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Journal of Threatened Taxa

10.11609/jott.2023.15.10.23931-24150

www.threatenedtaxa.org

26 October 2023 (Online & Print)

15(10): 23931-24150

ISSN 0974-7907 (Online)

ISSN 0974-7893 (Print)



Open Access





ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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Cover: Orange Oakleaf *Kallima inachus* with colour pencils and watercolor wash by Elakshi Mahika Molur adapted from a workshop by Lenin Raj.



Fine structure of sensilla on the proboscis of the Indian Honey Bee *Apis cerana indica* Fabricius (Insecta: Hymenoptera: Apidae)

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Abstract: Honey bees feed on flowers from which they collect nectar and pollen and their mouth parts are designed for fluid-feeding from flowers. The proboscis consists of a 'tongue' that includes a long glossa and ends in a spoon-shaped labellum, labial palp, galea and mandibles. The sensilla on the proboscis assists in nectar feeding. A study of the chemosensory hairs on the proboscis was carried out in *Apis cerana indica* collected from apiaries at the foot of Western Ghats, India. Light- and scanning electron microscopy were employed. In addition, silver staining was carried out to distinguish different types of chemosensilla. The glossa has 60 sensilla chaetica that stain by silver nitrate technique. The length (110 μ), width (2 μ) and spacing of microtrichia on glossa and forked hairs on the labellum are suited for the collection of nectar due to viscosity and to reduce leakiness while feeding. The length of the glossa being short suggests that *A. cerana indica* feeds on small-sized flowers that are not tubular. The labial palp has sensilla chaetica A and sensilla chaetica B distinguished by their length and sensilla basiconica, all of which are silver nitrate-positive and thus chemosensory in nature. Distal galea has sensilla basiconica, sensilla chaetica A and B and sensilla coeloconica. The maxillary palp is a mechanosensory structure. The bulge on the galea near the maxillary palp has chemosensory sensilla chaetica. Mandibular hairs did not stain with silver and are hence mechanosensory. The sensilla on proboscis in *A. cerana indica* is comparable to mouth part sensilla in *Apis mellifera* and *Apis florea*. The position of the chemosensilla at different regions suggests their role in tasting nectar, detecting the flow of nectar, and the dimensions of the flower and pollen.

Keywords: Basiconica, chaetica, coeloconica, epipharynx, hotspots, olfactory, sensory, silver-staining, taste, Western Ghats.

Editor: Tushar K. Mukherjee, Kolkata, West Bengal, India.

Date of publication: 26 October 2023 (online & print)

Citation: Krishna, A.G.S., S.V. Raghu & R.K. Patil (2023). Fine structure of sensilla on the proboscis of the Indian Honey Bee *Apis cerana indica* Fabricius (Insecta: Hymenoptera: Apidae). *Journal of Threatened Taxa* 15(10): 24054–24062. <https://doi.org/10.11609/jott.8548.15.10.24054-24062>

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Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: SK performed all the experiments; SVR and RKP analyzed the data and wrote the paper.

Acknowledgements: Electron microscopy facility at the DST-PURSE laboratory was used for the study and authors thank Dr. M Murari for SEM work. Facilities at SDM Medical College, Dharwar and P C Jabin College of Arts and Science, Hubballi were used for observations and microphotography. Dr. SV Raghu is grateful to Dept of Biotechnology (DBT), Govt of Indian for DBT-Ramalingaswami Re-Entry Fellowship.



INTRODUCTION

In the course of evolution, flowering plants have developed form, colouration, and nectar to entice bees for pollination. Visit to flowers is the major point of interaction between plants and bees, with the bee getting nectar and pollen and the flower getting pollinated. Palynological studies to deduce host plants' sources of nectar and/or pollen are a favored approach to identify the host plants (Lau et al. 2019) and to reveal the plants preferred by honey bees. The choice of food plants by honeybees is a deliberate process and involves learning, memory and nutritional requirements. The mouth part of honey bees can be expected to assist feeding and their structure can be related to flower morphology and location of nectar and pollen.

The Asiatic Honeybee *Apis cerana indica* F. is distributed in southeastern Asia and the Indian subcontinent (Jaffe et al. 2010). It is suitable for apiculture along the Western Ghats, one of the biodiversity hotspots. To protect honeybee production and conserve bees, there is a need to gain insights into the biology of *Apis cerana indica* F. and its principal sources of nectar and pollen. The distribution of *Apis cerana indica* and its pollen sources in the vicinity of Mangalore near the Western Ghats region has been surveyed by a palynological analysis (Krishna & Patil 2019). The principal pollens found in honey samples in the study were *Areca catechu*, *Cocos nucifera*, *Hopea* sp., *Ixora coccinea*, *Mimosa pudica*, and *Psidium guajava*. *A. cerana indica* is a short-range forager and its foraging is restricted to a radius of ~500 m from the hive (Punchihewa et al. 1985). While sensilla on the galea of honeybees respond to salts, sugars (Whitehead & Larsen 1976a) and umami (taste of amino acids) as reported by Lim et al. (2019), they have a limited number of taste receptors (Sanchez et al. 2007). It is believed that honeybees have limited gustatory abilities (Monchanin et al. 2022). They may, therefore, be unable to detect and avoid pesticides and hence vulnerable to exposure to pesticides. It is essential to understand the gustatory abilities of honey bees and there is a need to survey the chemosensilla on their mouth parts, which is the focus of the present study.

Drinking nectar and gathering pollen is a specialized mode of feeding in insects. They need structural specialization of mouth parts (Krenn et al. 2005) and their operation. Secondly, chemoreception is crucial for foraging and feeding. The broad types of sensilla in insects and in honey bees are described by (Esslen & Kaissling 1976). Preliminary studies on the olfactory

capabilities of honeybee *A. cerana indica* have been carried out wherein the ultrastructure of the antenna and electroantennogram has been studied (Bhowmik et al. 2016). The proteome of antennae of *A. mellifera linguistica* and *A. cerana* drones and workers suggest differences in the olfactory capabilities of the two species (Wolstedji et al. 2012) suggesting differences in olfactory senses. Such mechanisms may also affect exclusive food preferences. Work on contact chemoreceptors on the mouthparts of *Apis mellifera* has been carried out by (Galic 1971) and (Whitehead & Larsen 1976a,b) and on *Apis florea* (Kumar & Kumar 2016). While studies on *A. florea* focused on sensilla types only, Whitehead & Larsen (1976b) studied the number and innervations of sensilla using both transmission and scanning electron microscopic studies. The response properties have been studied by electrophysiology by Whitehead & Larsen (1976a) and shown to sense salts and sugars but not water. Galic (1971) studied the chemoreceptors in the epipharynx and hypopharynx. Study of insect feeding behavior requires knowledge of its taste repertoire and distribution of taste hairs on the mouth parts is necessary for this purpose. The form of mouthparts in honey bees is a determinant of the type of flower that the honey bee feeds on. The present study focuses on the distribution and organization of sensilla of the mouthparts of *A. cerana indica* F. We used scanning electron microscopic and silver nitrate staining approaches (Babu et al. 2011) to characterize the sensilla and its distribution. The current study reliably differentiates chemoreceptor sensilla from mechanoreceptor sensilla and describes their functional role in nectar feeding.

MATERIAL AND METHODS

Microscopic Observations

Asian worker honey bees *Apis cerana indica* were collected from apiaries in Puttur (12.7687° N, 75.2071° E, 87 m elevation), Karnataka, India and the region is located at the base of the Western Ghats. Fifty honey bees were collected from colonies and seven bees were randomly selected for SEM analysis. The bees were dissected with the help of fine forceps, the mouthparts were carefully excised under a dissecting microscope and later fixed in 4% glutaraldehyde (in 1 M phosphate buffer) for four hours. The dirt on the bees was cleaned by ultra-sonication (Sidilu Ultrasonic, Bangalore) for 5 s. The mouth parts were washed further in distilled water followed by serial dehydration. The whole mounts were prepared and mounted using DPX. The number of

Table 1. Chemosensory hairs were found on different regions of mouthparts of *A. cerana indica* (present study). The numbers in *A. mellifera* were reported by (Whitehead & Larsen 1976b). The numbers are comparable, except in the region of glossa where *A. cerana indica* has less sensilla chaetica A. Sensilla coeloconica occurs at the distal end of galea and these sensilla on galea have not been reported so far in honey bees. Sensilla chetica A (SC A), Sensilla chetica (SC B), Sensilla basiconica (SB) and Sensilla coeloconica (S CO) have not been reported earlier in honeybees (*Apis mellifera* and *Apis florea*)

	<i>A. Mellifera</i>		<i>A. cerana indica</i>					Sample size
	SC (A)	SC (B)	SC (A)	SC (B)	S. Ba	S.CO		
Region								
Glossal tip	12 (6 + 6)		12 (6 + 6)					7
Distal glossa	66–78	0	21 ± 3	4				7
Labial palp segment 1	0	0	0	0				7
Labial palp segment 2	4–6	8–13	3	6				7
Labial palp segment 3	10	11–15	10	4				7
Labial palp segment 4	7–9	9–12	8 ± 1	7 ± 1				7
Distal galea	12–16	10–16	11 ± 1	22 + 3	4	12 ± 2		7
Adjacent to maxillary palp	0	43–47	0	33 ± 2				7

samples observed is provided in Table 1.

Scanning Electron Microscopy

The samples were fixed in absolute alcohol or Karnovsky's fixative (Karnovsky 1964). Specimens were dehydrated by incubating through a series of alcohol grades. After a final wash in acetone, the specimens were gold-coated at 5 nm thickness using a Class I gold sputtering system and observed using a field emission scanning electron microscope (FESEM, Carl Zeiss Ltd., Germany). The images were processed using Adobe Photoshop. The sensilla are named based on the description provided by Callahan (1975) and that of Whitehead & Larsen (1976b) for *Apis mellifera*.

Silver Nitrate Staining

Porous chemosensilla were stained with silver nitrate following the method described by (Babu et al. 2011) and observed using an Olympus BX51 microscope. Anesthetized bees were washed with 1% acetone and incubated in 1% silver nitrate (AgNO_3) for five minutes. Subsequently, they were washed thrice for two minutes each to remove AgNO_3 . The specimens were treated by soaking the samples in photo/film developer for five–seven minutes. Then the specimens were immediately rinsed in 3% acetic acid for one minute. The specimens were dehydrated using 70%, 80%, 90%, and 100% alcohol (10 minutes each). The dehydrated samples were washed in methyl salicylate. The antenna/maxillary palp was mounted on a slide using DPX.

Nomenclature of Sensilla

Sensilla is named based on the descriptions by Callahan (1975) and of Whitehead & Larsen (1976b) for *Apis mellifera*. The naming of sensilla by (Kumar & Kumar 2016) in *A. florea* is ambiguous.

RESULTS

Sensilla on the segments of the mouth parts, viz., glossa, labellum, labial palp, galea including maxillary palp are described here. Glossa and labellum together measure 1.8 ± 0.1 mm in length and 120 ± 8 μm in width. The labellum is oval measuring 120 ± 7 μm and 80 ± 6 μm . The glossa bears hairs to collect nectar by surface tension and capillarity. The labellum has forked hairs on the dorsal side whereas the ventral surface is bald (Image 1). The grooved labellum assists in sucking the nectar and also helps draw in nectar due to capillarity. The glossa is sheathed by labial palp and galea. The various types of sensilla that were observed are:

1. Sensilla chaetica, type A and type B, based on their length.
2. Sensilla basiconica, and
3. Sensilla coeloconica.

The base of the labellum has six sensilla chaetica on either side, slightly curved, measuring 40 ± 4 μm in length. They are stained by the silver staining technique and are therefore chemosensory and possibly gustatory in function (Image 1). Along the distal two-thirds of the glossa, there are sensilla, measuring 30 μm , thus shorter in length. They are silver-positive. A large number of thin

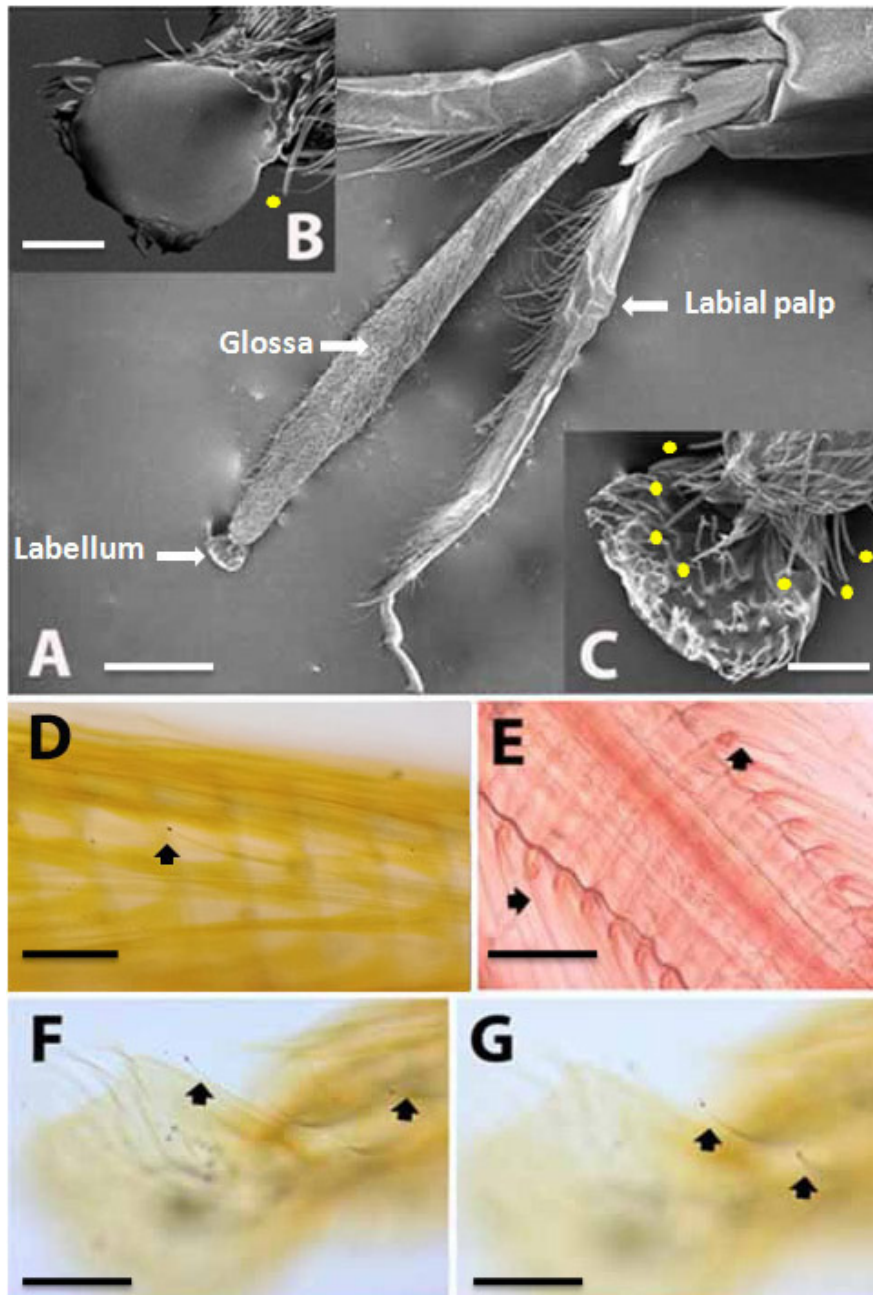


Image 1. Glossa and labial palp of *A. cerana indica*. Galea is removed for clarity and shown in Image 3: A—the elongated glossa measures 1.9 mm long with an oval labellum measuring (100 μ) | B—dorsal side of the labellum has forked hairs (arrowhead) | C—the ventral side of the labellum, taste hairs are indicated by asterisk | D—glossa has annular arrangement with microtrichia and silver stained trichoid sensilla (arrow) | E—there are about 90 annuli on the rim of which microtrichia are arranged with interspersed trichoid sensilla (arrow) | F & G—taste hairs at the distal region at the base of the labellum are stained with silver nitrate and are indicated by arrow. Scale Bar: A—100 μ m | B–G—20 μ m. © Rajashekhar Patil.

microtrichia ($120 \pm 9 \mu\text{m}$ long and $2 \pm 0.12 \mu\text{m}$ wide) cover the glossa facilitating the collection of nectar. They are placed at a distance of $20 \pm 1.1 \mu$ from each other on the annulus.

The labial palp has four segments with segments 3 and 4 protruding out of the galea that en-sheath them (Image 2). The length of segments 1 and 2 helps

the segments protrude out of the galea and segments distal regions segment 2, segment 3, and segment 4 have chemosensilla. There are three broad types of sensilla – sensilla chaetica A, sensilla chaetica B, and sensilla basiconica. Their absolute numbers are provided in Table 1. These are clearly stained by silver staining, predominantly at their tips.

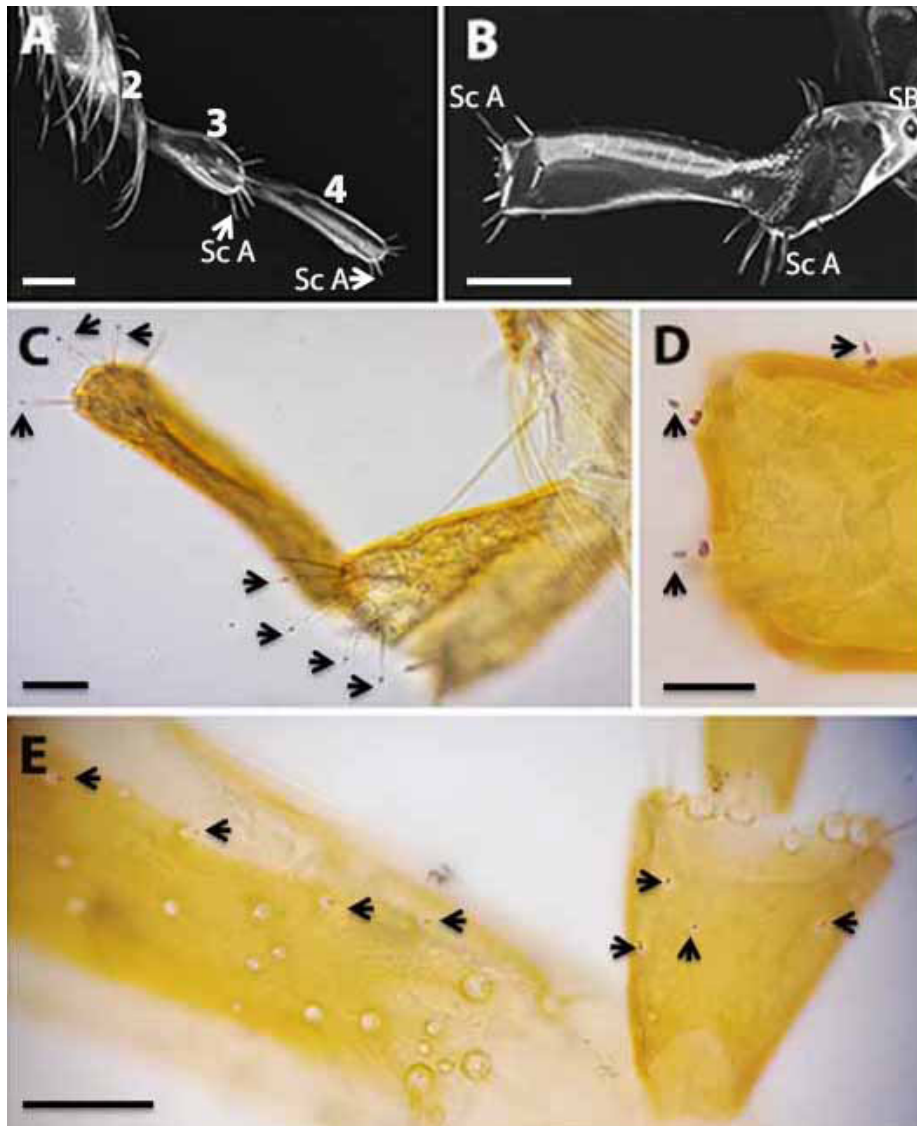


Image 2. Labial palp of *A. cerana indica*: A—dorsal view of the 2nd, 3rd, & 4th segments | B—ventral view of segments 2 & 3, showing sensilla chaetica A (ScA), and sensilla basiconica (SB). Their actual numbers are given in Table 1. A cobble-stone-like cuticle is seen at the joints in which is supposed to provide lubrication while folding of joints due to dipping of labial palp in nectar. The arrangement reduces drag in a high-viscosity fluid. Silver-stained chemosensilla are seen in C, D, & E | C—sensilla chaetica (ScA) (arrow) | D—Sensilla basiconica are marked with arrow | E—Silver-stained sensilla chaetica B on segments 2 & 3. Scale Bar: A, B & C—40 μ m | D—10 μ m | E—20 μ m. © Rajashekhar Patil.

The galea is tapered and en-sheaths the labial palp and the glossa. It bears 12 sensilla coeloconica on the proximal region and sensilla chaetica A measuring $12 \pm 1 \mu\text{m}$ and sensilla chaetica B measuring $20 \pm 1.8 \mu\text{m}$ (Image 3). The ventral surface has four sensilla basiconica which have been described hitherto in *A. mellifera* or *A. florea*. Sensilla chaetica are identified to be chemosensory by being permeable to silver nitrate. The second segment of the maxillary palp bears nine mechanosensory hairs measuring $12 \mu\text{m}$ to $24 \mu\text{m}$ that are not porous. The bulged surface of the galea at the base of the maxillary palp bears a group 34 ± 2 sensilla basiconica measuring

$2 \pm 0.2 \mu\text{m}$ that is permeable to silver (Image 4). The mandibles bear numerous microtrichia measuring $10 \mu\text{m}$ and are possibly mechanosensory as they are not stained by silver.

DISCUSSION

Feeding in honeybees is well-studied by several authors as summarized by (Düster et al. 2018). A recent study shows that honeybees can switch from lapping to sucking mode when needed (Wei et al. 2023). While

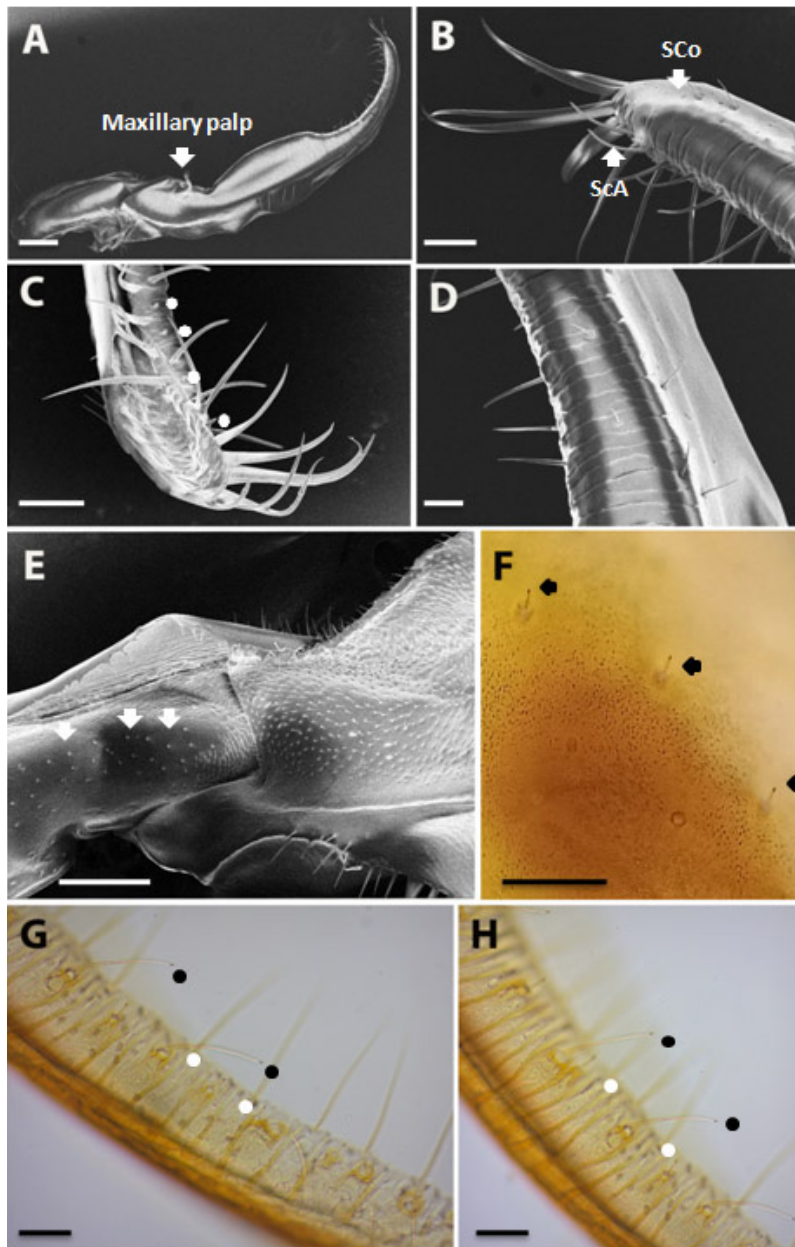


Image 3. Scanning electron micrographs of galea: A—entire galea in lower magnification. The arrangement of sensilla on the distal region is shown in B, C, & D | B—arrangement of Sensilla chaetica A, Sensilla chaetica B, Sensilla coeloconica on the dorsal side of distal region | C—ventral side of distal region showing Sensilla basiconica (asterisk and inset). D—Medial region | E—the bulge adjacent to maxillary palp (Sc A—Sensilla chetica A | Sc B—Sensilla chetica B | SCo—Sensilla coeloconica) | F—Silver stained (asterisk) corresponding to sensilla shown in E suggesting them to be chemosensory which number about 33. Scale bar: A—100 μm | B—D—20 μm | E—200 μm | F—H—20 μm . © Rajashekhar Patil.

feeding nectar, the erectable microtrichia gets extended due to the viscosity of the nectar. This event helps to collect nectar by surface tension. Nectar is held between microtrichia due to the length of the microtrichia and is influenced by the viscosity of nectar (He et al. 2020). The taste hairs observed in the present study are shorter than microtrichia and may provide information about nectar contents. The taste hairs are largely hidden among the microtrichia and cannot be observed by

scanning electron microscopy. Silver staining and transmitted light microscopy help reveal taste hairs (Image 1). The glossa is a long structure with an oval labellum. It is made up of annular segments which number about 90. The hairs are arranged on the margins of the annuli. Sensilla chaetica, possibly taste hairs and stained at the tip by silver are found along with setae. There are taste hairs for providing chemosensory input during feeding. The present work excludes the sensilla

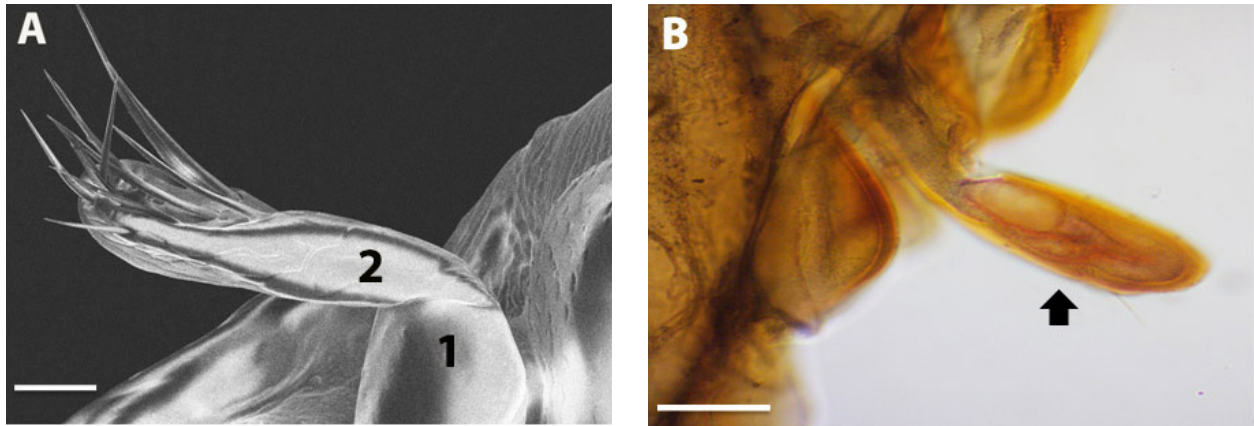


Image 4. Maxillary palp on the galea of *A. cerana indica*: A—SEM image of maxillary palp showing mechanosensory hairs (segments 1 & 2) | B—these hairs are silver-negative suggesting them to be mechanosensory. Scale bar: A & B—10 µm. © Rajashekhar Patil.

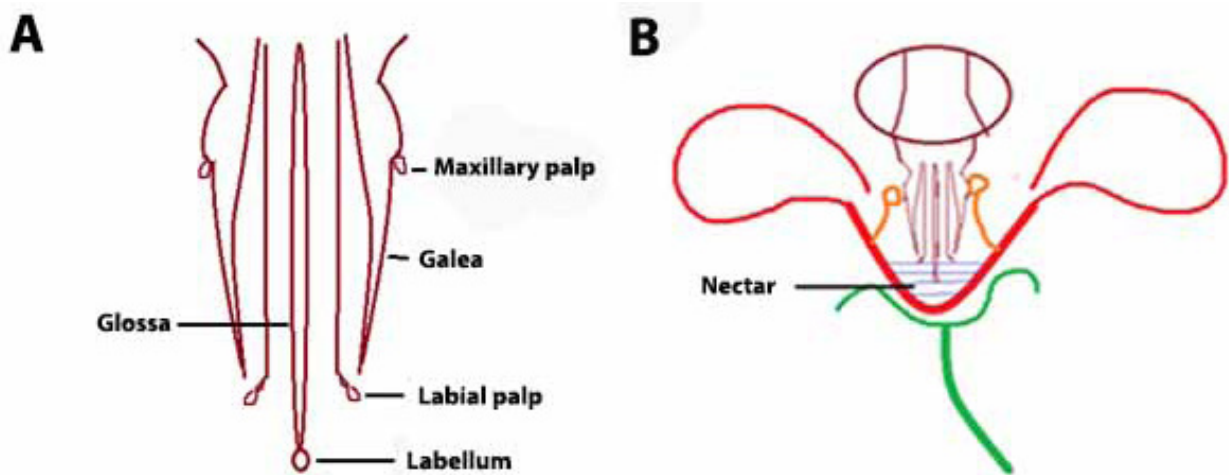


Image 5. A—schematic diagram of the proboscis of *A. cerana indica* | B—the position of different appendages of mouth parts in relation to parts of a flower indicate the possible role played by sensilla in feeding. The distal half of glossa dips into nectar. The taste hairs on glossa, labial palp and galea may help sense nutrients and flow of nectar. The labial palp is also designed to reduce drag encountered in viscous nectar. Maxillary palp is mechanosensory and may provide information on dimensions of flower (Duster et al. 2017). A patch of chemosensory hairs posterior to maxillary palp are suitably placed to come in contact with the anther. Length of the glossa suggests that the proboscis is designed for non-tubular polypetalous flowers (Style μ and stigma not shown). Flowers of: B—*Cocos* | C—*Areca* | D—*Mangifera*, frequently visited by *Apis cerana indica*. © Rajashekhar Patil.

in the groove identified by (Whitehead & Larsen 1976b). The taste hairs at the distal regions protruding out from among the microtrichia are comparable to the distal taste hairs of *A. mellifera* (Table 1) and *A. florea*. The number of taste hairs along the length of the glossa is lesser in *A. cerana indica* than in *A. mellifera*. A Sensilla number is suggested to enhance sensitivity. However, the difference between the two species (*A. mellifera* and *A. cerana indica*) is not large. The forked hairs of the labellum may increase the ability to take up and retain nectar.

Taste hairs occur on the distal region of segments 3 and 4 of the labial palp. They are positioned to come in contact with nectar/food while feeding. Their number and types are comparable to labial palp hairs found to occur in *A. mellifera* and *A. florea*. Segments 1 and 2 are long to ensure that they protrude beyond the envelope of galea. The arrangements of micro-protuberances seen in the articulations of segments 3 and 4 of the labial palps help reduce resistance during feeding in *A. mellifera* (Ji et al. 2017). This region has been wrongly attributed as plate sensilla by Kumar & Kumar (2016) in *A. florea*. The sensilla basiconica occur on the surface of segments 2, 3, and 4 and may also act as olfactory hairs.

The sensilla chaetica A of the galea are long and are less in number than sensilla chaetica B. The shorter sensilla chaetica B are 12 in number and the two types are well positioned to be contact chemosensory hairs (Image 3). These hairs may also help sense the occurrence of pollen grains and the stage of floral development. The maxillary palp appears to be mechanosensory in function as none of the hairs are stained with silver (Image 4). Their function as mechanosensory is suggested by (Whitehead & Larsen 1976a). Similarly, the mandibles have hairs that do not stain with silver and one sensillum was found to innervate them by TEM studies (Whitehead & Larsen 1976a), suggesting them to be mechanosensory. The observations in the present study using silver nitrate and the previous TEM study corroborate each other. Honeybees have adapted to exploit different types of nectar/pollen sources. The length of glossa in *A. mellifera* (3.3 mm measured from illustrations of Zhu et al. (2016) and *A. cerana indica* (1.8 mm, present study) render them suitable for foraging small-sized flowers. Bees such as *Euglossa championi* (glossa length 11.25 mm) and *Euglossa imperialis* (glossa length 22.25 mm) forage on long, tubular orchids. The length of an orchid bee proboscis is three–five times more than that of honey bees. Despite the differences in lengths, the types of sensilla in the three species of *Apis* (Whitehead & Larsen 1976b; Kumar & Kumar 2016;

present study) and in Euglossini (Düster et al. 2018) are comparable. The observations that the number of sensilla does not differ much and their positions are comparable has prompted (Düster et al. 2018) to make interesting suggestions summarized below: (1) The sensilla of galea and labial palp provide information on nectar availability and quality, (2) The flexed segments 3 and 4 of labial palp helps detect whether flowers are open and (3) The hairs on inner galea, labial palp and between microtrichia of labellum detect the flow of the nectar. The mouth parts of honey bees are thus crafted and endowed with strategically placed sensilla to probe the morphology, nutrient quality and the stage of blooming of flowers for feeding.

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

October 2023 | Vol. 15 | No. 10 | Pages: 23931–24150

Date of Publication: 26 October 2023 (Online & Print)

DOI: 10.11609/jott.2023.15.10.23931-24150

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– Lal Tlanhlu, Margaret Lalhlupuii, Sanatombi Devi Yumkham & Sandhyarani Devi Khomdram, Pp. 24135–24139

Notes

First sighting record of Western Reef-Heron *Egretta gularis* (Bosc, 1792) (Aves: Pelecaniformes: Ardeidae) from Jammu & Kashmir, India

– Parvaiz Yousuf, Semran Parvaiz, Nisheet Zehbi, Sabia Altaf, Showkat Maqbool, & Mudasir Mehmood Malik, Pp. 24140–24143

Rare desmid genus *Bourrellyodesmus* Compère (Chlorophyceae: Desmidiaceae) in India with description of a new species (*Bourrellyodesmus indicus* Das & Keshri sp. nov.) from eastern Himalaya, India

– Debjyoti Das & Jai Prakash Keshri, Pp. 24144–24147

Threats faced by *Humboldtia bourdillonii* Prain (Magnoliopsida: Fabales: Fabaceae), an endangered tree endemic to the southern Western Ghats, India

– Jithu K. Jose & K. Anuraj, Pp. 24148–24150

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