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Comparative morphology and distribution pattern of antennal sensilla of banana weevil pests, *Cosmopolites sordidus* (Germer) and *Odoiporus longicollis* Oliver

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ABSTRACT: Types and distribution of antennal sensilla were studied in two important pests of banana *viz.,* pseudostem weevil *Odoiporus longicollis* Oliver, 1807 and rhizome weevil *Cosmopolites sordidus* (Germer, 1824) using scanning electron microscopy. Morphometric analyses showed that the antennae of male and female weevils within the species are similar in the morphological structure but vary only in size. Four categories of antennal sensilla namely sensilla chaetica, sensilla trichoidea, sensilla basiconica, and sensilla coeloconica were found in these banana weevils. Though the types of sensilla among the banana weevils were found to be same, differences were noticed in their distribution and density. The morphometrics and the antennal sensilla distribution were explained in detail, and differences across the sexes within and between the species were compared. This study not only helps in the better understanding of the antennal morphology of banana weevils and but also assist for future electrophysiological studies.

Keywords: Antennae, sensilla types, sensilla chaetica, sensilla trichoidea, weevils

INTRODUCTION

Insect behavioural research studies have clearly established the fact that the crucial behaviours like egg laying and mate selection majorly depend on odour cues that are perceived by sensory structures (= sensilla) situated on antennae that plays a pivotal role in insect olfactory perception (Wang *et al.,* 2010 and Mamidala *et al.,* 2013). The antenna serves as a crucial sensory organ for insects with multiple sensilla on it supporting olfaction, mechanoreception, hygroreception and thermoreception, hence termed as "feelers" (Schneider, 1964; Gao *et al.*, 2007 and Zacharuk, 1985). The antennal sensilla abundance and their morphological as well as distribution complexity forms the functional basis for varied behavioural and ecological aspects among the insects (Faucheux *et al.,* 2006). Antennal sensilla morphology, pattern of distribution and electrophysiology were studied in several coleopterans and have been proved to be crucial for the selection of habitat, host, mate and also in predator detection (Schneider, 1964; Ploomi *et al*., 2003; Merivee *et al*., 2005 and Onagbola *et al.,* 2008). In case of the specialist coleopteran herbivores like banana weevils [pseudostem weevil *Odoiporus longicollis* Oliver; rhizome weevil *Cosmopolites sordidus* (Germer)] which feed on specific parts of same host plant (like pseudostem and rhizome), it will be interesting to know that whether their specific feeding habits greatly influence the diversity and abundance of antennal sensilla as these were mentioned as functional basis for behavioural ecology of insects. However, in case of banana weevils, very limited information is available on the antennal sensilla morphology and a detailed investigation of sensory structures involved in stimuli perception of these weevils will greatly improve our understanding about their odour mediated olfaction. The present study explains the antennal sensilla typology and the number as well as their distribution pattern in both the sexes of banana weevils, *O. longicollis* and *C. sordidus* through Scanning Electron Microscope (SEM) studies.

MATERIALS AND METHODS

The studies were carried out at the Division of Crop Protection ICAR-Indian Institute of Horticultural Research (IIHR), Bangalore (13.1348° N, 77.4960° E). Both the species of weevils (*O. longicollis* and *C. sordidus*) were collected from the infested banana plants in the experimental orchards of IIHR. The collected weevils were brought to the laboratory in plastic containers ($20 \times 25 \times 40$ cm^3) and maintained on cut pieces of pseudostem and rhizome parts of banana plant as the case may be. The containers were placed at ambient conditions and checked periodically for changing food. These weevils were used for SEM studies.

SEM studies

The weevils were anesthetized by placing them in a freezer for brief period. From these weevils, antennae were dissected out from the antennal sockets using surgical scissors under the stereomicroscope (Leica M2050), and washed with 70% ethanol twice. Antennae were cleaned using brush to remove the debris. Later, antennae were allowed to dehydrate for 20 min successively in series of ethanol dilutions (30, 50, 70, 90, and 100% twice). Finally, antennae were dried and glued onto copper pin mounts having a double-sided carbon adhesive tape and were examined with a scanning electron microscope (Hitachi Tabletop TM3030).

Image analysis

The images taken in the scanning electron microscope were processed using ImageJ software (v. 1.44p). The length of antennal segments and various sensilla were measured on multiple samples (n =5) and the mean was calculated. A total of five replicates were used to estimate the mean sensilla size and numbers. Sensilla were classified as per the descriptions in earlier studies (Schneider, 1964; Zacharuk, 1980; Ryan, 2002; Hix *et al.,* 2003; Shields, 2004 and Hu *et al*., 2009).

Statistical Analysis

The data were analysed using student *t*-test and Analysis of Variance (ANOVA). Measurements of antennal segments as well as each of sensillum type were compared between the sexes by the Least Significant Difference (LSD) with $P \leq 0.05$ after ANOVA using Graphpad Prism (Ver 7.0).

RESULTS

General structure of antennae

The distinctly elbowed and folded antennae of adult weevils, *C. sordidus* (Fig.1) and *O. longicollis* (Fig.2) consisted of a long, broad and extended scape, stout pedicel, and six non-soldered flagellomeres with the last segment forming a rigid, oval club like structure. The antenna attached to the head by scape, in the front portion covering the anterior margin of the compound eye. First flagellomere is slightly triangular, but the rest are spherical. The basal part of each segment is narrower and broadened gradually towards the distal end. Each segment had a granular surface on which the different types of sensilla are distributed. The types of sensilla on the various segments of antenna were similar in both the sexes of weevils except for their size with no apparent sexual dimorphism. Total length of the antenna was longer in females than those of the maleweevils. Further, the length of antenna was found significantly longer in *O. longicollis* compared to the *C. sordidus*. The measurements of mean total length of antenna, length and breadth of each antennal segment are given in Table 1.

The maximum length of *O. longicollis* antenna measured about 4.02 mm with a mean of 3.74±0.35 mm in case of male weevils, and it is shorter than the mean length of female antenna (4.02±0.36 mm). Similarly, the maximum length of antenna in the *C. sordidus* measured 3.59mm with a mean length of 3.43±0.18 mm in male weevils which is shorter than the female antennae (Mean, 3.59±0.12 mm). In both the species of weevils (*O. longicollis* and *C. sordidus*), the density of sensilla on the antennal segments like scape, pedicel, and five flagellomeres was sparse compared to the copiously-covered club where the sensilla were compactly distributed throughout. Further, the antennal segments of both the male and female weevils of *O. longicollis* and *C. sordidus* showed similar pattern and general organization of sensillae. The sensilla type, number and their measurement on the various segments of antenna were similar in both the sexes of weevils with no apparent sexual dimorphism. However, differences in sensilla topography between the two species was noticed (Tables 2 and 3).

The mean abundance of different types of antennal sensillae of *O. longicollis* and *C. sordidus* revealed no significant difference (P>0.05) in the mean number of sensillae between female and male weevils within the species. However, significant differences were found in the abundance of sensilla types namely sensilla chaetica 1, sensilla trichodea, sensilla basiconia 1 and 2 between the species (Table 3).

Types of sensilla

As mentioned earlier, there were more numbers of sensillae on the antennal club than the rest of the antennae. There were no significant changes in the types of sensillae present in antennae between the male and female weevils in both the species (*O. longicollis* and *C. sordidus*). The major types of sensillae that were present in these weevils broadly include sensilla chaetica (s. ch), sensilla trichoidea (s. tr), sensilla basiconica (s. ba) and sensilla coeliconica (s. coe).

Sensilla chaetica

They are long straight bristles with longitudinal grooves which are wider at base and tapering towards the tip around the surface. They are characterised with a sharp tip and an apical pore. The base of sensilla is inserted in a wide socket and the sensilla projects from the antennal surface at an inclination of 90°. Sensilla chaetica has grooved cuticular wall ending in a sharp tip, enclosed in a wide tight socket. Their length ranged from 30 to 70 µm. It is the most abundant type of sensilla on the antennae of banana weevils and is present on all segments except scape. The numbers of sensilla increased towards the tip of the antenna in both the species and mainly two types, sensilla chaetica 1 and sensilla chaetica 2, were prevalent (based on the length).

Sensilla chaetica 1 (s. ch1) is the longest among the chaetica subtypes. They have slightly longitudinal grooved cuticular wall with a wider base and tapering apex. The length of s. ch1 did not show any significant difference between males and females in both the species. They are present in all the antennal segments except scape in both the species. Sensilla chaetica 2 (s.ch2) was shorter than the s.ch1. However, its cuticular wall is deep, longitudinally grooved with a tight socket. Further, s.ch2 was noticed only on sixth flagellomere. There were no significant differences among the male as well as female weevils in both the species for the length and diameter of the sensillae.

Sensilla trichodea

Sensilla trichodea is a straight bristle like hair and shorter. The length of sensilla trichodea (s. tr) ranged from 13 -16 µm and there was significant difference between males and female weevils in both the species. They were also seen to be present on the sixth flagellomere along with the s.chaetica (s. ch) and s. coeloconica (s. coe).

Sensilla basiconica

These sensillae were straight, smooth walled peg without any longitudinal grooves and blunt tipped with a distinct droplet shape at the tip. They were seen on both the antennal regions namely scape and apex region (= club of the antennae) amidst the sensilla coeliconica and sensilla trichoidea. They were less in number compared to the other types of sensillae.

Two forms of sensilla basiconia namely s. ba1 (sensilla basiconica 1) and s. ba2 (sensilla basiconica 2) were noticed in banana weevils. Among these, s. ba1 is longer with a tapering base immersed slightly in a socket and seen only on the scape consistently in both the species. No significant difference was found for parameters like length of the sensillae and diameter at the base among the male and female weevils. The second type of sensilla namely s. ba2 was noticed in the apex region and it is shorter than the former (s. ba1) with bulged base. The length of the sensilla was 7 -11µm.The peg projected from the antennal surface at an angle of 50-70^{o}.

Sensilla coeliconica

These sensillae are straight, smooth walled without any longitudinal grooves and found to be bifurcated at the tip. They are present only on the apex region or club of the antennae amidst the sensilla chaetica and sensilla trichoidea in both the species. They are maximum in numbers compared to the other sensillae types. However, they were not seen in any other antennomeres other than the club in both the species of banana weevils. The length of the sensillae ranged from 9µm-14µm.

DISCUSSION

Insects antennae usually comprise of numerous types of sensilla that have a functional role while perceiving their immediate environment like location of host plants, mating partners and also in assessing the predation risk through odour perception (Hu *et al.*, 2009). The present study describes the number and types of sensillae in *C. sordidus* and *O. longicollis* that can serve as ready reckoner for future chemical ecology studies involving electrophysiological techniques and also to understand the olfaction ability of these dreaded weevils to detect the diverse host cues.

The present study highlighted that in general, female weevils comparatively have a greater number of antennal sensilla than the male weevils in the species studied viz., *C. sordidus, O. longicollis*. The reason for this gender bias in the density of antennal sensilla might be due to the longer antennae noticed in female weevils compared to that of males in both the species. However, no other obvious sexual dimorphism was noticed either with antennal sensilla typology or with their distribution. With respect to the various types of sensilla noticed in both the species of banana weevils (*C. sordidus* and *O. longicollis*), sensilla chaetica, s. ch1, a long, robust and bristle like with sharp tips was found in abundance. Previous studies also described that sensilla chaetica had been the most common type that covers the entire antennal surface in a large number of coleopteran species (Merivee *et al*., 2005). For example, these sensillae are reported in several weevils like *Polytes mellerbogi* Boheman (Yin *et al*., 2016), *Sitophilus granarius* L. (Abd El-Ghany and Abd El-Aziz, 2017), ground beetle, *Bembidion lampros* Hbst (Ploomi *et al*., 2003), *Naupactus xanthographus* Germer (Vera and Bergmann, 2018). Further, the sensillae chaetica have been reported as olfactory receptors in several curculionids antenna (Wang *et al*., 2012; Yen *et al*., 2011; Yin *et al*., 2016). The sensilla chaetica, s. ch1 is the longest among all the sensillae, hence it may come in touch with substratum first as they were placed perpendicular to the antenna surface. Thus, the external shape, large measurements, specific location of this sensilla makes it act as taste sensillae for host plant detection and host plant selection (Yin *et al.,* 2016).

The second type of sensilla chetica, s. ch2 is also long, robust and bristle like sensilla similar to s. ch1 and typically placed at an angle of 20° to the antennal surface. Similar to s.ch, these sensillae were also reported in small banana weevil, *P. mellerbogi* (Yin *et al*., 2016), granary weevil *Sitophilus granaries* (Lin.) (Abd El-Ghany and Abd El-Aziz, 2017), *B. lampros* (Ploomi *et al*., 2003), grape weevil *N.xanthographus* (Vera and Bergmann, 2018). Sensilla chaetica type 2 has a wide articulated socket and a pointed tip and differs mainly in its length and distribution pattern. The presence of a flexible socket and an apical pore in these sensilla indicates that they mainly serve as contact chemoreceptors (Merivee et al., 2004). Previous electrophysiological analyses also revealed that s. ch2 is a contact chemoreceptor and responded to the p^H variations of the salt solutions in *Pterostichus aethiops* (Panzer) and *Pterostichus oblongopunctatus* F. (Merivee *et al.,* 2005). Further, researchers opined that s. ch is mostly comparable with s. tr but distinctly identified by their thick walls and blunt apexes. Further, they found to arise from sockets (Abd El-Ghany and Abd El-Aziz, 2017).

The other group of sensilla noticed in the banana weevils is s. tr, that are quite common across the insect kingdom and known to serve as olfactory receptors (Bleeker *et al.,* 2004). However, previous studies also highlighted their role in mechanical function and found that single sensory neurons innervate these sensillae (Zacharuk, 1985). In sensilla trichoidea group, s. tr has been suggested to have a tactile function of either mechanical or in some occasions not at all receptive, there by playing protective role (Mustaparta, 1973, Bartlet *et al.,* 1999). This sensilla is also reported very commonly in many beetles and weevil pests like *B. lampros* (Ploomi *et al*., 2003), *Phyllophaga* sp. (Lopez and Moron, 2013), *Rhyncophorus ferrugineus* (Oliver) (Mahmoud *et al.,* 2013), *Tomicus* spp. (Wang *et al*., 2012), *Hylastinus obscures* Marsham (Palma *et al*., 2013), *P. mellerbogi* (Yin *et al*., 2016), *S. granarius* (Abd El-Ghany and Abd El-Aziz, 2017), and *N. xanthographus* (Vera and Bergmann, 2018). The role of s. tr has been established as pheromone receptors in the beetles *Hylobius abietis* Linn. (Mustaparta, 1973) and *Agriotes obscures* L. (Merivee *et al.,* 1997) through electrophysiological methods.

The other group of sensilla that were found in banana weevils were s. ba that are smooth walled and seen less in numbers compared to other sensillae. These are reported to be typically small, with a greater number of wall pores compared to s. tr (Shields, 2004). These sensillae are reported in other coleopterans like *Psylliodes chrysocephala* L. (Bartlet *et al.,* 1999), *Dastarcus helophoroides* (Fairmaire) (Ren *et al*., 2012), *P. mellerbogi* (Yin *et al*., 2016), *S. granarius* (Abd El-Ghany and Abd El-Aziz, 2017). Numerous pores on the cuticular shaft of these sensilla suggest their olfactory functions (Lopes *et al.,* 2002). Previous studies using GC-SSR studies (Gas Chromatography Coupled to Single Sensillum Recording) established that s. ba respond to the host plant odors (Sun *et al*., 2014; Zhang *et al.,* 2015). Studies also proved that each subtype of sensillae could be specialized for a specific host and non-host volatiles (Lopes *et al*., 2002). Further, studies revealed that these sensilla function could be related to the number of neurons present within it. For example, sensilla with four neurons often related to contact chemoreception and with one neuron suggested to have a role in mechano-reception (Zacharuk, 1985; Merivee *et al*., 2002).

Another group of sensilla that were noticed in banana weevils were s. coe. They were not found abundantly throughout the antennae but only in the club portion amongst the other sensillae. Previous studies also reported that these sensillae are not usually found in all coleopterans but rarely reported in few species like *Trogoderma variabile* Ballion (Chunyan Wei *et al.,* 2019). Usually, they present in the dense areas of antennae with their tips assembled in the form of flower buds (Chunyan Wei *et al*., 2019). However, their functions are yet to be described. The possible functions of each type of antennal sensilla, described in this paper are based on the previous reference studies related to SEM analyses of similar sensilla. The current study characterizes and compares the antennal sensilla of banana weevil pests namely *C. sordidus* and *O. longicollis* is first of its kind. This study emphasizes for further research to understand the detailed olfactory functions of the reported antennal sensilla using transmission electron microscopy coupled with electrophysiological techniques like Single Sensillum Recording. Such studies will not only confirm the functions of the different sensilla but also expands our knowledge on these notorious pests. Besides, these results will further support the investigations to understand the niche adaption in these monophagous banana weevils. In depth understanding of the functional roles of various types of antennal sensilla in sensing pheromones, host and non-host volatiles will help us to gain deeper insights in to the underlying olfactory mechanisms, which could ultimately lead to develop an efficient semiochemical-based management strategy against these weevils.

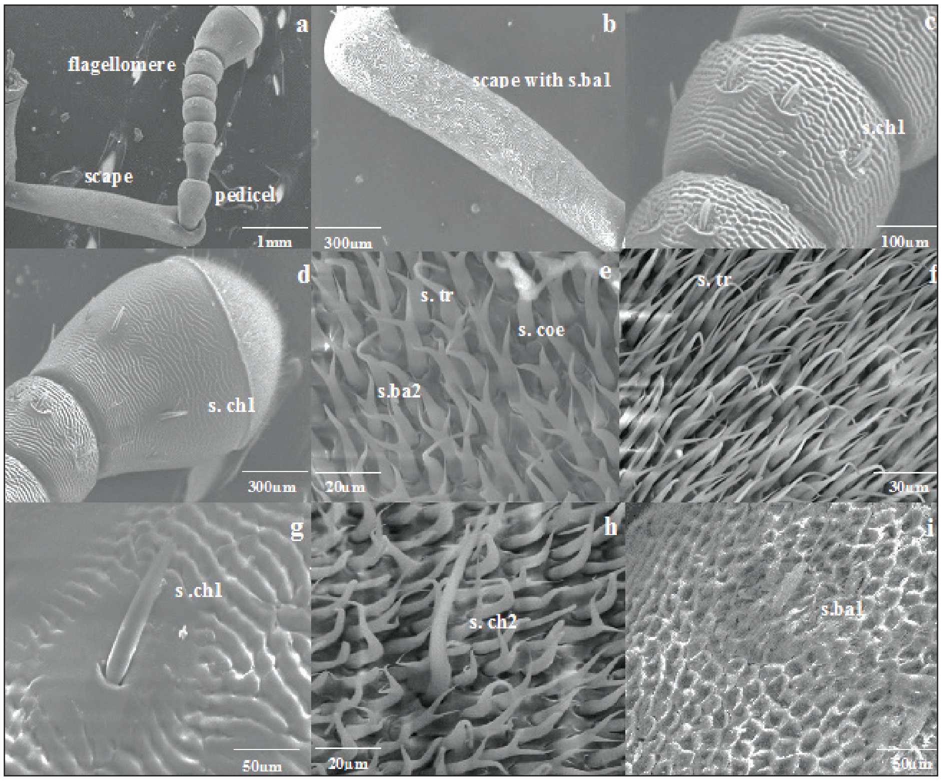
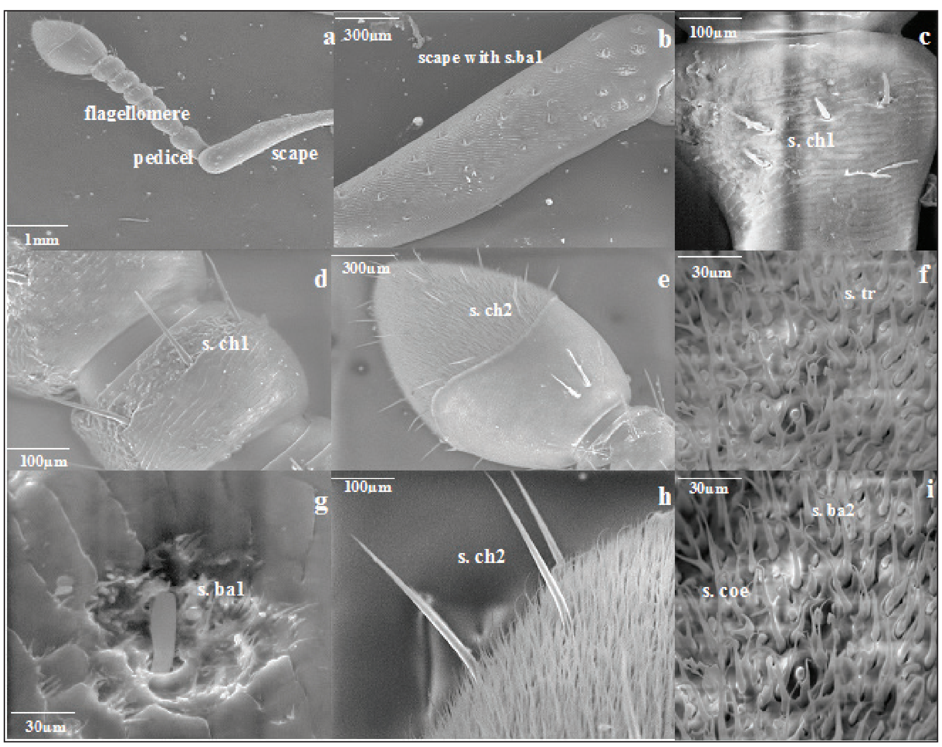


Fig.1. (a) Antennae of *C. sordidus,* consisting of scape, pedicel, and six flagellomeres. (b) scape with s.ba1, (c) segment with s.ch1 (d & g) flagellomere with s.ch1.(e,f, h) diffferent sensillae in the final segment (i) s.ba1in scape



scape with s.ba1. (c & d) segment with s.ch1.(e,f,h &i) different sensillae in the final segment. (g) s.ba1in scape

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