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Occurrence of multiple nest entrance in the stingless bee *Tetragonula travancorica* (Hymenoptera: Meliponini)

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ABSTRACT: Multiple nest entrance occurs in the stingless bee *Tetragonula travancorica* Shanas & Faseeh at a low frequency (2.3%). Different types of multiple nest entrances are described and its significance discussed.

KEY WORDS: stingless bee, nest architecture, Kerala, India.

INTRODUCTION

The stingless bees (Hymenoptera, Apidae, Meliponini) are the most diverse eusocial bees in the tropical regions of the world. They are 60 times more speciose than the *Apis* bees. Nesting habits and nest architecture vary greatly not only among genera but also among species within this genus. The nesting habitats of stingless bees vary widely from cracks and crevices, walls, tree hollows, the base of trees, bird nests, underground structures, limestones etc. According to Roubik (2006) the nest entrance is normally associated with physio-chemical regulation, maintenance of microclimate inside the nest, foraging activity and defence of a colony and it is the check point at which the individual stingless bees entering the nest are recognized. He also mentioned the possibility of occurrence of innovations in the nest architecture within a taxon.

Attributes of nest structure and nesting habits can be used as a tool for ecological, phylogenetic and taxonomical studies (Rasmussen and Camerago, 2008; Lima *et al.,* 2013). Kelly *et al.* (2014) observed that the nest entrance of stingless bees differs with genus. Most of the studies were concentrated on ecology, biology, morphology and pollination of these bees (Vijayakumar 2014). They use the waxy secretion from their dorsal body along with resins collected from plants for construction of nests (Virkar, 2014). Only limited information is available on the nesting behaviour of stingless bees in India (Roopa *et al*., 2015; Patel and Pastagia, 2016).

Shanas and Faseeh (2019) described three new species of stingless bees from south India and provided keys to the species of *Tetragonula* of the Indian subcontinent. They also established that, *T. iridipennis* (Smith, 1854) does not occur in India and *Tetragonula iridipennis*, mentioned in publications from south India most probably refers to *T. travancorica* Shanas & Faseeh. Multiple nest entrance in *Tetragonula travancorica* from Kerala is described and its significance discussed here.

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MATERIALS AND METHODS

The study was conducted during 2017-2018 in Kerala, India. Stingless bees were collected from different geographical regions in the state. The colonies were located at Manakkadavu, Kannur District (12^{0}13'12.21"N 75^{0}30'07.29"E, altitude 223 m), Peechi, Thrissur District (10^{0}30'56.06"N 76^{0}21'31''.35"E, altitude 115 m), Vellayani, Thiruvananthapuram District (8^{0}25'40.85"N 76^{0}59'05.68"E, altitude 29 m), Kadannamanna, Malappuram District (11^{0}01'53.58"N 76^{0}10'07.90" E, altitude 55 m), Ambanad, Kollam District (9^{0}00'50.99"N 77^{0}05'21.88"E, altitude 362 m), Thenmala, Kollam District (8^{0}57'45.58"N 77^{0}03'53.38"E, altitude 153 m). A total of 207 colonies were located during the study. The colonies with more than one entrance were recorded with the number of guard bees, shape of entrance mouth, length and width of entrance mouth, number of entrance tubes, length of each entrance tube, number of active entrances, height from the ground level and nest habitat. Each nest entrance was photographed with a 55mm macro lens mounted on a Nikon D80 camera. Latitude, longitude, and altitude of each colony were noted with the help of Google earth pro app.

Transparent bottles of 15-20 cm height were kept covering the mouth of the entrance and gently tapped around the nearby areas of nest which resulted in bees getting trapped inside the bottle. An average of 20 stingless bees was collected from each colony and no colonies were harmed during the entire collection. Collected bees were killed using ethyl acetate and preserved in 70% ethyl alcohol, few of which were pinned, dried and stored in insect boxes for further studies.

RESULTS AND DISCUSSION

The samples were collected from various locations in Kerala (comprising all geographical regions such as coastal areas, planes, and hills). Out of the 207 colonies, six colonies had multiple nest entrance openings, of which four were from the hill tracts and two were from the plains. The shape of entrance tubes identified were either round, oval or slit. Three of the nest entrances were oval and

Table 1. Nest entrance characters of colonies with more than one entrance

Character	Location*					
	M	P	V	T	A	K
No of entrance tubes	2	2	2	2	2	5
No of active entrance tubes	2	1	1	2	2	5
Shape of entrance	Round	Oval	Oval	Round	Slit	Round
No of guard bees	5+5	5	6	4+4	7+7	4+1+1+1+1
Length of entrance mouth (cm)	1.2	1.2	2.1	1	2.5	
Length of each entrance tube (cm) 1		2.1	7.6	5.4	7	04.3
2						3.8
3						2.3
4						5.4
5						5.4
Height from ground level (cm)	34	67	28.7	25.4	138	7.8
Nest habitat	Stone wall	Stone wall	Stone wall	Stone wall	Building wall	Stone wall

*(M-Manakkadavu, P-Peechi, V-Vellayani, T-Thenmala, A-Ambanad, K-Kadannamanna)

Fig. 1 Vellayani

Fig. 2 Thenmala

Fig. 3 Manakadavu

Plate 1. Multiple entrances recorded in *Tetragonula travancorica*

Fig. 4 Kadannamanna

Fig. 5 Peechi

Fig. 6 Ambanad

Plate 1. Multiple entrances recorded in *Tetragonula travancorica*

Fig.7 Arrows indicate the openings in the entrance tube at Thachampara, Palakkad (Host: Fig tree)

two of them were round and one was slit in shape. It was also observed that the shape of entrance mouth was similar for all entrance tubes of the same colony.

The number of guard bees varied between colonies, however, the number of guard bees were same in each active entrance tube of the same colony, except in the case of a single colony where in, out of five active entrance tubes, one entrance tube had 4 guard bees and the remaining entrance tubes were guarded by a single bee (Fig. 4).

The number of entrance tubes was two for colonies identified from Vellayani (Fig. 1), Thenmala (Fig. 2), Manakkadavu (Fig. 3), Peechi (Fig. 5), and Ambanad (Fig. 6) whereas five entrance tubes were found in a single colony from Kadannamanna (Fig. 4). The number of active entrances differed in each colony. Colonies from Thenmala, Ambanad and Manakkadavu had two active entrances, whereas colonies from Vellayani and Peechi were with only one entrance tube which was active and the other one was closed entirely or joined with the active entrance tube mouth (Vellayani). A colony from Kadannamanna had five entrance tubes (Fig.4) and all of them were active.

The length and width of entrance mouth were almost same for different entrance tubes in a single colony. Length of entrance mouth varied from 1.0to 2.5cm and width varied from 0.2 to 1.2 cm in different locations. The height of colony from ground level varied in different locations. All colonies were located at a height of less than 50cm, except the ones in Ambanad (138 cm) and Peechi (67 cm). Five colonies were found associated with foundations of buildings made of stones, and one colony was observed inside the wall of a building.

All the five colonies were having the entrance tubes one above the other presenting an overall look of vertical arrangement of entrance tubes. In one colony, the five entrances were arranged in a different manner: two pairs of entrance tubes arranged vertically (one below to the other) and the fifth, in line with the upper entrance tubes (three tubes were in one line and two tubes were in another line) (Fig.4).

In addition to these six colonies with multiple entrances, one more colony was identified from Thachampara (Palakkad dist) with multiple nest entrance openings. Three to four small openings were observed in the entrance tube, in addition to

opening on the apex. Except for the apex, all openings were non-functional and without any guard bees. The main entrance was guarded by 4-5 bees and the colony was located 186cm above the ground level on a fig tree (Fig.7).

Wille and Michener (1973) reported two species of stingless bees *Tetragonisca angustula* and *Trigona fulviventris,* those rarely form nests with two or three entrances. Roubik (2006) stated the existence of multiple entrances in some genera such as *Lepidotrigona, Scaptotrigona, Plebia, Tetragona*, and *Hypotrigona* and these multiple entrances cannot be considered as a consistent character in these genera. Benziger and Benziger (2010) observed two exceptional nests of minute stingless bees, *Lisotrigona cacciae* and *L. furva* having two nest entrances for a single colony. The nest entrances of *Pariotrigona klossi* obtained from calcareous rocks were having several tubelets (highest reported were with 300 tublets) arranged on interconnected clumps (Benziger, 2011). Such clumps were observed only in one of the five colonies (colony which had five entrance tubes originating from a clump) in the current study. The nests with two entrances had no clumps, however, each tube was very closely constructed or merged partially at the base. This may enhance the stability of the nest entrances as the number of entrance tubes increases. Divya *et al*. (2016) observed two nests of *Tetragonula iridipennis,* each with two entrances, from Poojappura and Tholicode in Kerala. Jose (2015) observed two separate entrance funnels in a *T. iridipennis* nest.

This behaviour of constructing multiple entrances can be considered as a defensive strategy of the stingless bees or to avoid the huge foraging traffic in the strong colonies. The building of a large, single entrance may result in variation in the microclimate of the bee nest, which may adversely affect the colony. This could be a plausible for the construction of multiple entrance holes instead of one big entrance. It is also difficult to manage the intruders as the size of the nest entrance increases and at the same time, an efficient distribution of guard bees can be done by dividing the nest entrance, and thus fortify the defence. All the nests mentioned in the current study were obtained inside stone walls of the foundations of buildings and all of them were free from harsh weather and other disturbances. Benziger *et al.* (2011) stated, in order to construct complex nest entrances they need to be protected well and the nests of *Pariotrigona klossi* obtained from crevices of limestones were protected by overhanging rocks.

Multiple exit holes and platforms were observed in *Tetragona clavipes* and the species also varied with broad and lamellate nest entrances (Roubik, 1979). Roubik (2006) reported construction of tubercles or hollow tubes around the nest entrance of *Lestrimelitta* and occurrence of dual entrances in colonies of *Meliponula ferrugenia* and *Lepidotrigona ventralis*. Jose (2015) reported two openings in a single entrance tube of an artificial hive in *T. iridipennis,* in which both the openings were guarded by bees. In the current study, we observed single entrance tube with multiple openings in a natural habitat (in feral colonies) however, none of them were functional except the opening at the apex of the tube (Fig.7).

The shape of nest entrance of *T. iridipennis* varied from slit-like, circular, oval, funnel-shaped and feral colonies are dominant with slit and circular nest entrance apex (Pavithra *et al*., 2013; Jose, 2015). The shape of entrance apex was similar with in a colony while it varied between colonies such as slit-like, oval or circular. The reported nest entrance length in *T. iridipennis varies* from 6 to 18 mm (Roopa *et al*., 2015), 10.6 to 102.6 mm (Jose, 2015) and 5 to 90mm (Ramya *et al*., 2015). *Tetragonula* colonies without an entrance tube were also reported from Kerala (Jose, 2015). The length of the entrance tube varied from 2.1 cm to 7.6 cm and among them, one colony was without a nest entrance tube and was with a slit-like opening. In addition to environmental cues, the pheromone-regulated nest mate interaction also acts as a stimulus for nest building, when these stimuli become more complex and vigorous, it changes the nest building behaviour and results in the formation of novel building action (Pavithra *et al.*, 2013).

The behaviour of *Tetragonula travancorica* in constructing nests with multiple entrance is

prevalent in different regions of Kerala, however, its frequency is low (2.3%). The factors behind this behaviour or instinct are not known. The knowledge on nesting and nesting site can be a useful tool for designing stingless beehives and their conservation for honey as well as pollination purpose.

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