs and herbs to collect nectar, pollen, wax, resins, oils and other plant substances [6]. By doing so, they indirectly transfer the pollen grains onto stigmas resulting in pollination of these plants which then lead to fertilization and eventually fruit and seed production. They also cause pollination result in high quality and quantity yields of fruits and seeds because of the frequency of their visits to the flowers and efficiency (ability to deposit pollen collected on body of bees on stigmatic heads) of pollinating [3].

Until now, most farmers use honeybees such as *Apis melifera* as pollinator in agricultural sites [16]. However, in recent years, the populations of *A. melifera* are decreasing, resulting in low yields of crops [7]. This problem occurred due to broad spectrum of insecticides usage that kill the colonies or pollute their hives [5].There are many natural enemies that consume the bees and too many logging activity that destroy the bee's habitat. Logging activities affect the bee as pollinators and resulting in decreasing of plant species and fruit yields in the forest. Therefore, stingless bee is the best alternative to be put into consideration to overcome the low number of *A. melifera*.

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The stingless bee is one of many valuable pollinators in natural habitats and sometimes for agricultural crops. Previous researches have shown that the richness and abundance of stingless bee may be related to various local forest conditions, overall habitat diversity, proximity of forest edges, or local disturbance history [9] [10] [11] [12] [13]. There are about nine species of stingless bee that are effective in pollinating. Other species may contribute to the pollination, but there is lack of knowledge to determine their overall importance or effectiveness. Stingless bee have been recorded from 20 crops plant in southeast Asia, but other evidence recommends that they do not have significant role because these plants are pollinated by other agents [14].

The continuously changes of the name of stingless bee make it harder to identify even for the expertise. The morphometric of stingless bees need to be determined because it is hard to identify a stingless bee without proper identification from their morphology. Lack of study on biology of stingless bee making the morphometric study of stingless bee harder if considering the evolution that always take place for certain type of insects which can also occur to stingless bee itself.

Thus the main objective of this study was to identify the diversity of stingless bees in urban and forest area around Penang state, peninsular Malaysia. In order to understand the variations that might occur among population of *H. itama* in urban and forest areas, the morphometrically study was conducted. *H. itama* was chosen for this study as this species is widely distributed in Penang.

2. Materials and Methods

2.1. Study Sites

The study was carried out at four different areas which characterized into urban and forest areas. The study areas selected were USM, Botanical Garden, Teluk Bahang and Balik Pulau all in Penang. Urban area is an area where there were high population density of human and various features such as buildings and houses. Forest was referred to an area with high density of trees or area with vegetation. Therefore, USM was categorized as urban area while Botanical Garden, Balik Pulau and Teluk Bahang were considered as forest areas except for Balik Pulau 3 and Teluk Bahang 1. Location of each sampling site was recorded using the Global Positioning System (GPS).

2.2. Sample collection and identification

Twenty stingless bees from each hive found in study areas were collected by using a sweep net. Sample of stingless bees from each hive was kept in separated universal bottles filled with 80% of alcohol for later identification. Stingless bees were identified until species level using published key from [15]. In addition, the height of the hive from the ground and its diameter were also been recorded (Table 1).

2.3. Morphometric Study

A sample of 20 individuals of *H.itama* was collected from all different areas. The length of the body, width of head including eye, width of thorax and width of abdomen were recorded. In addition, morphometric of body appendages such as the length of proboscis, length of forewing, length of femur, length of tibia, length of metatarsus, width of forewing, width of femur, width of tibia, width of metatarsus, inter ocellar distance and number of hamuli was also recorded. All morphometries data were recorded from each individuals using Image Analyser under Olympus Microscope.

2.4 Statistical Analysis

Means and standard deviation were calculated for the data of each species. The data was not normally distributed, thus non-parametric test (Kruskal-Wallis) was performed on morphometric data. All statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) software version 22.

3. Results and Discussion

There were seven hives found in urban area (USM, Balik Pulau 3 and Teluk Bahang 1) and the species were *H. itama, L.terminata* and *T. pagdeni* (Table 1). In USM, there were two species recorded: *H. itama* and *T. pagdeni*. In forest area, (Balik Pulau, Botanical Garden and Teluk Bahang), four species from eight hives were recorded and the species were *H. itama, L. terminata, T. laeviceps,* and *T. iridipennis*. In Balik Pulau, five species were recorded including *T. itama, L. terminata, T. apicalis, T. iridipennis* and *T. laeviceps*, while three species can be found in Teluk Bahang such as *T. pagdeni, T. iridipennis and T. laeviceps. H.itama, T. pagdeni* and *T. iridipennis* can be found both in urban and forest areas.

Table 2 shows the mean morphometrics of *H. itama*. The mean body length of *H. itama* collected from different places ranged between 4.96 ± 0.37 mm from urban area to 5.36 ± 0.06 mm in forest area. The width of the head including eyes ranged from 1.32 ± 0.07 mm (forest) to 2.34 ± 0.15 mm from urban area. Width of abdomen ranged from 1.97 ± 0.08 mm from urban to 1.06 mm in forest area. The highest mean length of proboscis was 0.90 ± 0.08 mm (forest) while the lowest was 0.32 ± 0.04 mm (urban). For width of forewing, the highest mean was 2.27 ± 0.04 mm while the lowest was 0.93 ± 0.10 mm. The Kruskal-Wallis test showed there is a significant difference between area for body length, $x2 = 21.8$, $\rho = 0.00$, width of abdomen $x2 = 9.94$, $\rho = 0.002$, length of proboscis , $x2 = 129.5$, $\rho = 0.00$ (Table 3).

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Table 1: Stingless bee collections at three different places around Penang Island.

Site	GPS coordinates	Place	Area	Hive height from ground (cm)	Hive diameter (cm)	Species
1	5°21'21.6"N 100°18'3.32"E	USM 1	Urban	266	4.7	*H. itama*
2	5°21'20.43"N 100°18'5.8"E	USM 2	Urban	232	3.5	*H. itama*
3	5°21'31.59"N 100°18'29.33"E	USM 3	Urban	184	4.4	*H. itama*
4	5°21'30.11"N 100°18'30.32"E	USM 4	Urban	222	1.1	*T. pagdeni*
5	5°21'40.24"N 100°18'17.98"E	USM 5	Urban	231	3.5	*H. itama*
6	5°26'23.8"N 100°17'07.3"E	Botanical Garden	Forest	243	3.4	*H. itama*
7	5°21'45.0"N 100°13'17.0"E	Balik Pulau 1	Forest	195	2.2	*L. terminata*
8	5°19'05.1"N 100°12'29.4"E	Balik Pulau 2	Forest	225	3.6	*H. itama*
9	5°20'58.8"N 100°13'56.7"E	Balik Pulau 3	Urban	257	3.7	*T. apicalis*
10	5°20'22.1"N 100°13'21.0"E	Balik Pulau 4	Forest	164	2.3	*T. iridipennis*
11	5°19'08.7"N 100°13'16.5"E	Balik Pulau 5	Forest	136	1.4	*T. laeviceps*
12	5°27'25.1"N 100°12'57.5"E	Teluk Bahang 1	Urban	98	2.3	*T. pagdeni*
13	5°26'55.5"N 100°12'57.7"E	Teluk Bahang 2	Forest	170	1.5	*T. laeviceps*
14	5°27'25.4"N 100°12'59.6"E	Teluk Bahang 3	Forest	160	2.1	*T. iridipennis*
15	5°26'55.2"N 100°12'57.6"E	Teluk Bahang 4	Forest	138	1.3	*T. laeviceps*

About 80% of flowering plants were pollinated by insect [1]. Stingless bee is considered as one of the important pollinators in peninsular Malaysia. Stingless bees collect nectar and pollen from wide variety of plants [6]. Four genera of tribe Meliponini found in this study were similar to the list of stingless bees reported from Kelantan [16].

Table 2: Morphometrics of the body of *H.itama* (mean ± SD) in urban and forest area in Penang Island.

No. of site	USM 2	USM 5	Botanical Garden	Balik Pulau 2
	Mean ± SD (mm)	Mean ± SD (mm)	Mean ± SD (mm)	Mean ± SD (mm)
BL	4.96 ± 0.37	2.54 ± 0.17	5.36 ± 0.06	5.34 ± 0.05
WoHE	2.34 ± 0.15	1.32 ± 0.07	2.22 ± 0.07	2.23 ± 0.06
WoThorax	2.00 ± 0.10	1.15 ± 0.07	2.04 ± 0.06	2.04 ± 0.06
WoAbd	1.92 ± 0.16	1.06 ± 0.06	1.96 ± 0.08	1.97 ± 0.08
LoP	0.86 ± 0.25	0.32 ± 0.04	0.88 ± 0.06	0.90 ± 0.08
FWL	5.63 ± 0.43	2.88 ± 0.03	5.87 ± 0.26	5.87 ± 0.26
FL	1.58 ± 0.14	0.81 ± 0.07	1.51 ± 0.03	1.49 ± 0.04
TL	2.19 ± 0.21	1.14 ± 0.08	2.26 ±0.06	2.26 ± 0.06
LoM	1.88 ± 0.17	0.85 ± 0.21	1.87 ± 0.04	1.86 ± 0.03
FWW	2.11 ± 0.20	0.93 ± 0.10	2.26 ± 0.04	2.27 ± 0.04
WoF	0.41 ± 0.03	0.19 ± 0.01	0.39 ± 0.03	0.40 ± 0.03
WoT	0.88 ± 0.06	0.39 ± 0.03	0.84 ± 0.03	0.84 ± 0.03
WoM	0.55 ± 0.05	0.16 ± 0.01	0.53 ± 0.03	0.53 ± 0.03
IOD	1.50 ± 0.11	0.80 ± 0.06	1.51 ± 0.02	1.52 ± 0.02
NoH	7.00 ± 0.00	5.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00

***Notes: BL (body length), WoHE (Width of head including eye), WoThorax (Width of thorax) and WoAbd (Width of abdomen), LoP (Length of proboscis), FWL (forewing length), FL (femur length), TL (tibia length), LoM (Length of metatarsus), FWW (forewing width), WoF (Width of Femur), WoT (Width of tibia), WoM (Width of metatarsus), IOD (Inter ocellar distance) and NoH (No. of hamuli).

From all listed species, *T. laeviceps* were only found in forest area. According to [17], T. laeviceps preferred *Hevea brasiliensis* or rubber tree but [18] stated that *T. laeviceps* was normally found in forest area. *H.itama, T. pagdeni* and *T. apicalis*, can be found in urban area while *L. terminata, T. iridipennis, H.itama* and *T. laeviceps* can be found in forest area. *Tetragonula pagdeni*, and *T. apicalis*, were only found in urban areas while *H. itama* can be found in both areas. Results from this study clearly shown that most of stingless bees favouring the forest area. This may due to a lot of flowering plant that favour these species. In urban area, usually the same type of flowering plants was planted along the roadside or near buildings as ornamental plants which probably less preferred by the stingless bees. Tree species such as *Swietenia macrophylla* and *Tabebuia pallida* with mistletoe shrubs (*Scurrula ferruginia* and *Dendrophthoe pentandra*) can be found commonly along roadside in Penang [19]. *H. itama* can be found in three different places therefore *H. itama* can be considered as the common species in either urban or forest area. Balik Pulau has the most diverse species of stingless bee because five different species were managed to be recorded in the area. Balik Pulau is located at southwest of Penang Island. Balik Pulau is a hilly area surrounded by four forest reserves (Bukit Genting Forest Reserve, Bukit Gemuruh Forest Reserve, Pantai Acheh Forest Reserve, Teluk Bahang Forest Reserve) makes this area was filled with various types of trees. Probably due to this, Balik Pulau recorded greatest stingless bee diversity compared to the other chosen sites with *Lepidotrigona terminata* was the less distributed species and only found in Balik Pulau. According to [11] and [12], distance to primary forest, proximity of forest edges and overall habitat diversity may be affecting the stingless bee richness and abundance. A study by [20] reported stingless bee were more common in primary and secondary forests than in disturbed sites. However, *H.itama* and *L. terminata* can be found in both urban and forest area. These two species probably do not have any preferred criteria where their hive will be built.

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Table 3: Morphometric measurements of *Heterotrigona itama* between study areas.

	Chi-Square	Asymp.sig.
BL	21.753	0.000*
WoHE	0.786	0.375
WoThorax	0.050	0.823
WoAbd	9.937	0.002*
LoP	129.503	0.000*
FWL	1.431	0.232
FL	0.522	0.470
TL	0.133	0.715
LoM	30.592	0.000*
FWW	10.770	0.001*
WoF	139.952	0.000*
WoT	96.527	0.000*
WoM	39.749	0.000*
IOD	44.637	0.000*
NoH	0.731	0.393

• significant at $p < 0.05$.

***Notes: BL (body length), WoHE (Width of head including eye), WoThorax (Width of thorax) and WoAbd (Width of abdomen), LoP (Length of proboscis), FWL (forewing length), FL (femur length), TL (tibia length), LoM (Length of metatarsus), FWW (forewing width), WoF (Width of Femur), WoT (Width of tibia), WoM (Width of metatarsus), IOD (Inter ocellar distance) and NoH (No. of hamuli).

Stingless bees in forest areas seem to have larger appendages size compared to those in the urban area. The possible reason of this phenomenon maybe related to the abundance of food sources which come from the various flowering plants and trees in forest area. Also, body size of *Trigona binghami* found in a forest in Thailand [15] was recorded bigger than in urban area. In the same way, [22] have reported that *Trigona iridipennis* from hilly zone in Karnataka, India were bigger than the urban area with mean body length of 5.05 mm. They attributed this variation to the availability of diverse floral resources in the area [22]. There is a distinct difference in morphometric of the *H.itama* individuals between USM2 and USM5 even though both are from urban area. *H.itama* hive at USM2 was located near bushy area (Lembah Burung) with many types of trees including fruit trees such as durians (*Durio zibethinus*) and rambutans (*Nephelium lappaceum*) while USM5 hive was found at Bungor tree (*Lagerstoemia spiciosa*) near School of Biological Sciences building. The differences of body size from the same species may due to the influence of food abundance [20] and food availability [22] [23]. Abundance of food sources affected the foraging distance of stingless bee and indirectly also affected the body size of stingless bee [21]. A stingless bee in forest area has slightly larger body and appendages in size because they don't need to travel further distance foraging for food compared in urban area. In addition, larger individuals can explore larger areas allowing to areas not used by other competitors [23]. Specimens collected for this paper only from small part of Penang island do not provide full geographical coverage for Penang or peninsular Malaysia and further studies are required.

4. Conclusion

In this study, a total of six species of stingless bees were recorded. The dominated species of stingless bee was *H. itama* due to their hive can be found almost in all places. The diversity of stingless bee was more diverse in forest area due to number of species of stingless bee found. Four species of stingless bee can be found in forest area including *H.itama*, *T. terminata*, *T. iridipennis* and *T. laeviceps a*nd the abundance of flowering plants and fruit trees probably attract more stingless bee species to come and accumulated in forest area. In urban area, three stingless bee species were found including *H.itama*, *T. pagdeni* and, *T. apicalis*. Furthermore, stingless bees, *H.itama* in forest areas have larger body size compared to those in the urban area based on the morphometric study.

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