

Antennal Sensilla of Cashew Stem and Root Borers *Plocaederus ferrugenus* and *P.obesus* (Coleoptera: Cerambycidae)

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Abstract: *The morphology of antennal sensilla of cashew stem and root borers Plocaederus ferrugenus L. and P.obesus Gahan (Coleoptera: Cerambycidae) were described by using scanning electron microscopy. Seven morphological types of sensilla were identified. Sensilla basiconica were found in abundance in the basal antennomeres of males of both the species, sensilla coeloconica on the F5 – F8 in male P.ferrugenus, sensilla gemmiformea in both the sexes of P.ferrugenus on the outer surface of the antenna, and filiform sensilla in the antenna of female Plocaederus spp. Sensilla trichoidea were abundant in P.obesus female. Sensilla chaetica in P.ferrugenus. Placoid sensilla in P.obesus male. The possible functions of the above sensilla types are discussed in light of previously published literature.*

Keywords: Plocaederus species, scanning electron microscope, antennal sensilla.

1. Introduction

Cashew stem and root borers namely, *Plocaederus ferrugenus* L., and *P.obesus* Gahan (Coleoptera: Cerambycidae) constitute main impediment in maintaining the optimum tree population in all cashew (*Anacardium occidentale* L.) growing zones of India. The incidence of these pests has been reported from India (Ayyar, 1942) and other cashew growing countries of the world, such as, China (Liu Kangde et al., 1998), Cambodia (Krishnamurthy 2007), Nigeria (Asogwa et al. 2009) and Vietnam (Renkang Peng et al., 2011).

The adults of *P. ferrugenus* are chestnut red colored, body length ranges from 35 – 45 mm while adults of *P. obesus* are straw colored with a body length of 40 – 45mm. The feeding stage (larva) is more prevalent from Feb – Aug in all the cashew growing regions of India. The larvae of these pests feed on the vital bark of stem, and primary roots of cashew trees, thereby causing hindrance in the flow of plant sap, leading to premature canopy yellowing, leaf fall and drying of the twigs followed by gradual death of infested cashew trees. (Ayyanna and Ramadevi, 1986) The death of pest infested trees causes substantial reduction in cashew nut productivity.

The response of these pests to an earlier infested tree was reported to be more than a fresh infestation. (Mariamma Daniel, 1991) The chemical ecology and reproductive behaviour of mate and host recognition by these pests is a less studied area.

2. Literature survey

The antennal sensilla of insects are pivotal sensorial organs mediating the perception of internal and external stimuli and enabling chemical communication to recognize host and mate. Studies in this regard to reveal the mode of communication was conducted earlier by many workers, using scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Scanning electron microscope studies of the antennal sensilla was reported in eucalyptus borer viz. *Phoracantha* spp. to understand the role of antenna in host and mate recognition. The role of olfaction in host plant recognition is well established in cerambycid borers viz. *Phoracantha semipunctata* (Fauchex 2011). The types of sensilla in Anoplistes species were recorded (Xin-Hai-Liu et.al, 2012). Lopes et.al, (2002) studied the fine structure of antennal sensilla of *Phoracantha semipunctata* and its role in detection of plant volatiles. It was reported that copulation occurred in cerambycids only after antennal contact with the body of the mate (Lopes et. al., 2005). Spikes et.al., (2010) reported on *Mallodon dasystemus* (Say) (Coleoptera: Cerambycidae), that, all males tested for mating behaviour, attempted to mate with females only after contacting females with their antennae.

Cheng (2006) reported that, the sensilla of longicorn beetles are distributed mainly on the antenna, maxillary palp and the labial palp, played an important role in activities such as feeding, mating and oviposition. Ma and Du (2000) reported that, the sensilla on the antennae played significant role on the detection of semiochemicals. The management of pests based on behavioral ecology studies has been considered as a new means of control (Allison et. al., 2004).

3. Previous work

General morphometrics of the antenna of cashew stem and root borers were reported by Vasanthi and Raviprasad (2013). There has been no study of the antennal sensilla of *Plocaederus* species in India to date.

The present study is aimed at examining the antennal sensory apparatus of these species using scanning electron microscopy, which will help to elucidate the receptive mechanisms and role of antenna in host and mate recognition by these species through the antenna.

4. Materials and Methods

4. a.

The test beetles were obtained by laboratory rearing of the cashew stem and root borers on host bark using the method standardized by Raviprasad and Bhat (2000).

4. b. Processing the Antenna

The antennae of newly emerged CSRB beetles of both sexes were carefully excised from the antennal sockets and washed thoroughly in distilled water, treated with a lipid solvent tetra chloro ethylene (C₂Cl₄) to digest lipid before dehydration. The antennae were transferred individually to vials containing tetrachloro ethylene (C₂Cl₄) which was then brought to a boil inside a smoke hood. After 30 sec., the fluid was renewed and boiled again. This was repeated 4- 5 times, after which the antennae were dehydrated sequentially in acetone at increasing concentrations (50%- 70%- 80%- 90% - 100%), one hour in each concentration. The antennae were observed under stereomicroscope to reveal the general morphology and the length of individual segments using an ocular micrometer. Antennae of thirty individuals of both sexes of *P. ferrugineus* and *P. obesus* each were used for the stereomicroscopic study and mean length were estimated. The lengths of flagellomeres of both sexes of the two species of beetles were compared.

c. The SEM observations

The SEM observations of antennae from male and female individual of both the species were done. The samples were mounted on specimen holder and coated with gold-palladium in a JFC1100 sputter coater and then examined under JEOL-JSM-6380LA Analytical Scanning Electron microscope at National Institute of Technology Karnataka, Surathkal, Karnataka. The forms of different types of sensilla on the antenna of different species and both the sexes were observed. The types were named by referring to the naming system cited in the reference.

5. Results and Discussion

a. General description of the antennae (Gross morphology)

As in most longicorn beetles, the antennae of *Plocaederus ferrugineus* L. and *Plocaederus obesus*

Gahan were longer (males) or as long as their body (females). In both the spp. antennae were filiform, located in the mortar like antennary fosse on the inner side of the kidney shaped compound eye on the head. (Slightly serrated in case of *P. obesus*). The antennae consist of scape (Sc), pedicel (Pe) and nine flagellomere (F1 to F9). There were multiple honey combed scale striae on the surface of these antennae. The scape is the segment which set and articulated to the head by a ball joint. The pedicel is, and is articulated to the hollow cavity of the scape. These two basal flagellomeres present very considerable mobility. The first flagellomere is fixed tight to the pedicel; it is the stout of all other flagellomere which from the 2nd to 9th tend to be longer and narrower in case of *P. ferrugineus*, but, slightly boat like in *P. obesus*. Except for flagellomere 1, the others F8-F9 have bulbous base without sensilla which is articulated in the distal cavity of the previous segment. F1-F6 are cylindrical but the distal F7-F9 is flattened. At rest, the antennae are bent backwards along the body and the flagellomere F7-F9 are bent outwards. When the beetle is active, the antennae are stretched forwards. On the antennae F1-F5 in both sexes possess distal spine located on the posterior border. Variations in the length of spine were seen between individuals. The outer side of the flagellum was ciliated in both the sexes. The integument surface had a structure of scales which was perfectly visible in the zones where sensilla were rare. Comparison of the morphometrics of the antenna of both the species was reported earlier by Vasanthi and Raviprasad (2013).

The relative length of each section of antenna of both the sexes of both the species is present in Fig 1.1.

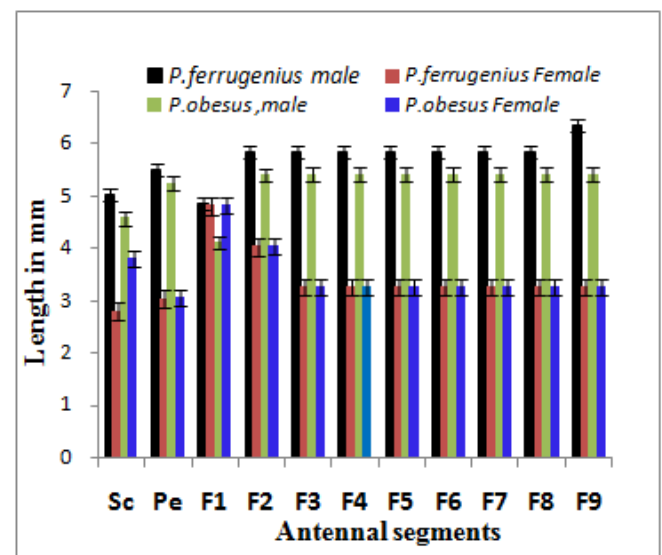


Figure 1.1: Length of each segments in the antenna of *P. ferrugineus* and *P. obesus* adults

Table 1: Mean length of the antenna in two species of *Plocaederus*

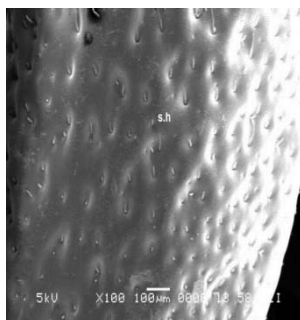
Body length (mm)				Antenna length(mm)			
<i>P.ferrugineus</i>		<i>P.obesus</i>		<i>P.ferrugineus</i>		<i>P.obesus</i>	
F	M	F	M	F	M	F	M
34.7	33.7	33.5	38.8	35.69	60.69	32.9±	72.7
± 3.7	± 4.6	±	±	± 2.8	± 7.2	2.6	±
		3.6	5.3				12.8

b. Types and forms of antennal sensilla

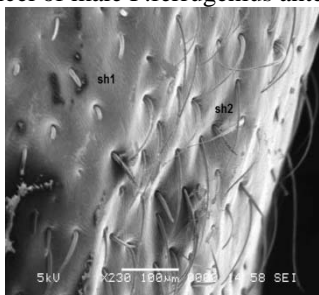
The antennae of these species were densely covered with sensilla, both on the inner and on the outer surface, which were more numerous in both sexes on the outer surface than on the inner surface. The types of sensilla varied among the sexes in both the species. The sensilla were identified to be seven morphologically different types, based on size, external form (outgrowth), length and shape. They were sensilla basiconica (s.ba), coeloconica sensilla, chaetica sensilla (s.ch), trichoidea sensilla (s.t), sensilla filiformia (s.f), gemmiformea sensilla (s.g.) and placoid sensilla (s.pl). Majority of the sensilla were located in pits on the surface of the antenna. The junction between the flagellomeres was with lesser density of sensilla.

5. b. 1. Mechanosensory and chemosensory hairs

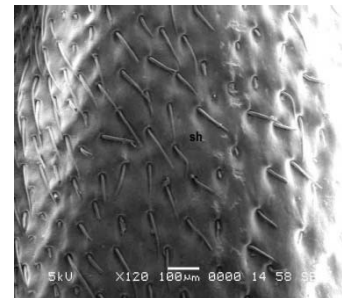
These were thin slender mechanosensory hairs (s.h.) of varying length, observed on the pedicel in pits and found in both the species in both sexes. However the length and form of these hairs were different. In *P.ferrugineus* male they were short (<20µ) (Fig 1a) whereas in female they were long, slender and filiform (300 µ<) (Fig 1b) or bent with sharp tips (<50µ). In *P.obesus* male they were straight and sharp tipped lying on the surface of the antenna (Fig 1c). The *P.obesus* female pedicel was covered with grooved sensory hairs. (Fig 1d).



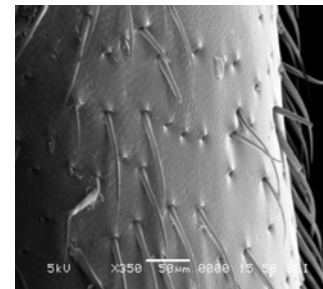
Pedicel of male *P.ferrugineus* antenna



Pedicel of female *P.ferrugineus* antenna



Pedicel of *P.obesus* male

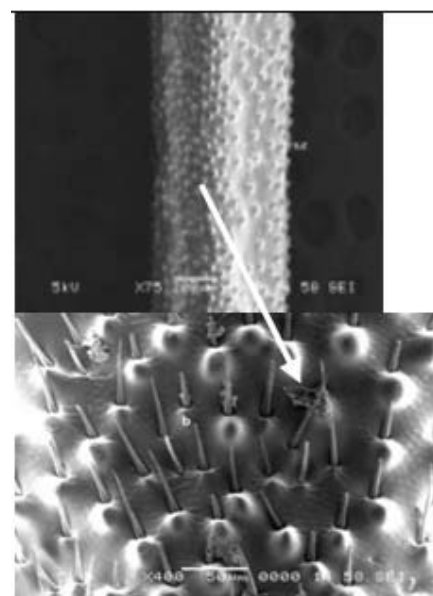


Pedicel of female *P.obesus*

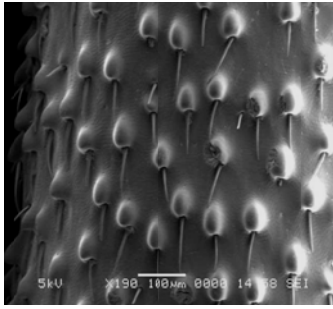
Figure 1: Mechano and chemosensory hairs in the pedicel of *Plocaederus* spp.

5. b. 2. Sensilla basiconica

These were located at the base of mound like raised structures lodged in the pits on the inner surface of the antenna. There were two types according to length and morphology: type 1- as long as 50 µ and type2: length ranging from 150 - 200µ is slanting to the surface of the antenna. This kind of sensilla was observed only on the male individuals of *Plocaederus* species. (Fig2 a and b, Fig 3) Sensilla basiconica were characterized by straight and blunt tipped sensory hairs. These were observed to vary in morphology in both the species. This type of sensilla were characterized by grooved surface and straight pegs with blunt tip and were with bulb - like structures projecting from a depression in the center of raised area of cuticle. The probable function of these sensilla is chemo or thermo- hygro reception.



a. Sensilla basiconica of *P.ferrugineus* male



b. Sensilla basiconica of P.obesus male

Figure 2: Basiconica sensilla on the inner surface of 1st to 4th flagellomeres of Plocaederus male

5. b. 3. Sensilla coeloconica

On the inner surface of the 5th -8th flagellomere of P.ferrugineus male there were coeloconica sensilla with the peg like sensory structures in sockets were observed. The outer surface of these segments were found o be with sensory setae and gustatory sensilla (Fig4).

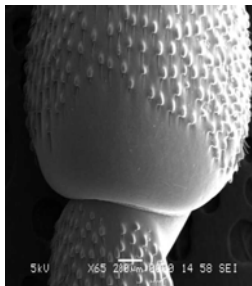


Figure 3: Junction of two basal flagellomeres in male P.obesus

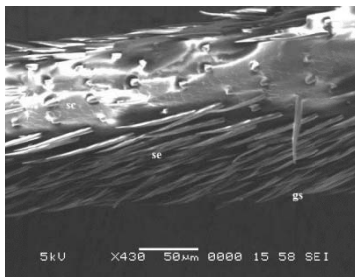
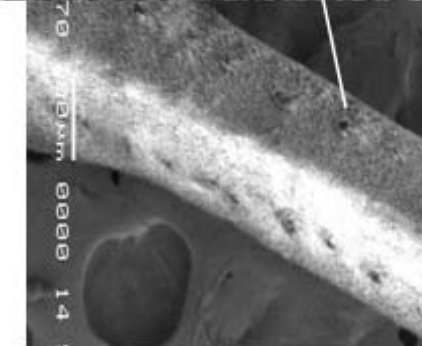
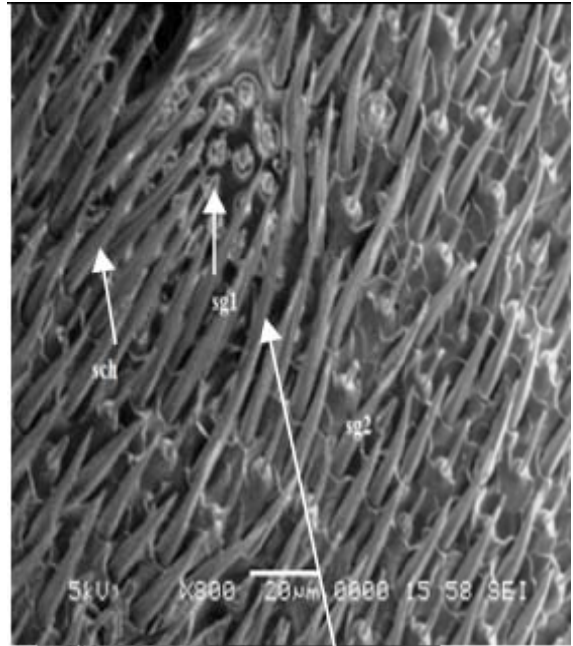


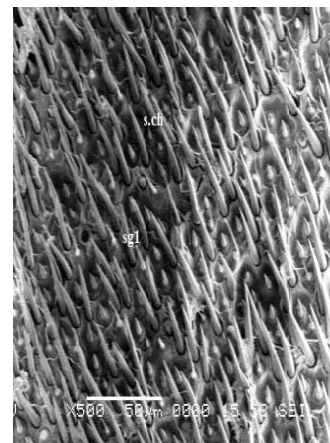
Figure 4: sensilla coeloconica in P.ferrugineus male

5.b.4. Sensilla gemmiformea

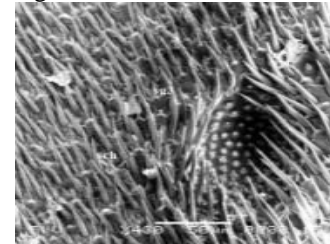
These sensilla appeared like buds that are seen on germinating seeds. There were of two types – 1.single (Fig.5a) and type 2- multiple buds in the same pit (Fig.5b) which were fairly open. These were richly found on outer surface of antennae of P.ferrugineus female. In male P.ferrugineus a third type sensilla were seen, resembling concealed buds in a common pit (Fig.5c).



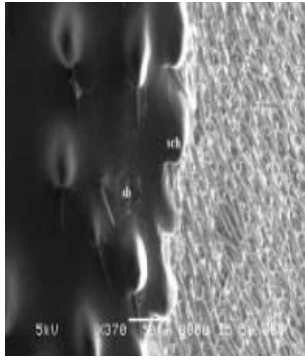
a. Sensilla gemmiformea of P.ferrugineus female



b. Sensilla gemmiformium and sensilla chaetica



c. Sensilla gemmiformea on the outer surface of long segments of P.ferrugineus male antenna



d. The density of sensilla on the inner and outer side on the long segments of the antenna of male P.obesus

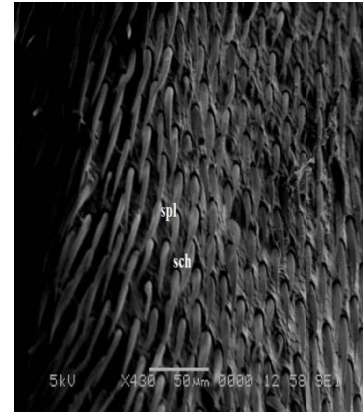


b. Sensilla trichoidea on the outer surface of female P.obesus

Figure 5: Arrangement of chaetica sensilla on the outer and inner surface of the antenna

b. 5. Sensilla chaetica

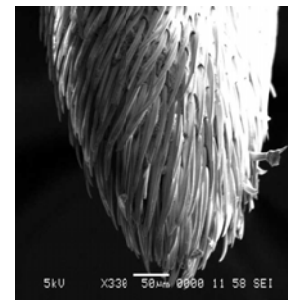
These were densely distributed, setiform, with flexible sockets, located on the outer surface of the antenna erectly or slantingly of varying length. Three sub-types of sensilla chaetica were identified: Ch1: These were short (100μ) and with blunt tips slanting on the surface of the antenna with the base on small projecting knob of pit in the antenna surface.(fig 5b) Ch2: They were sharp tipped slantingly placed (Fig 5a and 5c). Ch 3: longer $80 - 360\mu$, sharp tipped or blunt of homogenous thickness. This kind of sensilla were observed in individuals of both the species. (Fig 5d, 6c)



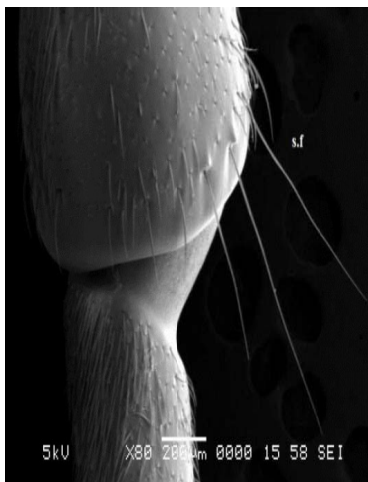
c. Placoid sensilla on the flagella of P.obesus antenna

5.b.6. Placoid sensilla

These were flat plates level with the general surface of the antenna (Fig 6c). Observed richly in male P. obesus. The tip of the flagellum was densely packed with sensilla.(Fig 6d)



d. Tip of the antenna of P.obesus.

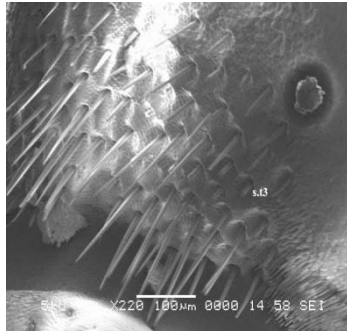


a. Sensilla filiform of in P.obesus female

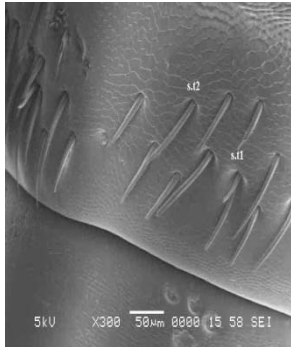
Figure 6: Details of sensilla on the flagellomere of female and male Plocaderus spp.

5.b.7. Sensilla trichoidea

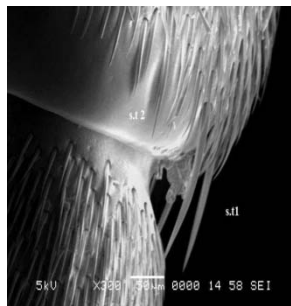
These were straight (s.t1 and s.t2), sharp tipped, and were of varying length ($100-200\mu$) (Fig 7a) and blunt tipped, presumably uniporous ($30-40\mu$) observed in P.ferrugenius female. Long trichoid sensilla were observed to be present densely at less than 100μ distance, on the outer surface of the flagellomeres in P.obesus female fig 7c and D). There were long sensory hairs, filiform on the junction of flagellomere in female of both the species (Fig 6a and b). Observations revealed that, sensilla trichoidea (s.t) were sharp-tipped hairs, nearly straight or slightly curved toward the antennal shaft. They were inserted into a wide socket. These might indicate that they have olfactory and gustatory function. (Chapman, 1998).



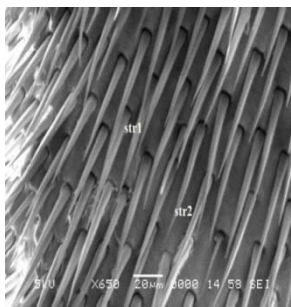
a. Trichoid sensilla on the inner side of the 5th flagellomere of female *P. ferrugenus*.



b. Trichoid sensilla *P. ferrugenus* female



c. Sensilla filiform of *P. obesus* female



d. Trichoid sensilla of *P. obesus* female.

Figure 7: Various types of trichoid sensilla on the antenna of *Plocaederus* spp.

Comparison of Sensilla in two species of *Plocaederus*

The morphological observations on the antenna revealed some common features of the antennal sensilla in these pest species. The junction between flagellomeres was with less number of sensilla (Fig3 and 6a). The basal segment of the antenna was with less density of sensilla. The number and density increased towards the tip. The 9th flagellomere was densely packed with sensilla (Fig. 6d).

The unusual features of *P. ferrugenus* male antenna was occurrence of coeloconica sensilla (Fig 4) on the outer side of the F5, with different types of chaetica sensilla.

The antenna of *P. obesus* male showed the presence of placoid sensilla towards the tip, which were observed to be slanting along the antennal axis (Fig.6 c.) and were not observed in antenna of both the sexes of *P. ferrugenus* and female *P. obesus*.

The serial arrangement of grooved sensilla chaetica and gemmiformea on the outer surface of the antenna was seen in *P. ferrugenus* female (Fig. 5a). The straight tipped, densely packed sensilla trichoidea of different length were rich in *P. obesus* female (Fig 7 d). The basiconica sensilla were common in males (Fig .2a, 2b), but the filiform sensilla were observed only in females of both the species (Fig.6a, b). The tip of flagellum was densely covered with chemoreceptor sensilla. (Fig 6d) Crook et. al., (2008) reported that, olfactory chemoreceptor sensilla increased toward the distal tip of the antenna in emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). The observations matched with this study.

The mechanoreceptors at the basal segments of the antenna would aid in considerable mobility and rotation of the antenna. Schneider (1964) reported the location of the mechanoreceptors sensilla on the basal segments of the antenna of insects.

The larger trichoidea sensilla on the outer surface of the flagellomeres were believed to respond to touch and taste. (Zacharuk, 1985). Orlando Lopes (2005) reported the role sensilla trichoidea on the antennal flagellum was for contact chemoreception and in mate recognition in case of *Phoracantha semipunctata* (Coleoptera: Cerambycidae). They were distributed on the entire flagellum, especially along margins and at the tip of the distal flagellomere in *Plocaederus* female. The males were reported to respond to females only after antennal contact (Spikes et.al. 2010). The present study revealed several common morphological types of sensilla on the antenna of both the species. The male specific sensilla might help to perceive the species specific semiochemicals of female. The sex specific filiform sensilla in females of both the species presumably, play a role in mate and host recognition for oviposition by the mated females.

Earlier reports by Xian-Hai Liu (2012) revealed species specific difference in the pattern and distribution of sensilla on the antennae of two subspecies, of *Anoplistes* (*Anoplistes halodendri halodendri* and *Anoplistes halodendri ephippium*). The present observations also revealed species specificity of sensilla. There were common sensilla which were observed in both the sexes of the species namely, trichoidea and chaetica sensilla, might aid in perception of aggregation pheromones. The sensilla found in both the species might help in the host identification.

6. Conclusion

In conclusion, the present study revealed communication via olfaction was predominated in these species, because of the variety and abundance of sensilla. This study herein supposedly stand to be the first attempt to describe the antennal sensilla of *Plocaederus* species using scanning electron microscopy and might be considered as the first step towards future investigations of the odorant receptors in this stem borer. More detailed studies on the functional morphology of the antennal sensilla using transmission electron microscopy (TEM) coupled with electrophysiological recordings needs be conducted for confirmation of the function of the different sensilla. A more exhaustive study will allow a full understanding of the role of antennae in the particular behavior of these long horned beetles.

7. Acknowledgement

The first author is indebted to the University Grants Commission for providing teacher fellowship under Faculty Development Programme for Ph.D. studies, to the Director, DCR. (ICAR), Puttur, Karnataka for providing the necessary facilities to carry out this work and to Dr. K.R. Udupa, Head of the Department of Metallurgy, NITK Surathkal for providing the Scanning Electron Microscope facility. Acknowledgements are also due to Dr. T.R Rupa and Dr. Kalaivannan of soil science laboratory DCR, for supporting the work. It is our duty to extend our gratitude to Dr. Sylvia Anton, RCIM Angers France and Dr. Thomas. A. Kiel of Max Plank Institute for their kind suggestions.

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