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NEST ARCHITECTURE OF STINGLESS BEES *TETRAGONULA IRIDIPENNIS* AND *LEPIDOTRIGONA VENTRALIS* IN NAGALAND

AVINASH CHAUHAN* AND H.K. SINGH

Department of Entomology
School of Agricultural Sciences and Rural Development
Nagaland University, Medziphema, Nagaland
*Email: avi_shimla@hotmail.com

ABSTRACT

Stingless bees are important pollinators and from India, eight species have been reported so far. Out of them, four species viz., *Tetragonula iridipennis*, *Lepidotrigona ventralis*, *Tetragonula laeviceps* and *Lophotrigona canifrons* are commonly present in the northeast, especially in Nagaland. *Tetragonula iridipennis* and *Lepidotrigona ventralis* are the dominant. Keeping in view the problems faced by the large number of beekeepers in the state, the present study explores the nest architecture of *T. iridipennis* and *L. ventralis* so that new hives could be designed for efficiency under protected conditions. Surveys were conducted in the forest and village areas of Dimapur district. A total of 15 colonies were located and reestablished in the department Apiary. The activity of the bees at the nest entrance was observed fortnightly. Five colonies of each species were dissected to know the colony architecture. The results revealed that size of the brood cells (queen, male and worker) in *T. iridipennis* was smaller as compared to *L. ventralis*. The pollen and honey cells in *L. ventralis* were bigger than *T. iridipennis*. The internal architecture of the nest was different: in *T. iridipennis* brood was not covered fully with involucrum while *L. ventralis* was covered in the involucrum connected with honey and pollen pots with tunnels supported by cerumen and resin pillars. Size of queen, drone and worker were also measured.

Key words: Stingless bees, nest architecture, *Tetragonula iridipennis*, *Lepidotrigona ventralis*, dimensions, castes, size, queen, drone, worker

Northeast India provides better opportunities to pollinators to thrive. Propagation of bee colonies contributes to conservation of biodiversity (Watanabe, 1994; Buchmann and Nabhan, 1996; Kearns and Inouye, 1997; Nabhan et al., 1998; Cane and Tepedino, 2001; Villanueva et al., 2005; Steffan-Dewenter et al, 2005; Biesmeijer et al.,2006; Goulson et al., 2008). Pollination is an essential component of the ecosystem service that results in the increase of food security and improvement in livelihoods (Costanza et al., 1997). Bees play a very important role in the pollination of flowering plants resulting in increased quantity and quality of fruits and seeds. About 70% of crops that account for 35% of all agricultural production depends on pollinators (Klein et al., 2007 ; Kearns and Inouye, 1997). Like other bees, stingless bees are members of the class Insecta, family Apidae, subfamily Meliponinae, tribe Meliponini. The total number of species within the Meliponini is estimated to be about 400 (Silveira et al., 2002). The presence of nesting sites is essential for survival, maintenance and reproduction of stingless bees (Hubbell and Johnson, 1977; Batista et al., 2003; Eltz et al., 2003; Roubik, 2006). Although their nesting habits are variable, most of the stingless bee species build their nests in several substrates ranging from subterranean cavities to arboreal ant nests (Campos, 1987; Roubik, 2006). The main building material is cerumen, a mixture of bee wax and plant resins (Rasmussen, 2013).

The nest entrance of stingless bees varied in shape, length, and colour. For example, nest entrance of *Heterotrigona itama* is of funnel shape, whereas, in *Geniotrigona thoracica* is of round mount-like shape (Syafrizal and Yousuf, 2014; Kelly et al., 2014). The common structure of stingless bee nests consists of entrance tunnels, brood cells, food storages (honey and pollen cells), cerumen and batumen layers (Sakagami et al., 1990; Michener, 2007). Within the nest the brood chamber is always clearly separated from the area of food storage. There are two cell types: brood cells and storage pots (Vijayakumar, 2014). Storage pots in most species several times larger than the brood cells and pots containing honey are generally intermixed with those that contain pollen (Kwapong et al., 2010). Brood cells are arranged horizontally in most species or in a cluster, however in others such as *Dactylurina* cells are vertically from the storage compartment. Brood cells in a cluster arrangement were not surrounded by an involucrum. This allows them to fit into irregularly shaped cavities (Sommeijir, 1999).

In Nagaland four species of stingless bees viz., *Tetragonula iridipennis, Lepidotrigona ventralis, Tetragonula laeviceps and Lophotrigona canifrons* are reported. Among them *T. iridipennis* and *L. ventralis* are major species dominating the stingless bee fauna. Keeping in view the potential of stingless bees as a pollinator, present studies were undertaken to investigate the nest architecture of *T. iridipennis* and *L. ventralis* so that new scientific hives could be developed for the better manipulation of these bees for honey production and as pollinator of crops grown under open and protected conditions.

MATERIALS AND METHODS

A survey was conducted in the forest and village areas around the Dimapur district of Nagaland during 2018. Location of feral stingless bee habitat was identified at the forest along with their nesting height preferences. Stingless bee nests were extracted from the fallen trees. Later both the ends of the cylindrical hive having colony were closed and transported to the experiment site in the evening after 1730 hr. The colonies brought to the apiculture field and placed there for five months to settle and develop. The activity of the bees at the nest entrance was recorded fortnightly and when the activity was noticed maximum, these colonies were dissected to know their nest architecture and colony structure. External features such as nest entrances were observed and recorded by taking their dimensions. Entrance funnels being one of the most diagnostic field traits for distinguishing stingless bee species. Internal nest structure was observed. The measurements were taken for brood cells, honey and pollen area of *T. iridipennis* and *L.ventralis*. Size, shape and type of cells (brood, honey and pollen) were measured using vernier callipers along with the involucrum structure of the colony. The different materials used in the colony were also recorded.

RESULTS AND DISCUSSION

Total 15 colonies were identified, and of these five colonies each of *T. iridipennis* and *L. ventralis* were extracted and re-established in the Apiary. The colonies of *T. iridipennis* were found at a mean height of 77.1±4.2 cm (range 65.4 to 120 cm) above the ground while *L. ventralis* were observed at 168±14.4 cm (range 133.2 to 213 cm). Similar findings had been observed by Nayak et al. (2012), who with *Trigona* observed that 47% preferred 330 to 450 cm elevation range. However, Suriawanto et al. (2017) observed a height of 1.18 cm-321 cm for the nests of *Tetragonula* spp.

The external features of nests were observed such as type of entrance funnel, shape of funnel, material used for the construction of funnel and number of guards at the entrance (Figs. 1-4). It was recorded that the entrance funnel of *T. iridipennis* was dark brown to black with an opening dia of 1.81 ± 0.14 cm, while *L. ventralis* funnel was white with an opening dia of 3.52 cm and length ranging from 4.16- 22.00 cm with mean length of 11.65 ± 2.54 cm. The entrance funnel was made up of resins and wax in *T. iridipennis* while in *L. ventralis* it was constructed with wax only. On an average, 9.66 guard bees were recruited at the entrance funnel in *T. iridipennis* whereas in case of *L. ventralis* it was 6.79 workers. The narrow nest entrance of *Melipona* and other genera allows the nest to be defended by one or only a few guards positioned in the mouth of the entrance tube and variation of nest entrances related to defense and foraging activities of stingless bees (Biesmeijer et al., 2006). The results are in confirmation with the observations of Syafrizal et al. (2014) and Kelly et al. (2014). They revealed that nest entrance of stingless bees varied in shape, length and colour in *Heterotrigona itama* and *Geniotrigona thoracica*. It is funnel shape in *H. itama* whereas, in *Geniotrigona thoracica* is round mount-like shape. Barbosa et al. (2013) studied the nest architecture of *Geniotrigona subterranea* and they reported that the stingless bee nest entrance was circular in shape having a dia of 0.85-1.20 cm.

Foraging activity of *T. iridipennis* was observed at the nest entrance from early morning till late evening, with maximum activity being at 1000 hr (24.04 bees) while minimum at 0600 hr (10.98). Similar trend of bee activity at nest of *L. ventralis* was observed where maximum nest activity was recorded in morning hours (21.08 and 20.94) while minimum at late noon hours (11.30 bees). The incoming activity was maximum at evening hours and outgoing was maximum during late morning hours of the day in both the stingless bee species (Table 1). Similar results were recorded by Jaapar et al. (2017) while studying the foraging behaviour of *Heterotrigona itama* at nest entrance.

The nest architecture studies revealed that the nest entrance in both the species was oriented to south.

Table 1. Foraging activity of *T. iridipennis* and *L. ventralis* at nest entrance- September, 2018

Day hours (h)	*Tetragonula iridipennis*			*Lepidotrigona ventralis*		
	Incoming bees/ 5 min	Outgoing bees/ 5 min	Mean	Incoming bees/ 5 min	Outgoing bees/ 5 min	Mean
0600	9.56	12.41	10.98	15.31	19.81	17.56
0800	19.22	25.78	22.50	20.22	21.95	21.08
1000	18.55	29.54	24.04	18.79	23.10	20.94
1200	17.70	12.91	15.30	12.90	15.95	14.42
1400	11. 66	14.66	13.16	9.95	12.66	11.30
1600	20.10)	13.58	16.84	17.99	18.90	18.44
1800	24.16	7.92	16.04	21.80	10.16	15.98
Mean	17.13	16.82		16.70	17.50	
SE ±	1.59	1.81		1.82	2.91	

The involucrum was incomplete and made up of wax, cerumen mixed with mud in *T. iridipennis*. The brood cells were located in the centre stacked over one another in horizontal manner, and size of queen cell was 7.15 x 4.84 mm; worker and male cells were smaller (4.16 x 3.14 mm and 4.06 x 3.09 mm). The honey pots (13.31 x 12.91 mm) and pollen pots (14.25 x 13.95 mm) were bigger than other cells.

Similarly, stingless bee, *L. ventralis* has queen cells (7.96 x 5.20 mm) bigger than the worker (5.44 x 4.05 mm) and male cells (5.04 x 4.58 mm). The honey pots (23.15 x 19.09 mm) and pollen pots (24.66 x 21.85 mm) were present in rear side of the brood. The whole colony (brood cells, honey and pollen pots) was covered in the involucrum made up of cerumen and wax and sealed with resins. Inside this covering, two holes were present that served as entrance and exit for the bees supported by resin and wax pillars. In front part of the fully covered brood, pollen pots (49± 9.60) were present while the honey pots (total 174± 24.12) were present in the rear side connected with tunnels to the brood. The pollen weight/ pot was found to be 2.67 ± 0.68 g while 2.14 g of honey was available in each sealed wax cell. The brood was covered fully with an involucrum made of wax and cerumen. It was arranged horizontally (23 ± 2.34 layers) in layers one above the other.

The different cells present in *L. ventralis* colonies were bigger as compared to *T. iridipennis* colonies (Table 2). Similar observations were made by Barbosa et al. (2013) in *G. subterranea*.

The sizes of different castes i.e. queen, males and workers differ in the species. The queen has an yellowish brown swollen abdomen and wings were not covering it fully, however in workers and males, the wings covers the whole abdomen in *T. iridipennis*. While in *L. ventralis*, the colour of the castes was dark brown to blackish. The length of queen (7.46 mm), male (4.02 mm) and worker bees (4.10 mm) was less in *T. iridipennis* as compared to queen (8.16 mm), male (5.21 mm) and (5.59 mm), length in *L.ventralis*, respectively. Tej et al. (2017) studied the morphometry of *T. iridipennis* with fourteen measurements and identified the bees as *T. iridipennis*. Body size differed considerably among Melliponini species and ranged from minute (*T. pipoli*, 2.5-3.0 mm body length)

Table 2. Dimensions of cells and castes in the nests of *T. iridipennis* and *L. ventralis*

Types of cells	*T. iridipennis*		*L. ventralis*	
	Length±S.E.(mm)	Width±S.E. (mm)	Length±S.E.(mm)	Width±S.E.(mm)
Queen cells	7.15±0.21	4.84±0.27	7.96 ±0.49	5.20±0.17
Worker cells	4.16±0.19	3.14±0.17	5.44±0.66	4.15±0.73
Male cells	4.06 ± 0.11	3.09±0.08	5.04 ± 0.14	4.58 ± 0.21
Honey pots	13.31±0.25	12.91±0.23	23.15±0.48	19.09±0.63
Pollen pots	14.25 ± 0.69	13.95 ± 0.77	24.66 ± 0.22	21.85 ± 0.41
Castes	Length±S.E.(mm)	Length±S.E.(mm)		
Queen	7.46 ±0.09	8.16 ±0.10		
Worker	4.10 ±0.12	5.59 ±0.19		
Male	4.02 ±0.14	5.21 ±0.06		

to medium 9-10 mm body length in *P. occidentalis* (Michener, 2007; Jarau and Barth, 2008).

Nest architecture of *T. iridipennis* and *L.ventralis* revealed the variation in their nest preferences and different materials (cerumen, wax, mud and resins) stingless bee use for nest construction. The thick layer of cerumen and wax serves as an insulator for maintaining the temperature in the nest. In *T. iridipennis* the brood was surrounded with pollen and honey cells in an involucrum while in *L. ventralis* the brood was covered by many layers of cerumen and wax and is totally separated from the honey and pollen cells. Small tube connections were present between the brood chamber and the food reserves. The honey and pollen pots were bigger in size as compared to *T. iridipennis*. The size of the different castes (queen, males and workers) was bigger in *L. ventralis* as compared to *T. iridipennis*. The nest architecture studies of dominant stingless bee species, *T. iridipennis* and *L.ventralis* revealed the complex and differentiated nest building in stingless bees which in future will help in designing of scientific hives which further helps to the farmers in better extraction of honey without causing any harm to the stingless bee brood and colony development.

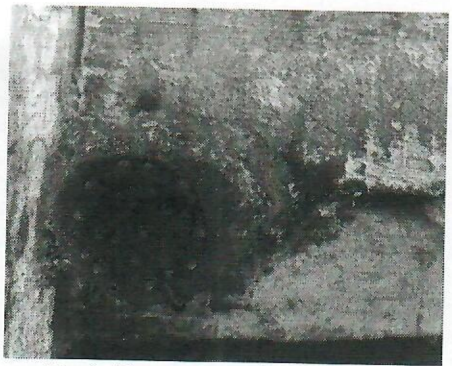

Fig. 1a. Nest entrance of *T. iridipennis*

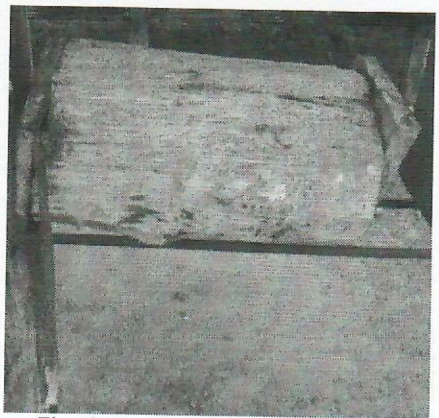

Fig. 1b. Nest entrance of *L. ventralis*

Fig. 2a. *T. iridipennis* guard bees at entrance

Fig. 2b. L. ventralis guard bees at entrance

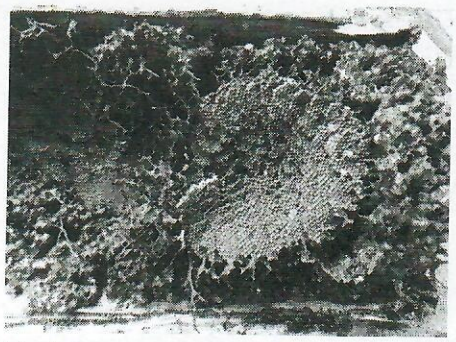

Fig. 3. Internal structure of *T. iridipennis* colony

Fig. 4. Internal structure of *L. ventralis* colony

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