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A study on antennal sensilla of the female worker castes of *Tetragonula iridipennis* (Smith) (Hymenoptera: Apidae)

***Martin J. Babu and Sujitha C.R.**

Department of Zoology, St. Berchmans College, Changanassery, Kerala-686101, India.

(Email: martinbabu25@gmail.com)

Abstract

Tetragonula iridipennis **are a group of stingless bees which are effective pollinators of the tropics. They are believed to be endowed with efficient chemo (olfactory and gustatory) and visual sensory systems. Sensory perceptions in these insects are understood minimally, so the primary sensory organ – the antenna of the insects was probed to understand the sensilla diversity and distribution. Antennal sensilla profile of an insect often reflects the sensory repertoire of the insect. Light and scanning electron microscopic studies were carried to understand the antennal sensilla organization. Sensilla were typified by their morphological features. Sensilla basiconica, four subtypes of sensilla trichodea, sensilla trichodea curvata, sensilla placodea (pore plates) are the major types of antennal sensilla we could identify from *Tetragonula iridipennis*. The hygroreceptive sensilla ampullacea and CO_2 receptive sensilla ampullacea were conspicuously absent on the antenna of *Tetragonula iridpennis* suggesting differences in the sensory ecology of this bees.**

Keywords: *Stingless bees, antenna, chemosensory system, sensilla, pore plates.*

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Introduction

Diverse sensilla on the antenna of insects help them to perceive vital sensory signals from the surroundings they live, which is a crucial process for their survival and success. Sensilla provide the most important interface for insects with the surroundings as multimodal sensory stimuli like olfactory, mechanical and others like temperature, O_2 and humidity etc. are perceived through these sensory structures. In case of social insects like ants and honey bees volatile and non-volatile chemical signals are the language for communication between conspecifics which is perceived through different types of sensilla on the antenna (Li *et al*., 2013). Foraging, mating and recognizing conspecifics are behaviors in social insects which are predominantly olfactory driven and sensed through antennal sensilla. Though antenna is considered as an olfactory organ endowed with numerous olfactory sensilla, mechano sensory and gustatory sensilla are also present in high numbers. Phylogenetic and life style based differences are reflected in the antennal sensilla types and their distribution of each insect species. Further, in social insects the antennal sensilla shows variations in relation to caste, life stage, sex and behaviours (Herzner *et al*., 2003; Li *et al*., 2013; Carvalho *et al.*, 2017; Galvani *et al.*, 2017). For example in the ant *Oecophylla smaragdina* the major worker castes have a significantly larger number and more diverse profile of antennal sensilla than that of minor workers; sensilla basiconica and sensilla trichodea which are present in high numbers on the major worker antenna are associated with chemosensory perception, crucial for foraging and defense duties they are involved in (Babu *et al*., 2011).

T. iridipennis (Stingless bees) are commonly known as dammer bees and are the smallest of the honey producing bees. Commonly found nesting in gaps of walls, logs, crevices and other concealed areas. They are mainly Neotropical, with certain species reported from tropical areas also (Makkar *et al*., 1854; Rasmussen, 2013; Rahman *et al.,* 2015). Though they are important pollinators, knowledge about their sensory ecology and organization of the chemo sensory systems are still not comprehensively understood. Although literature on the antennal sensilla of other honey bees (Slifer and Sekhon, 1961), *Mellipona spp.* (Ravaiano *et al.,* 2014; Carvalho *et al.*, 2017), Corbiculate bees (Fialho *et al*., 2014) are extensive, but studies pertaining to the stingless bees of Indian terrain in particular *T. iridipennis* is missing. Therefore the present study aims to understand the sensilla types and distribution of sensilla on the antenna of *T. iridipennis* – the most common stingless bee of India. The study could help us to get insights into the peripheral sensory system of the insect in a comparative perspective. Understanding the antennal sensilla organization can give us fair comprehensions about the sensory perceptional capabilities of the insect, say for example whether it relies more on the light or depends on chemo sensory signals for social life based activities. Knowledge about the sensory structure and its organization is important as these insects are economically important and play a crucial role in the ecosystems as pollinators and as ecosystem service providers.

Materials and Methods

T. iridipennis was collected from nearby areas as they are easily available from nests they build on the exterior foundations and walls of houses. They were collected by aspirator and brought to the lab. They were etherized/cold anaesthetized before subjecting to experimental protocols. The antennae were carefully separated by using needle and forceps under a stereo microscope.

Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out to study the detailed sensilla morphology of *T. iridipennis.* The procedure for the method is as follows:

1. Isolated antennae of *T. iridipennis* were immersed in 70% acetone.
2. The specimens were then dehydrated in different grades of acetone.
3. The antenna were then mounted on a brass stub and gold sputtered for 2 minutes (SPI-Module Gold Sputter Coater).

Observations were made using a JEOL JSM - 5800VL scanning electron microscope.

Results

Based on the shape and the other morphological features we could identify six types of sensilla from the antennae of *T. iridipennis,* majority of which appear to be chemosensory suggestive by their shape and structural features. The geniculate antenna of *T. iridipennis* has a flagellum which is approximately 76µm in length with 10 flagellomeres, scape and pedicel. Flagellomeres of the antenna are nearly equal in their length and breadth (Table 1). The characteristic club of flagellomeres seen on the antennae of many social insects is not conspicuous in *T. iridipennis,* however distal most flagellomere is the longest. Flagellomeres are endowed with different types of sensilla (sensory hairs) and sensilla placodea (pore plates) which are the units of the sensory interface for the insect. The distal flagellomeres bears diverse sensilla in comparison to the other flagellomeres. We noticed different types of mechano and chemo sensilla on the antenna. Placodea sensilla, a class of olfactory sensilla was found to be the most prominent type of sensilla on the antenna in terms of their greater number and distribution. A higher number of sensilla indicate a higher degree of sensory perception (Chapman, 1982).

Chemosensilla are predominantly present on the antenna of *T. iridipennis*. In addition, presence of many sub types of mechano sensilla suggests the reasonably well developed sensory repertoire of *T. iridipennis.* Sensilla placodea, sensilla basiconica, and subtypes of sensilla trichodea were observed in the present study (Table 2).

1. Sensilla placodea

(Pore plate sensilla) is characteristic of many bees in particular and is believed to be olfactory in function. It appears oval in shape and has a diameter of 10µm in and distributed on each flagellomeres in varying numbers (Figs. 1b, 2a). We counted on an average 25 sensilla placodea on the distal flagellomeres. However, a reduction in the number of these sensilla was noticed on other flagellomeres indicating a descending pattern in distribution of these sensilla towards the basal flagellomeres.

2. Sensilla basiconica

They projects straight from the antennal surface and are approximately 10µm long with a sensillar shaft which ends bluntly. Apparently, they are abundantly distributed on the tip and lateral sides of the distal flagellomere (flagellomere 10). However, they are conspicuous by their absence on other flagellomeres.

3a. Sensilla trichodea type 1

This sensilla is approximately 12µm in length with a sensillum shaft that tapers gradually and ends with a sharp tip. The base of the sensilla arises from a circular depression below the antennal surface. We noticed this sensilla on the distal most flagellomeres, however the sensilla was absent on other flagellomeres (Fig. 2a). Generally, these sensilla are considered to be mechano sensory in function.

3b. Sensilla trichodea type 2

These sensilla are present on all flagellomeres. They are approximately 15µm in length and have a shaft that has uniform width throughout its length. The basal part of the shaft arises from a small dome like structure of the antenna surface (Fig. 2a). The structure of these sensilla suggests a mechano receptive function.

3c. Sensilla trichodea type 3

These sensilla are sparsely distributed on the flagellomeres. The most striking feature of these sensilla is the highly curved appearance of their shaft. The sensillum has a length of 12µm and is a putative mechano receptor (Figs. 2a and 2b).

3d. Sensilla trichodea type 4

These sensilla area present abundantly on the distal flagellomere, it is approximately 5µm in length with a shaft of uniform width from its base to the tip (Fig. 2b).

4a. Sensilla trichodea curvata type 1

These sensilla are 3µm in length with a sparse distribution as they are distributed on the flagellomere number 1-3. They appear to be chemosensory (Fig. 3a).

4b. Sensilla trichodea curvata type 2

They are present on all flagellomeres except the 8^{th} and 9^{th} flagellomeres. They have a characteristic curved shape of the sensilla shaft. They are 6µm in length. Unlike other sensilla which are distributed in a single direction on the antenna, sensilla trichodea curvata are found to be oriented in different directions on the antenna (Fig. 3b).

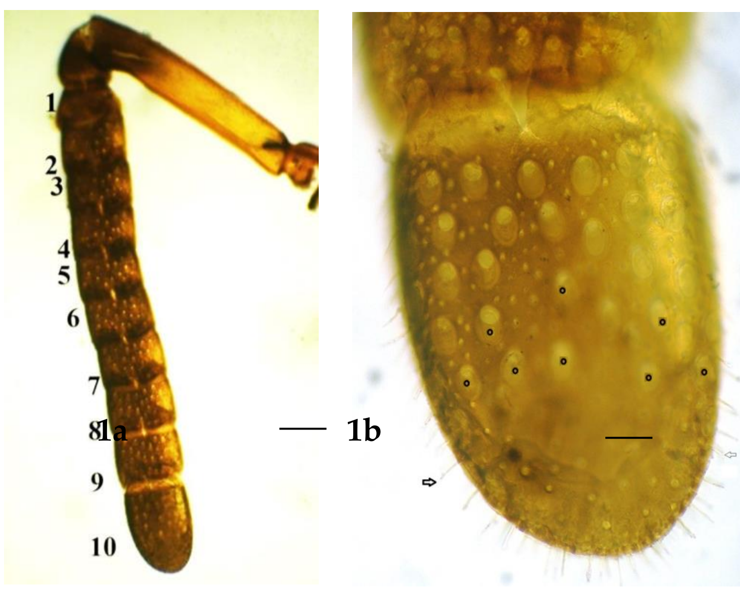

Figure 1: a. Microphotograph of the antenna of *T. iridipennis*; **b.** enlarged view of the distal flagellomere (Flagellomere no: 10) the arrow indicates the sensilla basiconica. Black dots indicate sensilla placodea. Scale bar: 100 µm for fig. 1a and 50 µm for fig. 1b.

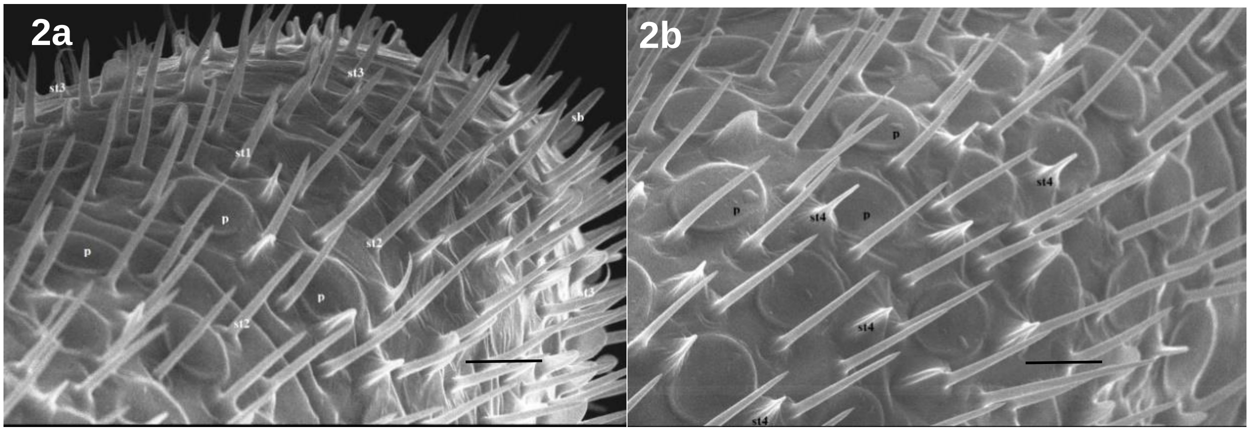

Figure 2: a. SEM image of the flagellomere number 10. p- sensilla placodea, st1- sensilla trichodea type 1, st2- sensilla trichodea type 2, st3- sensilla trichodea type 3. sb- sensilla basiconica; **b.** Scanning electron microscope (SEM) image of the flagellomere 9. p- sensilla placodea, st4- sensilla trichodea type 4. Scale bar 10 µm.

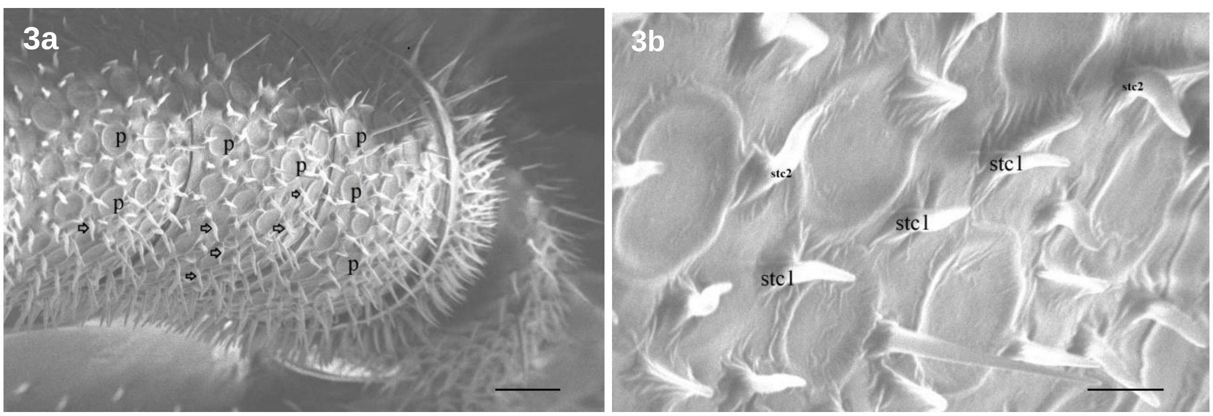

Figure 3: a. SEM image of the flagellomere nine of *T. iridipennis*; p- sensilla placodea, arrow indicates sensilla trichodea curvata, st4- sensilla trichodea type 4. Scale bar 20µm; **b.** SEM image of the lateral area of the flagellomere number 3, 2 and 1; stc1- sensilla trichodea curvata type 1, stc2-sensilla trichodea curvata type. Scale bar 5 µm.

Table 1: Morphometry of antennal flagellomeres of *T. iridipennis*

Flagellomere number	10	9	8	7	6	5	4	3	2	1
Length (µm)	15	15	8	7	8	8	8	7	7	8
Width (µm)	11	11	11	11	11	11	11	10	10	10

Table 2: Characteristic features of the antennal sensilla of *T. iridipennis*

	Sensilla type	Length diameter (µm)	Morphological features
I	**Sensilla basiconica**	10	Long, having blunt end with pores on the shaft and tip pore
II	**Sensilla trichodea 1**	12	Long, straight with pointed tip
	Sensilla trichodea 2	15	Broader base, not very sharp tip
	Sensilla trichodea 3	12	Elongated with curved, prominently pointed tip
	Sensilla trichodea 4 (engraved)	5	Shaft of uniform width
III	**Sensilla placodea**	10	Oval, flattened, and distributed throughout the flagellomeres
IV	**Sensilla trichodea curvata 1**	3	Sparse distribution
V	**Sensilla trichodea curvata 2**	6	Comma shaped with a broad base and tapering and shaft towards the tip

Table 3: Number of sensilla placodea on the flagellomeres

Segment No.	No. of sensilla placodea (pore plates)
1	**12±3**
2	**14±3**
3	**12±6**
4	**10±6**
5	**10±4**
6	**12±8**
7	**24±6**
8	**26±4**
9	**32±6**
10	**34±6**

Discussion

T. iridipennis shows general patterns to other stingless bees based on the types of sensilla they possess and their distribution on the antenna (Month-Juris *et al.*, 2020). However, significant differences are suggestive of the life style influenced changes (Johnson and Howard, 1987). Distal most flagellomeres of *T. iridipennis* bear (flagellomeres: 10, 9 and 8) the most number of diverse sensilla (Figs.1b, 2a and 2b). We observed that the dorsal part of the distal flagellomeres bear sensilla abundantly compared to other flagellomeres. Further, sensilla diversity and the total number of sensilla shows a clear pattern of decrease as one goes from the distal most flagellomere to the basal ones towards the scape. Sensilla placodea are the most abundant sensilla on the antenna of *T. iridipennis*, and this seems to be a prominent pattern in the sensilla distribution which has been corroborated from previous studies on different species of stingless bees. However, the absence of sensilla coeloconica and sensilla ampullacea in *T. iridipennis* is noteworthy; these sensilla being hygro receptive and CO_2 receptive and found to be present in many social insect including some species of stingless bees, their absence in case of *T. iridipennis* indicates difference in the sensory ecology. It is also interesting to note that *T. iridipennis* has a conspicuous reduction in the number of sensilla basiconica on the antenna. Sensilla basiconica is found to be the chief olfactory sensilla of many hymenopterans including ants, and their reduction in numbers suggest a change in the chemo sensory perceptional mechanisms. Sensilla placodea playing the key role as the primary olfactory sensilla in *T. iridipennis* is quite likely.

We could also identify the presence of three unique sensilla on the antenna of *T. iridipennis*, putatively chemo sensory by their appearance: the disheveled sensilla basiconica, the engraved sensilla trichodea and the bifid sensilla. However, electro physiological studies needs to be conducted to ascertain their functional properties.

Sensilla profiles often reflect the sensory ecology of the insects. Our studies suggest that *T. iridipennis* shows many similarities to other stingless bees and in general to other hymenopterans with respect to their antennal sensilla profiles. However, sensilla profile of insects differs in relation to sex, caste and adaptations (Ravaiano *et al*., 2014). Sensilla profile especially the total sensilla number and the types of sensilla an insect possess are probable reflections of an insects interface with the environment. For example, a comparison of the antennal sensilla of *Drosophila* and honey bees reflects phylogenetic and life style based differences of these insects (Stocker, 1994; Kropf *et al*., 2014).

The many subtypes of sensilla trichodea types and the absence of sensilla ampullacea and sensilla coeloconica and the presence of very few campaniform sensilla are the prominent features of antenna sensilla profile of *T. iridipennis.* Sensilla placodea are the most abundant one with the highest number of these sensilla observed on the distal most flagellomeres and a consequent reduction in their numbers in the subsequent flagellomeres towards the basal flagellomeres. This is observed to be a common feature of the chemosensilla distribution noticed in many hymenopterans, where a skewed distribution of certain types of sensilla (Elgar *et al.*, 2018). It is the chemosensory sensilla basiconica which is the most abundant sensilla whereas in many cases of stingless bees it is the sensilla placodea as in the case of *T. iridipennis*.

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