

# CHARACTERIZATION OF NEST STRUCTURE AND FORAGING ACTIVITY OF STINGLESS BEE, *GENIOTRIGONA THORACICA* (HYMENOPETRA: APIDAE; MELIPONINI)

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## Abstract

Meliponiculture is the activity of stingless bee keeping, where the colony of stingless bees extracted from wild for the purpose of domestication. The study of stingless bee nest architecture of selected species brings benefit to this newly booming industry. *Geniotrigona thoracica*, is one of the largest stingless bee in Malaysia and has potential used in meliponiculture. The objectives of this study are to determine nest structure and growth, foraging activity and morphometry of worker bee. Colonies (n=3) of *G. thoracica* were used to study the nest structure and growth, foraging activity and morphometry of worker bee. Sampling was done for a period of one year from July 2013 until June 2014 for nest growth and foraging frequency. For morphometry study, worker bees were sampled from each nest. Result showed external nest structure formed in brown mount shape and the internal nest formed by three components, horizontal brood combs that surround with honey pots and pollen pots. The nest dynamics of *G. thoracica* changes according to the season. The peak time foraging frequency of *G. thoracica* was in between 1000 hrs until 1200 hrs. From morphometry study of worker bee, result for flight cost and aggressive cost were  $8.48 \pm 0.440$  and  $0.06 \pm 0.004$  respectively.

Keywords: Stingless bee, nest, structure, meliponiculture, *Geniotrigona*

## Abstrak

Meliponikultur adalah aktiviti menternak lebah kelulut, di mana koloni lebah kelulut diekstrak dari luar dan ditenak. Kajian mengenai sarang lebah kelulut daripada spesis yang terpilih memberi manfaat kepada industri yang baru berkembang. *Geniotrigona thoracica*, merupakan lebah kelulut terbesar di Malaysia dan mempunyai potensi untuk digunakan dalam meliponikultur. Objektif kajian ini adalah untuk mengenalpasti struktur sarang dan tumbesaran sarang, aktiviti keluar masuk dan masa aktiviti keluar masuk lebah kelulut. Koloni (n=3) *G. thoracica* digunakan dalam kajian struktur sarang dan tumbesaran, aktiviti lebah kelulut keluar dari sarang dan kajian morphometri lebah pekerja. Persempelan telah dilakukan pada Julai 2013 hingga Jun 2014 bagi tumbesaran sarang dan kekerapan lebah kelulut keluar dari sarang. Bagi kajian morphometri, sampel lebah pekerja diambil dari setiap sarang. Keputusan menunjukkan struktur luaran dan dalaman sarang serta tumbesaran sarang *G. thoracica* menunjukkan pertambahan luas saiz pada bahagian telur. Kekerapan maksimum kelulut keluar dari sarang adalah pada April hingga Julai dan waktu puncaknya dari 10 a.m sehingga 12p.m. Kajian morphometri lebah pekerja pula menunjukkan keputusan bagi kos penerbangan dan kos agresif adalah seperti berikut  $8.48 \pm 0.440$  dan  $0.06 \pm 0.004$ .

Kata kunci: Lebah kelulut, sarang, struktur, meliponikultur, *Geniotrigona*

## 1.0 INTRODUCTION

Stingless bees are social bees that lack a functional sting and belong to the tribe Meliponini [1]. They are able to produce honey by collecting and storing nectar in a honey pot [2]. This Malaysian stingless bee has potential for commercialization through

meliponiculture or commercial cultivation of stingless bee. Though there were many stingless bee species in Malaysia, not all species have economic value. In Australia, there are two species of stingless dominated in meliponiculture industry which are *Tetragonula carbonaria* and *Austroplebeia australis* [3]. Meanwhile, in Thailand there are four species of

economic value, which are *Trigona pegdeni* Schwarz, *Tetragonula laeviceps*, *Lepidotrigona terminata* Smith and *Lepidotrigona ventralis doipaensis* Schwarz, where these species of stingless bees used for pollination services [4]. Recent study showed that, there are five species of stingless bees have commercial value in Malaysia. They are *Geniotrigona thoracica* Smith, *Heterotrigona itama* Cockerell, *L. terminata* Smith, *Tetragonula fuscobalteata* Cameron and *T. laeviceps* [5]. In order to sustain the meliponiculture industry in Malaysia, research on their nest biology and behaviour of stingless bees were needed. There still lack of information regarding the nest structure of economic potential of stingless bee species in Malaysia.

Stingless bees live in perennial colonies, nesting in cavities within old walls, logs, crevices and such other concealed places [2,6]. Generally, the internal nest structure of stingless bee consist of different shapes and arrangements of brood cells and food storage containers, where honey and pollen are stored in separate pots while the ripened honey is stored in nest cavity together with the pollen, while some honey surround the brood area. Most Meliponini tribes arrange the cells in the nest in the horizontal comb and the cells in a cluster form [3]. Inside the nest, there is one laying queen, gynes (virgin queens), drones (males) and worker bees [4]. These gynes and drones do not participate in foraging; only workers do [6]. During foraging, stingless bees collect pollen, nectar, oils, water, resins, muds and sand particles. Most stingless bee species will communicate with each other on locations of forage sources by secreting pheromones [1].

The morphology of stingless bee can be divided into three main parts: head, thorax and abdomen. These morphological characteristics are important in differentiating the species of stingless bee and their caste. For example, the size of the virgin queen is larger than worker bee [8]. From morphological information, flight cost and aggressive of stingless bee can be calculated [9]. To determine nest structure and growth; and to analyse foraging frequency of *G. thoracica* throughout a year.

## 2.0 MATERIALS AND METHOD

Three colonies of *G. thoracica* were established in Agropark Universiti Malaysia Kelantan (UMK) Jeli (05° 44'N 101° 51'E). Two nest structure parameters (external nest and internal nest) were measured using digital callipers (Kern 6"/150mm). The growth of nest which includes the area of brood, area of honeypot, area of pollen pot and volume of honey were determined. About 70% of honey was collected from colonies and another 30% honey was left for colonies survival. Photo of nest growth were snapped and analysed using computer assisted digital image analysis (Image J). These nests growth and foraging activities data collected from July 2013 until June 2014.

Observations of foraging frequency of *G. thoracica* workers were made from July 2013 until June 2014. The foraging frequencies were recorded once a week throughout the study period. Each observation was made for five minutes at interval of two hours from 0800 hrs to 1600 hrs. The observations were focus on the foraging activity in terms of the number of foragers leaving their nest that assigned as "Outgoing Foragers", number of foragers leaving nest with garbage pallet assigned as "Outgoing Foragers with Garbage", number of foragers returning to their hives with pollen load assigned as "Incoming Foragers with Pollen Load" and without pollen load assigned as "Incoming Foragers". These observations were recorded by writing on notepad.

Then, total 150 samples of stingless bees collected randomly from three colonies from February 2014 until June 2014. The stingless bees were caught in individual plastic cup with cover [12.0 cm (h) x 9.0 cm (d)] for each colony and brought back to Biology Laboratory, Universiti Malaysia Kelantan. In the laboratory, the bee specimens were killed using chloroform, pinned [pin size No. 00] and oven dried at 40°C overnight. Once preserved, the specimens were observed under the stereo microscope at 10 x magnifications (Rax Vision MTS). Image of the specimens were captured and measured by using microscope camera (Dino capture 2.0). The morphometry parameters include length of a bee (LB), length of head (LH), width of head (WH), length of thorax (LT), width of thorax (WT), length of abdomen (LA), width of abdomen (WA), length of forewing (LF), width of forewing (WF), length of tibia (LT) and width of tibia (WT). Mean sizes of worker bee collected (February 2014 until June 2014) were calculated. Meanwhile, for flight cost and aggressive cost, monthly mean of morphometry parameters were used to insert into formula of flight cost (FC) and aggressive cost (AC) [9].

### Formula of Flight Cost (FC) and Aggressive Cost (AC):

$$FC = \frac{(BL)^3}{(FL)^2}$$

$$AC = \frac{(HW)^3}{(BL)^3}$$

FC = Flight Cost

AC = Aggressive Cost

BL= Body Length (mm)

FL= Forewing Length (mm)

HW= Head Width (mm)

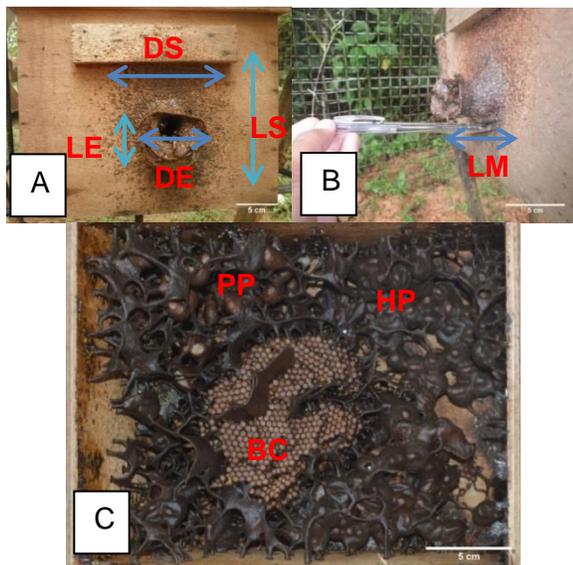
## 3.0 RESULTS AND DISCUSSION

### 3.1 Nest Structure and Growth

The nest structure of stingless bee consists of external or entrance nest structure and internal nest structure. The entrance nest of *G. thoracica* was formed in round with mount shape (Figure 1) and made up of resin. The colour of entrance can be found in light brown and this entrance of the nest was guarded by five to seven worker bees. The entrance of each

different nest varied in shapes and structures. For example, *Tetragonisca fiebrigi* showed the nest entrance in a tube shape, *L. ventralis* made thin soft funnel shape [10]. In Malaysia, it was reported that *G. thoracica* formed the widest entrance compared to *H. itama*, *L. terminata*, *L. scintillans* and *T. laeviceps* [5].

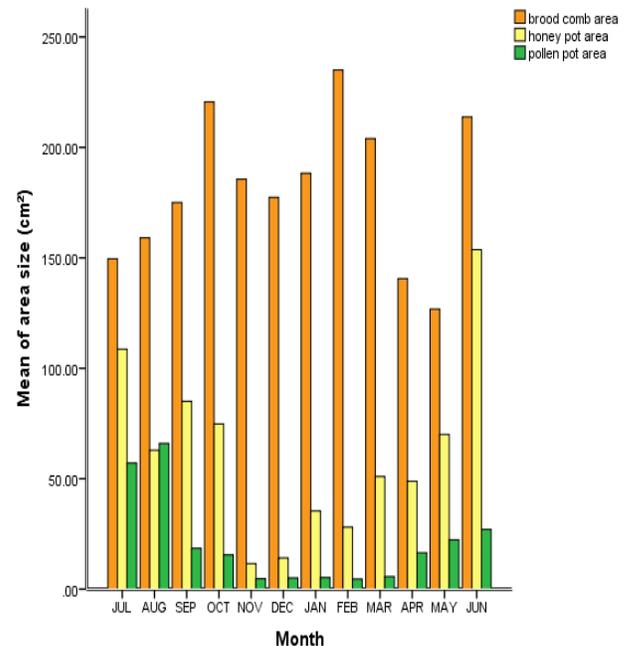
Meanwhile, the internal nest comprised of brood cells and layers, honey pots, and pollen pots. Honey and pollen were stored in separated pots, but these pots were often arranged side by side. The pollen and honey pots are oval shaped and are made from soft cerumen, a mixture of wax and propolis [7]. The brood cells are arranged in horizontal layer form. The sizes of brood cells were smaller than the pollen pots and honey pots (Table 1). The shape of brood cells was spherical to ovoid. These brood cells are covered by involucrum. The involucrum is a series of sheath made from cerumen. This involucrum functioned to protect the brood cells from intruders and to maintain the temperature surrounding the broods. The structure of nest entrance or the thickness of the resin enclosing the internal nest are influenced by the nest age, bee genetics and micro environment including predators, parasites, symbionts, rain, wind and sun [7].



**Figure 1** External and internal nest structure of *G. thoracica*. A- The front view of external nest (entrance); measurement parameters shown on picture were (LE) Length Entrance, (DE) Diameter entrance, (LS) Length spread of propolis, (DS) Diameter spread of propolis; B- side view of external nest (entrance) showed parameter (LM) Length mount of entrance; C- the view of internal nest measurement parameters shown on picture, (BC) Brood comb, (HP) honey pot, (PP) pollen pot.

**Table 1** Data of external and internal nest structure of *G. thoracica*

| Parameters (mm)                        | Mean $\pm$ SD     |
|--|-------------------|
| Diameter of nest entrance              | 50.15 $\pm$ 1.02  |
| Length of nest entrance                | 50.07 $\pm$ 1.11  |
| Length mount of nest entrance          | 20.17 $\pm$ 1.39  |
| Diameter spread of nest entrance       | 120.75 $\pm$ 5.46 |
| Length spread of nest entrance         | 12.58 $\pm$ 4.88  |
| Height of brood cell                   | 4.53 $\pm$ 1.27   |
| Diameter of brood cell                 | 3.61 $\pm$ 0.21   |
| Height of honey pot                    | 23.28 $\pm$ 4.48  |
| Diameter of honey pot                  | 11.91 $\pm$ 2.12  |
| Height of pollen pot                   | 21.18 $\pm$ 5.76  |
| Diameter of pollen pot                 | 12.57 $\pm$ 3.50  |
| Distance between brood layers (pillar) | 7.48 $\pm$ 1.63   |



**Figure 2** Nest growth of internal nest components (brood combs area, honey pots and pollen pots area) of *G. thoracica* by month

The growth of *G. thoracica* nest (Figure 2) showed through the increasing area size of brood, where the brood areas were increased within two months. Meanwhile, the area sizes of honey pot were increased from May until August, and the area of pollen pot showed less than honey pot. The honey harvested from closed storage pots from each colony (Figure 2). Mean honey production of *G. thoracica* was 37.59 $\pm$ 54.83ml (n=3) monthly (July 2013-June 2014). Honey production of stingless bee depends on the quality of environment and competition with other bees for special floral resources [14]. The production of honey was lower during rainy season. Rainfall was found to correlate with honey production, both direct and negative

impacts through the reduction of foraging activities [15].

### 3.2 Foraging Activity

The activities of foraging by workers or foragers were divided into four types which were outgoing foragers, outgoing foragers with debris, incoming foragers with pollen and incoming foragers. Based on four types of foraging activities, the highest activity done by foragers were outgoing and incoming to the nest. Remove the garbage pellet was the least activity that done by foragers.

Based on foraging activity, the highest frequencies for all foraging activities of *G. thoracica* were done on July 2013 and June 2014 and both months was the dry season (Table 2).

The lowest foraging of *G. thoracica* occurs during October 2013. This month was considered as raining season. Meanwhile for time of foraging, the outgoing foragers and incoming foragers with pollen were highest in period of 1000 hrs until 1200 hrs. The foragers outgoing with debris was highest in the early morning, 0800 hrs until 1000 hrs and incoming foragers of *G. thoracica* was highest on 1200 hrs until 1400 hrs. The lowest foraging times for *G. thoracica* were on 1600 hrs until 1800 hrs. The result of foraging frequency was similar with that of the pollination season of many Hymenoptera (ants, bees, and wasps), Diptera (flies), Heteroptera (bugs) and Lepidoptera (butterflies, moths) [13].

Most *Melipona* species began their flight activity when the optimal levels of relative humidity and temperature are between 70% to 90% and approximately 20 °C respectively [16]. The foraging activity was also influenced by colony condition where, weaker colonies had a shorter period of foraging than stronger colonies as in *M. bicolor* and in *Schwarziana quadripunctata* [17]. In addition, the foraging activities of *G. thoracica* showed less forager perform foraging activity during raining season. But *T. irridipennis* showed different result, where during monsoon season (June until September) significantly higher outgoing, pollen foragers and incoming foragers were observed [2].

*Lepidotrigona terminata* that domesticated in Besut, Terengganu showed the most effective foraging activity in the morning (0800-1100) and late afternoon (1400-1800)[18].

**Table 2** Analysis of foraging activity of forager

| Months/<br>Hours | Mean $\pm$ SD number of bees |                                |                                    |                     |
|------------------|------------------------------|--------------------------------|------------------------------------|---------------------|
|                  | Outgoing Foragers            | Outgoing Foragers with Garbage | Incoming Foragers with Pollen Load | Incoming Foragers   |
| Jul 2013         | 23.1<br>3 $\pm$ 0.85         | 1.80<br>$\pm$ 0.49             | 8.08 $\pm$ 0.31                    | 19.57<br>$\pm$ 0.32 |
| Aug 2013         | 19.9<br>4 $\pm$ 0.52         | 1.46<br>$\pm$ 0.86             | 4.86 $\pm$ 0.60                    | 20.02<br>$\pm$ 0.23 |
| Sep 2013         | 19.0<br>8 $\pm$ 0.84         | 0.98<br>$\pm$ 0.31             | 5.10 $\pm$ 0.39                    | 18.73<br>$\pm$ 0.85 |
| Oct 2013         | 13.0<br>3 $\pm$ 0.82         | 0.62<br>$\pm$ 0.07             | 2.32 $\pm$ 0.27                    | 13.85<br>$\pm$ 0.36 |
| Nov 2013         | 12.7<br>2 $\pm$ 0.12         | 0.63<br>$\pm$ 0.04             | 3.89 $\pm$ 0.62                    | 14.18<br>$\pm$ 0.81 |
| Dec 2013         | 16.0<br>8 $\pm$ 0.69         | 0.66<br>$\pm$ 0.02             | 5.44 $\pm$ 0.78                    | 13.78<br>$\pm$ 0.26 |
| Jan 2014         | 16.8<br>9 $\pm$ 0.06         | 0.81<br>$\pm$ 0.02             | 1.78 $\pm$ 0.75                    | 17.26<br>$\pm$ 0.39 |
| Feb 2014         | 15.0<br>0 $\pm$ 0.95         | 1.01<br>$\pm$ 0.05             | 3.85 $\pm$ 0.52                    | 13.09<br>$\pm$ 0.30 |
| Mar 2014         | 14.3<br>2 $\pm$ 0.65         | 0.86<br>$\pm$ 0.09             | 4.23 $\pm$ 0.45                    | 13.94<br>$\pm$ 0.08 |
| Apr 2014         | 18.5<br>8 $\pm$ 0.18         | 0.81<br>$\pm$ 0.07             | 6.06 $\pm$ 0.51                    | 16.78<br>$\pm$ 0.62 |
| May 2014         | 20.0<br>8 $\pm$ 0.52         | 1.09<br>$\pm$ 0.03             | 7.49 $\pm$ 0.15                    | 21.00<br>$\pm$ 0.83 |
| Jun 2014         | 22.0<br>3 $\pm$ 0.30         | 0.49<br>$\pm$ 0.05             | 7.79 $\pm$ 0.77                    | 23.61<br>$\pm$ 0.55 |
| 0800             | 19.1<br>2 $\pm$ 0.12         | 1.11<br>$\pm$ 0.81             | 6.70 $\pm$ 0.85                    | 16.26<br>$\pm$ 0.56 |
| 1000             | 21.9<br>5 $\pm$ 0.42         | 0.96<br>$\pm$ 0.06             | 7.51 $\pm$ 0.53                    | 19.52<br>$\pm$ 0.24 |
| 1200             | 20.5<br>9 $\pm$ 0.20         | 0.96<br>$\pm$ 0.07             | 5.72 $\pm$ 0.37                    | 21.74<br>$\pm$ 0.90 |
| 1400             | 15.2<br>2 $\pm$ 0.14         | 0.88<br>$\pm$ 0.06             | 3.27 $\pm$ 0.98                    | 16.64<br>$\pm$ 0.49 |
| 1600             | 10.9<br>8 $\pm$ 0.48         | 0.77<br>$\pm$ 0.05             | 2.16 $\pm$ 0.51                    | 12.52<br>$\pm$ 0.11 |

### 3.3 Morphometry Study

*G. thoracica* was one of the largest stingless bees found in Indo-Malayan areas with the range of body length 6.67 mm to 10.80 mm [19]. Compared with other stingless bees, the body length of *T. irridipennis* in the range of 3.92 mm to 4.12 mm [2].

From the data of morphometry study (Table 3), the flight cost and aggressive cost were calculated, flight cost (FC) of *G. thoracica* was  $8.48 \pm 0.440$  and aggressive cost (AC) was  $0.06 \pm 0.004$ . A high ratio of FC means that body weight is bigger than wing area and wing need more energy to fly and a high ratio of AC means head is larger to body size where and consequently showed a big mandible. Compared with research done by FRIM [6] the result of FC and AC of *G. thoracica* showed the in the middle range of AC and FC. These results of FC and AC were similar with large species of stingless bee, *Trigona canifrons* and *Trigona fimbriata* [6]. Usually, small species of stingless bee fly off a food patch or flower when threatened by dominant species, yet they quickly return to the same site or nearby flowers and

continue feeding as if indifferent to the aggressors [14]. A high ratio of FC means that body weight is bigger than wing area and wing need more energy to fly and a high ratio of AC means head is bigger to body size where and consequently showed a big mandible [20]. Usually, small species of stingless bee fly off a food patch or flower when threatened by dominant species, yet they quickly return to the same site or nearby flowers and continue feeding as if indifferent to the aggressors [15].

**Table 3** Data of morphometry study of worker bee of *G. thoracica*

| Parameters (mm)         | Range      | Mean $\pm$ SD   |
|-------------------------|------------|-----------------|
| Length of a bee (LB)    | 6.67-10.80 | 8.47 $\pm$ 0.84 |
| Length of head (LH)     | 0.52-2.67  | 1.43 $\pm$ 0.35 |
| Width of head (WH)      | 2.01-3.88  | 3.37 $\pm$ 0.26 |
| Length of thorax (LT)   | 2.10-3.76  | 3.17 $\pm$ 0.36 |
| Width of thorax (WT)    | 1.80-3.43  | 2.81 $\pm$ 0.27 |
| Length of abdomen (LA)  | 2.40-5.48  | 4.10 $\pm$ 0.64 |
| Width of abdomen (WA)   | 2.42-5.64  | 3.10 $\pm$ 0.40 |
| Length of forewing (LF) | 6.70-9.07  | 8.48 $\pm$ 0.43 |
| Width of forewing (WF)  | 1.68-3.66  | 2.98 $\pm$ 0.40 |
| Length of tibia (LT)    | 1.21-4.36  | 3.50 $\pm$ 0.43 |
| Width of tibia (WT)     | 0.91-3.81  | 1.27 $\pm$ 0.32 |

n=150 sample

#### 4.0 CONCLUSION

There were found that, the nest structure of *G. thoracica* having round mount sticky entrance and their internal nest having horizontal brood combs that surround with food storage pots (honey pots and pollen pots). There is significant difference in the growth of the nest structure of *G. thoracica* throughout year. The study showed the characteristics of internal nest components and pattern of internal nest component areas increased or decreased by month. The study of nest structure of *G. thoracica* result on information and guidelines for bee farmers in identify this species based on the characteristics of their nest. Based on nest dynamics of *G. thoracica*, it was shown that *G. thoracica* able to produce more honey than bee bread (= pollen) and also the productive season for *G. thoracica* occur during dry season. Foraging activity also showed the significant difference for each type of foraging activity. The peak time of foraging frequency for *G. thoracica* from 1000 hrs until 1200 hrs and these foraging frequencies were affected by climatic changes that decreased during raining season. From morphometry study of *G. thoracica* workers, showed that *G. thoracica* was the largest stingless bees as compared to other species from the literature. Besides, there were found that there is significant difference between the monthly parameters encounter in this study and the result from morphometry study, the flight cost and aggressive cost of *G. thoracica* were determined where high flight cost give advantage for *G. thoracica* in exploits food sources.

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