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Ultrastructure of the antennal sensilla of *Alabama argillacea* (Hübner, 1823) (Lepidoptera: Erebidae)

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ABSTRACT

Insects have several types of sensilla, the characterization of which has been fundamental to understanding the mechanisms of sensory perception in different species. This study aimed to describe the ultrastructure of the sensilla present on the antennae of *Alabama argillacea* (Hübner, 1823) (Lepidoptera: Erebidae), an important pest of cotton (*Gossypium hirsutum* L) crops, as well as their possible variation between sexes. To do this, the antennae of males and females of *A. argillacea* were analyzed using scanning electron microscopy. Sensilla morphometry was assessed using photomicrographs, from which the lengths and basal and apical diameters of sensilla were measured using the ImageJ program. Seven types of sensilla were identified on the antennae of *A. argillacea*: sensilla trichodea, sensilla chaetica, sensilla auricillica, sensilla coeloconica, sensilla styloconica, sensilla basiconica, and sensilla Böhm bristles. Differences between the sensilla of males and females were found in their lengths and basal diameters in the distal and proximal regions. This suggests that sensilla functionality may not only vary from one species to another, but also between sexes within the same species. Thus, further transmission electron microscopy and single sensillum recording studies may provide more detailed information on the sensilla of *A. argillacea* and their functions.

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Introduction

Over the course of their evolutionary history, insects have developed a great capacity for environmental perception, and show high sensitivity to climatic variations, as well as mechanical and chemical stimuli. The success of these adaptations is related to the sensorial structures present on their bodies, named sensilla (Altnér et al., 1983; Cossé et al., 1998; Krishnan, 2012; Wee et al., 2016). Sensilla are the basic structures involved in insect perception, and are composed of neurons, thecogen, trichogen, and tormogen cells, and cuticular structures (Keil, 1997). The lattermost of these is one the principal bases for the classification of the various sensilla present on insects (Zacharuk, 1980).

Sensilla vary morphologically, and as a result can be classified as trichodea, chaetica, coeloconica, basiconica, Böhm bristles, auricillica, and styloconica, among others. Every type of sensillum may be responsible for one or more functions, including acting as mechanoreceptors, chemoreceptors, hygroreceptors, thermoreceptors, or CO2 receptors (Schneider, 1964; Stange and Stowe, 1999). Sensilla function as an interface between the external and internal environments of insects, capturing different stimuli from the external environment and directing them to the central nervous system. This then triggers specific behavioral responses, such as: selection of a host for feeding and oviposition based on the perception of captured plant volatiles; or meeting a partner with whom to perform copulation after the capture of sexual pheromones (Schneider, 1964; Cossé et al., 1998; Smith, 2007; Park et al., 2013; Wee et al., 2016).

The types of sensilla on an insect’s antennae may vary from one species to another. Furthermore, differentiation within the same species may occur, with many species presenting sexual dimorphism (Castréjón-Gómez et al., 1999; Sukontason et al., 2007; Ravaiano et al., 2014; Ren et al., 2014). The number of sensilla may also vary due to selection pressure, wherein the size, sex, and feeding habits of species might have a considerable impact on the variation in their sensilla (Chapman, 1982).
Many studies have been performed to better understand how insects perceive the environment, and what function each kind of sensillum serves in this perception. The morphological and physiological characterization of these sensilla have been fundamental components of this research. Such studies have been done on species in the orders Diptera (Hempelchom et al., 2017; Pezzi et al., 2018), Hymenoptera (Zhang et al., 2015; Zhou et al., 2015), Coleoptera (Ren et al., 2014; Vera and Bergmann, 2018), and many species of Lepidoptera, considered important agricultural pests, such as *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Yan et al., 2017), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Diongue et al., 2013), and *Spodoptera littoralis* (Boisdulav) (Lepidoptera: Noctuidae) (Seada, 2015). The characterization of their sensilla may help future studies to understand the physiological mechanisms whereby the chemical compounds of particular plants have repellent or attractive effects on such pests, as well as how these species detect their own sexual pheromones.

In this context, the present study aimed to evaluate the ultrastructure of the antennal sensilla of *Alabama argillacea* (Hübner, 1823) (Lepidoptera: Erebidae), an important pest of cotton.
(Gossypium hirsutum L.) crops. This species is considered the main defoliating pest of this crop, as it has caused losses in Brazil ranging from 21 to 35% of the cotton lint yield (Ramilho, 1994; Miranda and Oliveira, 2006; Ramalho et al., 2014). The antennae of male and female *A. argillacea* were analyzed using scanning electron microscopy (SEM). This was done to describe the sensilla present on the antennae of this species in detail, as well as their possible variation between the sexes.

**Materials and methods**

This study was conducted in the Laboratory of Insects’ Physiology in the Department of Morphology and Animal Physiology (DMFA) of the Federal Rural University of Pernambuco (UFRPE) and in the Immunopathology Laboratory Keizo Asami of the Federal University of Pernambuco (LIKA), both in Recife, PE, Brazil.

**Alabama argillacea breeding**

Insects were obtained from the breeding stock of the Laboratory of Insects’ Physiology. They were kept at a temperature of 25.2 ± 1.4 °C, a relative humidity of 67 ± 0.7%, and a photophase of 12 h. The caterpillars were fed daily with cotton leaves of the cultivar BRS Safira. The adults were kept in a PVC cage that was 15 cm in diameter by 20 cm in length and was internally coated with sulfite paper as a substrate for oviposition. The adults were fed with a 10% honey solution, along with a 1:1 mixture of yeast and honey.

**Scanning electron microscopy (SEM) analysis**

The antennae of five 48-hours-old males and five females of the same age were pre-fixed in Karnovsky liquid (G.A.: 2.5%; PFA: 4%). The antennae were treated with three washes with a 0.1 M cacodylate buffer for 10 min each, all at a pH of 7.4. They were then post-fixed in 1% osmium tetroxide (O<sub>S</sub>O<sub>4</sub>) for one hour in the absence of light. The samples were then treated with another wash in a 0.1 cacodylate buffer at a pH of 7.4, and then two washes in distilled water, for 10 min each time. The samples were subjected to triple dehydration in a graded series of ethanol (30, 50, 70, 90, and 100%) for 15 min each, and subjected to drying by the critical point method using liquid CO<sub>2</sub>. Samples were later mounted in stubs, metalized with colloidal gold for one minute, and analyzed under a JEOL-5600LV microscope.

**Terminology and statistical analyses**

The terminologies used herein to classify the sensilla of *A. argillacea* were based on Schneider (1964), Chang et al. (2015), and Seada (2015). The sensilla of the proximal and distal regions, which were considered to comprise the 10th to 15th antennomeres from either the base or the apical extremity of the antennae, respectively, were analyzed separately. The lengths and basal and apical diameters of the antennal sensilla of *A. argillacea* were measured from the photomicrographs obtained by SEM using the ImageJ program. The differences in sensilla sizes between males and females, and
between the proximal and distal regions of each sex, were compared using Student’s t-tests in the SAS program (SAS Institute, 2002).

Results

Based on differences in their morphology, seven types of sensilla were identified on the antennae of both males and females of *A. argillacea*: sensilla trichodea (s. trichodea), sensilla chaetica (s. chaetica), sensilla auricillica (s. auricillica), sensilla coeloconica (s. coeloconica), sensilla styloconica (s. styloconica), sensilla basiconica (s. basiconica), and sensilla Böhm bristles (s. Böhm bristles).

**Description and morphology of the antennal sensilla of Alabama argillacea**

The results of morphometric analyses showed that the length and basal diameter of s. trichodea type I were significantly greater in males than of those in females. Males also had longer s. trichodea type II than females. However, females had longer s. auricillica in comparison to those of males. Differences in the basal diameters of s. styloconica were also verified, in that these were larger in males than in females. The sizes of the other sensilla did not show any differences between males and females (Table 1).

In terms of the comparisons between the sensilla on the proximal and distal regions of the antennae, in males differences were only observed in the lengths of the lateral s. chaetica and ventral s. chaetica, and in the basal diameters of s. basiconica type I (Table 2). In females, only the lateral s. chaetica and ventral s. chaetica differed in length between regions. Regarding the diameters of sensilla, only the ventral s. chaetica and s. trichodea I showed differences between regions in the diameter of the sensillum base (Table 3).

The s. trichodea were visibly more abundant on the antennae of *A. argillacea* than any of the other types of sensilla were (Fig. 1A), and were distributed on the lateral and ventral regions of the antennae. This sensillum type was hair-shaped, with the cuticle presenting annealing in almost all of its extensions (Fig. 1B). Due to its external characteristics, the s. trichodea were subdivided into three types: type I, II, and III (Fig. 1C). The s. trichodea type I each formed an ‘S’ shape perpendicular to the antenna, and were concentrated mostly in the ventral region (Fig. 1D). Type II showed slight curvature, each with a shape similar to a ‘C’ (Fig. 1E). Type III could be slightly curved, or not, and was usually positioned parallel to the antenna (Fig. 1F). Types II and III both occurred in the lateral and ventral regions of the antenna.

The s. chaetica had a shape resembling a long hair (Fig. 2A–D) with longitudinal grooves in the cuticular wall (Fig. 2E) arising from a doughnut-shaped base. A total of six s. chaetica were observed per antennomere, two of them dorsal, two lateral, and two ventral, except in the last antennomere, which had more of them (Fig. 2F).

The s. coeloconica were ‘well’ shaped, each presenting a peg in the center with grooves in the cuticle. This sensillum was
surrounded by 10–12 spines facing the interior of the sensillum (Fig. 3A and B).

The s. auricillica had a shape resembling a ‘rabbit’s ear’. The cuticular surface showed a shallow concavity, with small longitudinal grooves in the cuticle (Fig. 3C). This sensillum type showed morphological variation in whether or not the side edges were directed toward the sensillum (Fig. 3C and D). The s. styloconica were located in the ventral region of the antennae, on the distal extremity of each antennomere, with a single sensillum being observed per segment. This sensillum type showed an ornate cuticular wall, with the top of the smooth sensillum containing two pegs (Fig. 3E and F).

The s. basiconica had the form of a stake, and their cuticular walls presented striations resembling ‘fishbone’ patterns. This sensillum type tapered from the base to the apex, but the apical extremity presented a triangular shape (Fig. 4A and D). The s. basiconica were subdivided into two types, I and II, based on their sizes. The s. basiconica type I (Fig. 4A and B) were longer than those of type II (Fig. 4C and D), being this last, able to be curved at the base or not.

Finally, the s. Böhm bristles were present on the pedicel and scape of the antennae, near the articulations that join the head to the scape and the scape to the pedicel. This sensillum type had a cone shape, with a smooth cuticular wall (Fig. 4E and F).

**Discussion**

Insect behaviors are mostly guided by chemical cues present in the environment. For example, insects locate food sources and oviposition sites by seeking the volatiles emitted by their hosts, or try to find their partners for copulation through the perception of sexual pheromones (Schneider, 1964; Cossé et al., 1998; Smith, 2007; Park et al., 2013; Wee et al., 2016). These cues are perceived through the chemoreceptor sensilla that detect these odor molecules, causing these organisms to accurately locate their targets (Gullan and Cranston, 2012; Cribb and Merritt, 2013).

Due to their high importance, chemoreceptor sensilla are usually present in greater numbers on insects’ antennae than other sensilla types. In some lepidopteran species, a predominance of s. trichodea on the antennae has previously been reported (Zheng et al., 2014; Chang et al., 2015; Seada, 2015; Yang et al., 2017), as was also observed in the antennae of A. argillacea in this study. This sensillum type plays an important role in the perception of plant volatiles and sex pheromones. This is a result of the presence of pores in the cuticle of these sensilla that allow these molecules to enter the sensillar cavity, where they connect to their receptors and trigger specific responses to the stimuli (Cossé et al., 1998; Gullan and Cranston, 2012; Cribb and Merritt, 2013; Wee et al., 2016).
The s. trichoea of insects present morphological variations, and can be classified into several subtypes according to their external characteristics, such as their form and shape (Chang et al., 2015; Seada, 2015) or the number of sensory cells they contain (Hallberg et al., 1994). In the case of lepidopteran species, three subtypes of s. trichoea were previously observed for *Mythima separata* (Walker) (Lepidoptera: Noctuidae) (Chang et al., 2015), *H. armigera* (Diongue et al., 2013), *Catocala remissa* Staudinger (Lepidoptera Noctuidae) (Zeng et al., 2014), and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Ndomo-Moualeu et al., 2014), as well as for *A. argillacea* in the present study. However, in other species, including *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Ma et al., 2017), *Copitaris consueta* (Walker) (Lepidoptera: Noctuidae) (Castrejón-Gómez et al., 1999), and *S. littoralis* (Seada, 2015), the s. trichoea were divided into two subtypes. In others, such as *Parnara guttata* (Bremer & Gray), *Parnara bada* (Moore), *Pelopidas Mathias* (Fabricius), and *Pelopidas agna* (Moore) (Lepidoptera: Hesperidae), only one type of s. trichoea was found (Xiangqun et al., 2014).

The morphological variations observed in s. trichoea suggest that the different subtypes of these sensilla may perform different specific functions from one another in the uptake of odor molecules. Some authors have suggested that the s. trichoea type I (long s. trichoea) are related to the perception of sexual pheromones (Van Der Pers and Den Otter, 1978; Dolzer et al., 2003; Binyameen et al., 2012). In addition, this subtype of sensillum is present in greater numbers on the males of some species of Lepidoptera than on the females, including *M. separata* (Chang et al., 2015) and *S. cerealella* (Ma et al., 2017). Further, in species like *C. consueta* (Castrejón-Gómez et al., 1999), *P. interpunctella* (Ndomo-Moualeu et al., 2014), and *C. remissa* (Zheng et al., 2014), the s. trichoea type I was found to only be present on males.

In the case of females that have s. trichoea type I, some studies have shown that they can respond to the sexual pheromones emitted by other females. These responses may be involved in triggering distinct behaviors depending on the species involved, such as grouping to increase the chances of mating, repelling other females, or stimulating oviposition (Birch, 1977; Palanawamny and Seabrook, 1978; Saad and Scott, 1981). However, for *A. argillacea*, the functionality of this subtype of sensillum is still unknown. Still, the differences observed between males and females in the morphology of this subtype of sensillum in the present study may reinforce the importance of s. trichoea type I in the capture of sexual pheromones emitted by females, although more studies are needed to confirm this hypothesis. In the case of s. trichoea types II and III, there is still little specific information regarding their olfactory functions. These sensilla may possibly be involved in the perception of plant volatiles.

Contact chemoreceptor sensilla play an important role in deciding whether an insect accepts particular substrates for feeding and oviposition. An important sensillum type that performs this gustatory function is s. chaetica (Hallberg et al., 1994; Cribb and Merritt,
These sensilla differ from s. trichodea in that they present longitudinal grooves in the cuticle and each possess a single pore at the apical extremity, while s. trichodea present annealing and possesses numerous pores in the cuticle (Zheng et al., 2014; Chang et al., 2015). The s. chaetica distribution on the antennae follows a particular pattern in some species of Noctuidae, such as M. separata (Chang et al., 2015) and S. littoralis (Seada, 2015), in which a total of six sensilla occur per antennomere, but with the last antennal segment bearing a greater number of these sensilla. This pattern is the same as that observed in A. argillacea in this study. In this species, the lateral s. chaetica and ventral s. chaetica of the distal region were found to be longer relative to those of the proximal region, suggesting that the distal region of the antennae may be more active in the uptake of gustatory stimuli, as was suggested by Seada (2015) for S. littoralis.

Other types of chemoreceptor sensilla, including s. auriicillica, s. coeloconica, s. styloconica, and s. basiconica were also found on the antennae of A. argillacea. The s. auriicillica in this species presented a similar morphology to those of S. littoralis (Seada, 2015) and Calyptra thalictrii (Borkhausen) (Lepidoptera: Noctuidae) (Hill et al., 2010). In relation to the functionality of these types of sensilla, previous studies performed with Scoloptrix libatrix L. (Lepidoptera: Noctuidae) (Anderson et al., 2000) and S. littoralis (Binyameen et al., 2012) showed that they are involved in the perception of plant volatiles. Meanwhile, in Cydia pomonella (Walsingham) (Lepidoptera: Tortricidae) (Ebbinghaus et al., 1997) and Eriocrania spp. (Lepidoptera: Eriocridae) (larsson et al., 2002), the s. auriicillica of the males were found to respond to female sex pheromone components, while presenting functional variation depending on the species.

The s. coeloconica in A. argillacea were similar to those of S. littoralis (Seada, 2015), H. armigera (Diongue et al., 2013), M. separata (Chang et al., 2015), and Pseudaletia unipuncta (Haw.) (Lepidoptera: Noctuidae) (Lavoie-Dornik and McNeil, 1987). This sensillum type is also responsible for the perception of plant volatiles (Van Der Pers, 1981). Furthermore, some studies have shown that s. coeloconica are involved in hygroreceptor and thermoreceptor functions in insects, detecting changes in humidity and ambient temperature, respectively (Altner et al., 1977, 1983).

The s. styloconica of A. argillacea were found on the ventral region of the antennomeres, at the distal extremity of each segment. This pattern was previously observed in P. unipuncta (Lavoie-Dornik and McNeil, 1987) and S. littoralis (Seada, 2015). Besides their chemoreceptor function (Wall, 1978; Lavoie-Dornik and McNeil, 1987; Chang et al., 2015), this type of sensillum has been suggested to be involved in thermo-hygroreceptive functions in Bombyx mori L. (Lepidoptera: Bombycidae) (Steinbrecht and Müller, 1991).

The s. basiconica type I of A. argillacea were very similar to its s. trichodea, although when examined closely it was possible to distinguish them by their cuticle wall and apical extremity, which is more triangular in s. basiconica. The occurrence of s. basiconica has also been reported in other species of Noctuidae, such as C. consueta (Castrejón-Gómez et al., 1999), M. separata (Chang et al., 2015), and S. littoralis (Seada, 2015). A generally chemoreceptive function has been attributed to these sensilla in insects (Chang et al., 2015).

A type of mechanoreceptor sensillum found in the antennae of A. argillacea in this study was s. Böm bristles. These sensilla were restricted to the scape and pedicel of the antennae, next to the articulation of these segments, and did not present pores in their cuticle (Chang et al., 2015). Their function might be related to the detection of antennal positioning and movement, with them playing a mechanoreceptive role in insects (Schneider, 1964; Merivee et al., 2002; Krishnan et al., 2012).

Conclusions

The antennae of A. argillacea were found to bear seven types of sensilla, which are: trichodea, chaetica, auriicillica, coeloconica, styloconica, basiconica, and Böm bristles. The differences between the sensilla of males and females were related to their lengths and basal diameters in the distal and proximal regions. This suggests that the specific sensory functions of these sensilla may not only vary from one species to another, but also within the same species between males and females. Thus, further transmission electron microscopy and single sensillum recording studies may provide more detailed information on the sensilla of A. argillacea and their functions.

Conflicts of interest

The authors declare no conflicts of interest.

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