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Ultramorphology of antennal sensilla of open-nesting honey bees *Apis florea* F. and *Apis dorsata* F. (Hymenoptera: Apidae)

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Abstract: Antenna of honey bees is geniculate and has been known to possess a wide variety of sensilla. The sensilla are sensitive to odor, temperature, humidity, air pressure and gustatory stimuli. In the present investigation, ultrastructural study on the antenna of the two open-nesting worker honey bees- *Apis florea* F. - the dwarf honey bee and *A. dorsata* F. - the giant honey showed considerable amount of variation in the types and distribution pattern of sensilla in the two species. The antennal form as well as the sensilla arrangement has been suggested to be adapted to the pheromone perception need of regarding a particular species.

Keywords: *Apis florea*, *Apis dorsata*, Antenna, SEM, Sensilla

INTRODUCTION

South-East Asia is the centre of honey bee diversity and the evolutionary homeland of honey bees. *Apis florea* and *A. dorsata* are open-nesting bees which build single comb. The head appendages constitute an important morphological feature to study honey bee systematics. The antenna of honey bees is important for understanding behavior of honey bees. It consist of a basal scape, pivoted pedicel and a ten-segmented flagellum. Antennae are important for sensory perception. Various types of sensilla are observed on the segments of flagellum. According to Wigglesworth (1965), the antennae are the main sites of olfactory receptors in most insects. Six types of antennal sensilla have been observed on the flagellum of honey bee *A. mellifera* drone and worker. They have been referred to as a thick basiconic sensillum, a tapered basiconic sensillum, a trichoid sensillum, a placoid sensillum, a coeloconic or ampullaceal sensillum and a coelocapitular sensillum, formerly known as campaniform sensillum (Kuwabara and Takeda, 1956; Dietz and Humphreys, 1971). SEM studies on antennal sensilla of honey bee workers have been performed by Al-Ghamdi (2006) from Saudi Arabia. Suwannapong and Wongsiri (2004) described occurrence of sensilla trichodea types A, B, C and D on different segments of flagellum from Thailand. Therefore, the aim of the present study is to document the diversity of *Apis* species by using the scanning electron microscopic technique of an important morphological character *i.e.* the antenna.

MATERIALS AND METHODS

Study material: Two open-nesting species- *Apis florea* F. and *A. dorsata* F. were taken for the present study. *A. dorsata* and *A. florea* are found wild in nature. *A. dorsata* contributes towards large amount of forest honey and bees wax harvested by tribal people of India.

Study area and sample collection: *A. florea* was collected from nests located in a hedges and bushes in Panjab University Campus while *A. dorsata* was collected while foraging on flowers in Botanical garden, Panjab University, Chandigarh.

Preservation: The collected material of *Apis florea* and *Apis dorsata* was preserved in 70% alcohol and the protocol of Bozolla and Russell (1999) was followed.

Preparation of material for scanning electron microscopy: The antennae were carefully excised from the freshly collected worker bees of *Apis florea* and *Apis dorsata*. These were then washed with phosphate buffer. The samples were fixed in 5% gluteraldehyde for 2 hrs. Subsequently these were washed with phosphate buffer 2 to 3 times and then dehydrated through graded series of acetone and dried in a critical point drier. Dehydrated samples were mounted on slides in the desired orientation with the help of double side adhesive tape under binocular microscope. The samples were attached in such a way that they became visible from all sides. The stubs were placed inside the sputter for gold coating to overcome the problem of "charging" and "beam damage". The sputtered specimens were examined in Jeol

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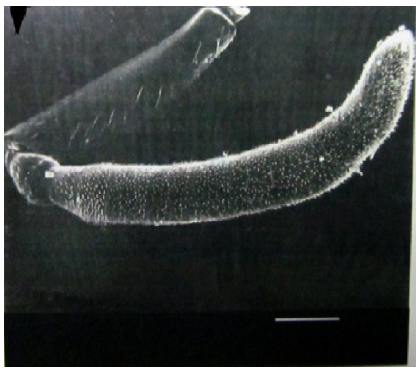

Fig. 1. *Antenna of Apis florea (Bar= 100 µm).*

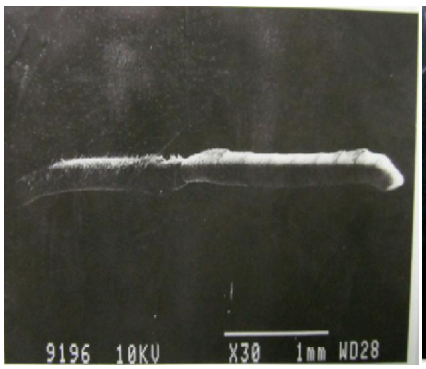

Fig. 2. *Antenna of Apis dorsata.*

Fig. 3. *SEM of scape of antenna of Apis florea (Bar= 100 µm).*

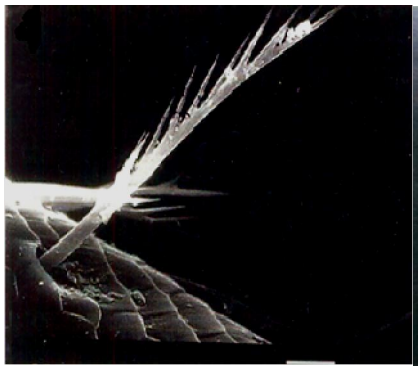

Fig. 4. *Higher magnification of first type (serrated) type of sensilla on scape (Bar=10 µm).*

Fig. 5. *Higher magnification of second type of sensilla on scape (Bar= 1 µm).*

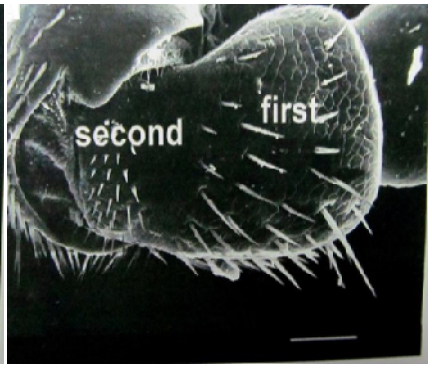

Fig. 6. *Higher magnification of pedicel showing two types of sensilla (Bar=1µm).*

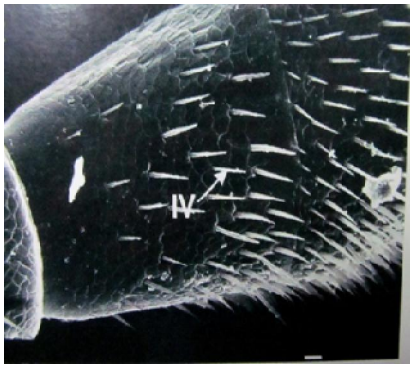

Fig. 7. *SEM of first segment of flagellum of antenna of A. florea showing S. trichodea of type IV (Bar= 10 µm).*

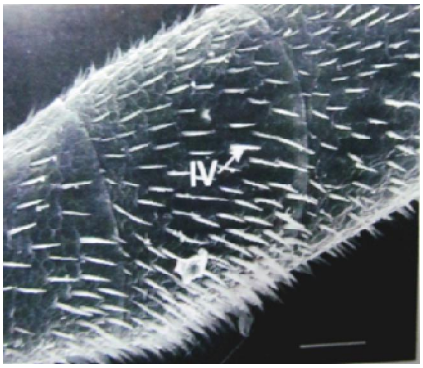

Fig. 8. *SEM of second segment showing S. trichodea type IV (Bar=100 µm).*

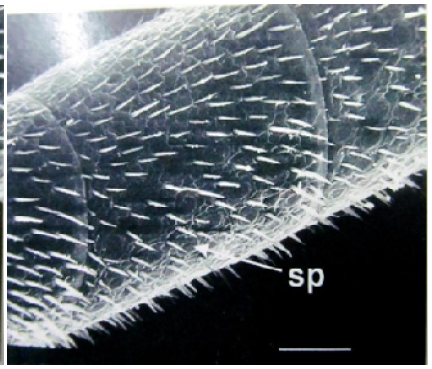

Fig. 9. *SEM of third segment showing S. trichodea type IV and S. placodea (Bar= 100 µm) sp= S. placodea.*

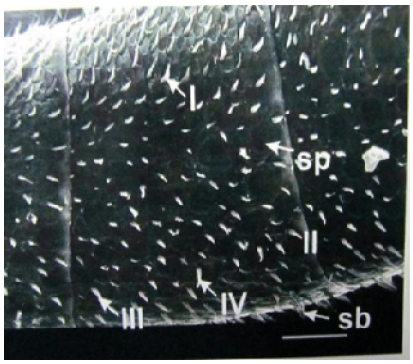

Fig. 10. *SEM of eighth segment showing S. trichodea type I, II, III, IV, S. placodea (sp) and S. basiconica (sb) (Bar=10 µm).*

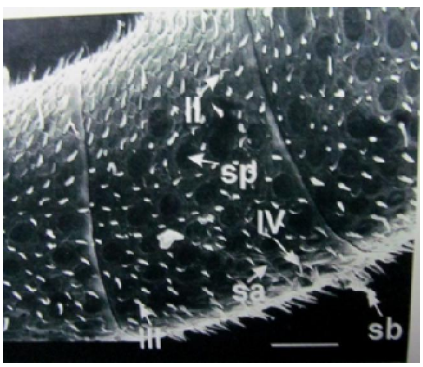

Fig. 11. *SEM of ninth segment showing S. trichodea type I, II, III, IV, S. placodea, S. ampullacea (sa) and S. basiconica (Bar= 10 µm).*

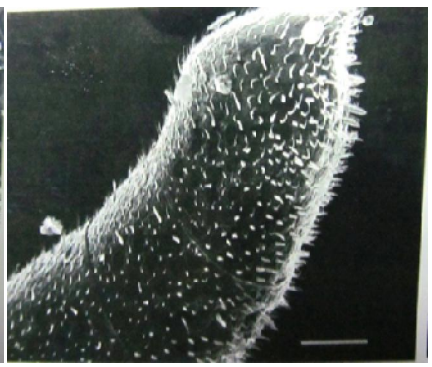

Fig. 12. *SEM of tenth segment (Bar= 100 µm).*

Fig. 13. *Magnification of tenth segment showing S. placodea and S. trichodea type II, III (Bar= 10 µm).*

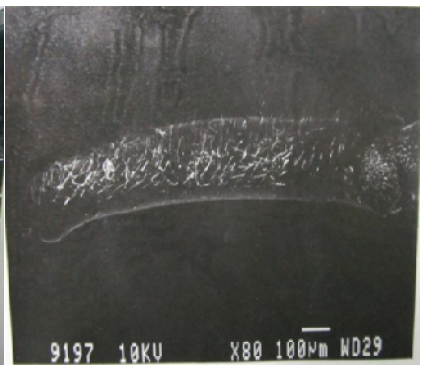

Fig. 14. *SEM of scape of Apis dorsata.*

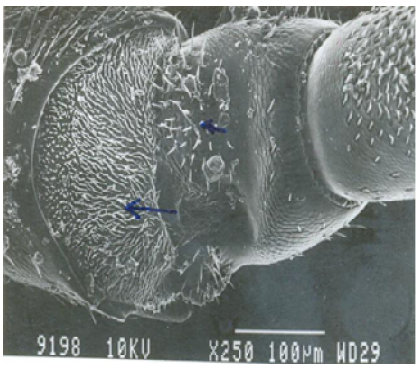

Fig. 15. *SEM of the pedicel of Apis dorsata showing sensilla type I (long arrow) and type II (short arrow).*

Fig. 16. *SEM of the pedicel of Apis dorsata showing type I sensilla at higher magnification.*

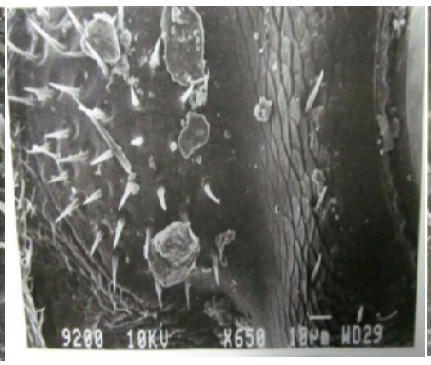

Fig. 17. *SEM of the pedicel showing type II sensilla.*

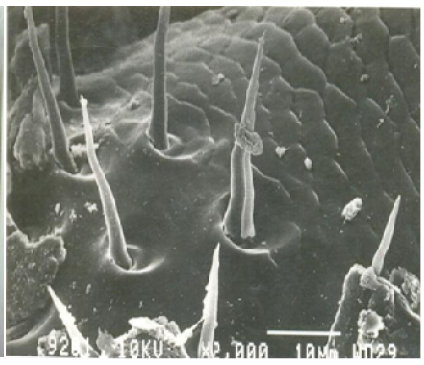

Fig. 18. *SEM of the pedicel showing type II sensilla at higher magnification.*

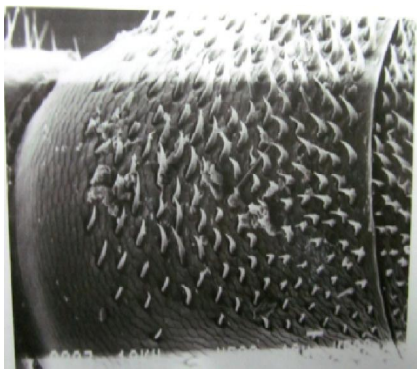

Fig. 19. *SEM of first segment of antenna of Apis dorsata showing sensilla trichodea type I.*

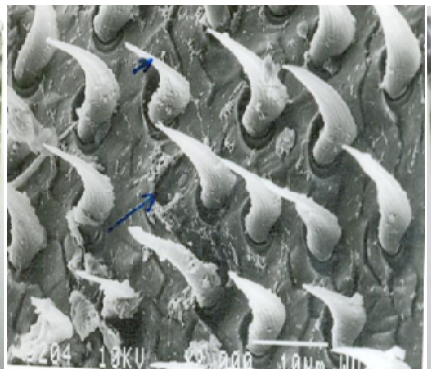

Fig. 20. *Higher magnification of the same segment showing S.trichodea (small arrow) and S. campaniformia (long arrow).*

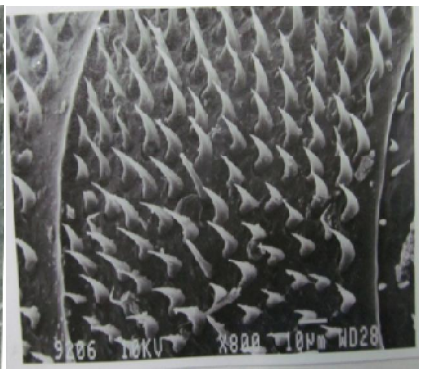

Fig. 21. *SEM of second segment of flagellum of Apis dorsata showing S. trichodea type I.*

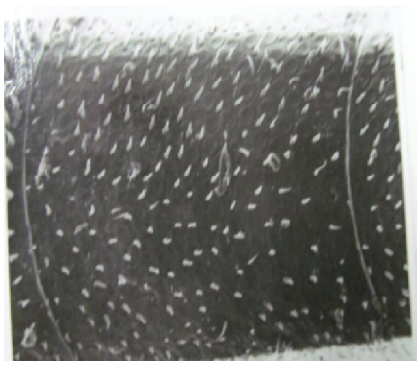

Fig. 22. *SEM of third segment of flagellum of Apis dorsata..*

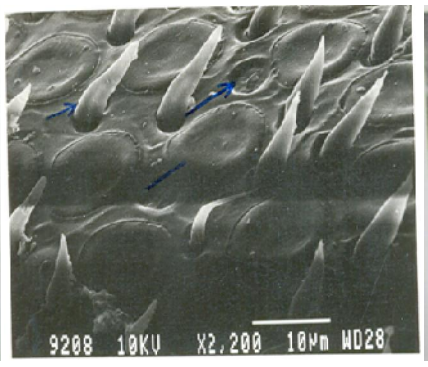

Fig. 23. *SEM of third segment at higher magnification showing S. trichodea type III (small arrow); S. campaniformia (long arrow); S. placodea (simple line).*

Fig. 24. *Still higher magnification of the same segment showing S. placodea.*

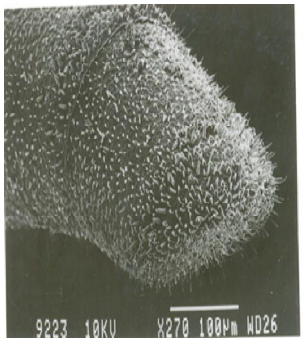

Fig. 25. *SEM of tenth segment of flagellum of Apis dorsata.*

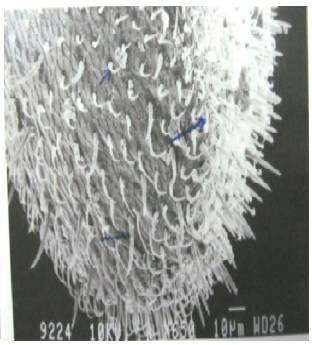

Fig. 26. *Higher magnification of the same segment showing S. trichodea type I (Long arrow), II (short arrow) and IV (straight line).*

JS-6100 scanning electron microscope operated at an acceleration voltage of 10KV at Regional sophisticated instrumentation centre, Panjab University, Chandigarh. The results of scanning were preserved as photographs used in this presentation.

RESULTS

The antenna of both the *Apis* species showed 3 distinct regions under the scanning electron microscope, the scape attached to pedicel which bears the flagellum (Figs. 1 and 2).

Apis florae: The scape, pedicel and segments 1st, 2nd, 3rd, 8th, 9th and 10th of flagellum were taken into account.

Scape: Two types of hair like sensilla were present on the scape (Fig. 3). One type was longer and present toward the outer surface of the scape. This showed distinct serrations (Fig.4). The 2nd type of sensilla were shorter and were restricted to the distal end of the scape. These were more abundant, characteristically spine like and appeared to arise from a pit. (Fig. 5).

Pedicel: It joined the scape to the flagellum. In *Apis florea,* it was distinctly elongated and showed 2 types of sensilla (Fig.6). Longer sensilla were more numerous and scattered over the pedicel. These were pointed at the tip. The other were shorter, fewer in number and arranged in a cup like depression on the pedicel. These were broader at the base and narrower towards the tip.

Flagellum: Segments 1 and 2 showed only one type of sensilla. Sensilla trichodea type IV which was long spinous could be identified (Figs. 7 and 8).

Segment 3 showed sensilla trichodea type IV and sensilla placodea which presented typical pore like arrangement with pit in the centre and characteristi-cally raised margins (Fig. 9).

Segments 8, 9 and 10 exhibited a rich variety of sensilla. Trichodea type I, II, III, IV, sensilla basiconica (peg like with blunt tip) (Figs.10-13) and sensilla placodea were observed while sensilla ampullacea (depressed structure with pore in center) were seen only on segment 9 (Fig. 11).

***Apis dorsata*:** The scape, pedicel and segments 1st, 2nd, 3rd and 10th of flagellum were considered in the present study.

Scape: Only one type of sensilla were present on it (Fig. 14).

Pedicel: The middle region of antenna which formed the joint between scape and flagellum was the pedicel. Two types of sensilla could be distinguished on it on the basis of arrangement and form (Fig.15). One type of sensilla present towards the basal end and were very thickly populated. These sensilla were broad at the base and pointed at the tip (Fig.16). The 2nd type of sensilla were present in the cup shaped depression of the pedicel which formed the elbow of the antenna. These occurred in a small group of 35 sensilla in this region (Fig. 17). The sensilla were distinctly seen to arise from a pit, were somewhat thick at the base and pointed at the tip (Fig. 18).

Flagellum: The flagellum of the antenna showed 10 distinct segments. The characteristic types and pattern of sensilla on 1st, 2nd, 3rd and 10th segments was studied. The first two segments showed the sensilla trichodea of type I which were peg-like and characteristic of these segments (Figs. 19, 20 and 21). In between these peg-like sensilla were present the sensilla campaniformia with a long arrow (Fig. 20).

The 3rd segment of antenna showed different types of sensilla (Fig. 22). There were present sensilla trichodea of type III, sensilla placodea which had slightly raised centres (Fig. 23) and sensilla campaniformia which were distinctly depressed structures with a mushroom-like body in the centre (Fig. 24).

The 10th segment was most thickly populated with sensilla. Three types of sensilla trichodea- type I, II and IV could be distinctly identified. The type I were peg-like, type II were typically bent and type IV were thin and long (Figs. 25 and 26).

DISCUSSION

During the present investigations, two types of sensilla were observed on the scape and pedicel of *A. florea* whereas one type of sensilla was observed on scape and two types were observed on pedicel of *A. dorsata*. Regarding the flagellum, sensilla trichodea types I, II, III, IV, sensilla basiconica, sensilla placodea, sensilla ampullacea were observed in *A. florea* whereas in *A. dorsata*, sensilla trichodea type I, II, III, IV, sensilla campaniformia and sensilla placodea were present. Gupta (1992) performed SEM studies on *Apis florea* and observed that scape and pedicel had only hair and was devoid of setae and sensilla. They reported that on first and second segment sharp-tipped setae were present whereas in the present study, sensilla trichodea type IV were present. Sensilla trichodea type IV and sensilla placodea were present on third segment. The sensilla trichodea type A, C and D were observed on third segment. In the present studies, eighth segment exhibited sensilla trichodea type I, II, III, IV and sensilla placodea and distinct pattern of sensilla

basiconica, sensilla trichodea type A, B and sensilla placodea. The tenth segment revealed sensilla placodea and sensilla trichodea type II and III. He also had observed sensilla campaniformia and sensilla ampullaceal on these segments.

***A. dorsata*:** The present findings with respect to *A. dorsata* are in confirmation with those of Anudeep and Kumar (2012) who also observed only one type of setae on the scape and two types on the pedicel. Sensilla trichodea type I and sensilla campaniformia were observed on first segment. They observed sensilla trichodea type A and B along with sensilla campaniformia on the same segment. They observed a wide variety of sensilla on third segment which included sensilla trichodea type A, B, C, D, sensilla placodea, sensilla campaniformia correspondingly in the present study sensilla trichodea type III, sensilla campaniformia and sensilla placodea are reported. On the tenth segment, sensilla trichodea type I, II and IV were observed in the present study and Anudeep and Kumar (2012) reported sensilla trichodea type A, B, C and sensilla placodea were present on this segment. Suwannapong *et al*. (2012) reported 8 types of antennal sensilla in *A. dorsata* which included sensilla ampullacea, sensilla basiconica, sensilla campaniformia, sensilla placodea and sensilla trichodea type A, B, C and D.

It was concluded that the presence of sensilla ampullacea and sensilla basiconica only in *A. florea* and not in *A. dorsata* can be regarded as an adaptation of the antenna with regard to the hygroreception and chemoreception which varies according to the species.

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