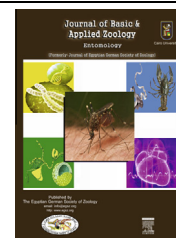




The Egyptian German Society for Zoology
The Journal of Basic & Applied Zoology

www.egsz.org
www.sciencedirect.com



Antennal morphology and sensillum distribution of female cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae)



Mervat A. Seada

Division of Entomology, Department of Zoology, Tanta University, 31527 Tanta, El-Gharbiya, Egypt

Received 20 October 2014; revised 22 December 2014; accepted 19 January 2015
Available online 24 February 2015

KEYWORDS

Spodoptera, Antenna;
Topology;
SEM;
Chemosensory;
Contact/mechanosensory;
Mechanosensory;
Sensilla

Abstract Female cotton leaf worm *Spodoptera littoralis* (Biosduval) is highly dependent on its antennal sensory structures for host location for feeding or oviposition. The external structure, number, and distribution of the antennal sensilla of female *S. littoralis* were examined with light and scanning electron microscopy. The antenna of adult female is filiform, composed of a scape, a pedicel and a flagellum. The flagellum has 65–80 segments densely packed with sensilla distributed on the ventral surface and lateral edges of the flagellum except the dorsal surface which is packed with scales. Eight types of sensilla; trichodea, basiconica, auricillica, coeloconica, uniporous peg, chaetica, styloconica, and squamiformia were detected. Total number of antennal sensilla varied among different antennal portions. Proximal segments showed significantly more short trichoid and basiconic sensilla than distal ones, while distal segments have the longest sensilla chaetica. Last antennal segment carries the higher number of sensilla chaetica than other segments and ends with an apical crown which has 1–3 branches, each is endowed with 4–6 apical aporous sensilla styloconica. The possible function of the antennal sensilla is discussed in relation to their morphology. © 2015 The Egyptian German Society for Zoology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Insects with a great diversity in form, development, habitat and habits, are expected to have a great diversity in their sensory modalities, both structural and functional. The structural and functional complexity of a sensillum is even greater in animals with an exoskeleton that is shed and replaced periodically (Zacharuk, 1985). Insects, such as moths, possess antennae

that are packed with a multitude of minute sensory structures (sensilla). These sensilla are the crucial interface between the outer world and the nervous system of the insect. The specialized receptor cells housed in these sensilla are designed to detect the environment and to transmit the information regarding the nature of surroundings to the central nervous system that drive behaviors such as host selection; including location, recognition, discrimination, and acceptance for feeding or oviposition (Byers, 1995; Schneider, 1964; Shields and Hildebrand, 2001). Antennae are the primary olfactory organs in insects (Byers, 1995; Wigglesworth, 1972). Whereas, antennal sensilla of insects present conspicuous morphological and

E-mail address: mervtseda@yahoo.com

Peer review under responsibility of The Egyptian German Society for Zoology.

<http://dx.doi.org/10.1016/j.jobaz.2015.01.005>

2090-9896 © 2015 The Egyptian German Society for Zoology. Production and hosting by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

ultrastructural differences. Zacharuk (1985) suggested that, the sensilla on insect's antennae are not randomly distributed. Their pattern may reflect the impact of many interacting selection pressures in which size of the individual, developmental stages, sex, feeding habits and habitats are of considerable significance (Chapman, 1982).

More comprehensive analysis of the morphology of the cuticular part of the sensilla, their arrangement and innervations allows using these data not only for studying the systematic position of the insect, but also advances in the evolution of species, family, and order (Akenteva and Chaika, 2007). Typical cuticular sensilla of insects consist of: a specialization of the cuticle, bipolar sensory neurons, supported and ensheathed by 3–4 accessory cells at the base, all are of ectodermal origin (McIver, 1975). The diversity of sensilla in insects has made it necessary to classify them. The classification of sensillum types in insects is done on the basis of cuticular morphology supported by studies of ultrastructure and electrophysiology. Various classifications of sensilla were made; the first one was suggested by Snodgrass (1935). Later, it was developed by Schneider (1964), and Schneider and Steinbrecht (1968). However, another classification of sensilla was suggested by Slifer (1961), the principle of this classification was associated with the ductance ability of the cuticular part of the sensilla. Nevertheless, in this classification it was difficult to evaluate the diversity in the morphology of the sensilla, in which, analogous changes in the structure of the cuticle can be seen in different groups of insects inhabiting similar environments. Furthermore, there are variations in the number of small pores on the cuticle of sensilla that are believed to be due to functional differences (Zacharuk, 1985). The pore numbers of sensilla vary from aporous, considered to be mechanosensory, to uniporous, considered to be gustatory though often with possible mechanosensory innervation, to multiporous, which are considered to be olfactory (Schneider, 1964; Zacharuk, 1985; Mitchell et al., 1999). Schneider's (1964) classified the insect sensilla into 10 morphological types; there may also be functional differences among the sensilla in a single morphological category.

Zacharuk (1962) was the first who, in his work dedicated to investigation of sensory structures on the head and its appendages in larvae of click beetles (Coleoptera: Elateridae). Thereafter, morphology of the antennal sensilla has been described for many orders of insects. Therefore, some important features could be inferred from the descriptive studies of the antennal sensilla.

Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is a serious pest of a large variety of crops in many parts of the world. It is mainly found in Africa, the Middle East and Southern Europe. Its host range includes at least 87 species of economically important crops; cotton, Lucerne, soybeans, *Trifolium* and other vegetables (Salama et al., 1984).

Female moths, like *S. littoralis*, find their host plants mainly through odor-mediated behavior. They detect volatile chemicals that indicate host plant suitability and also the presence of potential competitors or co-habitants (Hansson et al., 1995). In moths, the volatile cues are detected by olfactory sensory neurons (OSNs) enclosed in sensilla distributed across the antennal surface (Hallberg et al., 1994; Shields and Hildebrand, 1999). Research on antennal sensilla of the moths in relation to their host preferences furthers our understanding of the evolution of taxonomic groups with specialized hosts. In

this study, we examined the type, number, external morphology, and distribution of antennal sensilla of female *S. littoralis* with both light and scanning electron microscopy (SEM). This reveals possible structure–function relationships, which might lead to a better understanding of behavioral mechanisms of the female moth in host selection.

Materials and methods

Insects

The *S. littoralis* used in the experiments originated from a laboratory culture. Since then, field collected moths have been introduced into the culture on a yearly basis. Larvae were reared on an artificial diet. Pupae were collected, sexed and then kept separated. For all experiments, 2–3 day old females were used. All developmental stages were kept at 25 °C, 70% relative humidity and at a light:dark cycle of 16:8 h.

Fine structure and scanning electron microscopy

For light microscopy, antennae were dissected from moth's heads. For better investigation of the antennal sensilla, scales of the dorsal edges of the antennal segments were gently removed by rolling the antenna on double sided sticky tapes, then antennae were mounted for temporary storage onto a microscope slide with a piece of double sided sticky tape. Thereafter, antennae and their sensilla were examined and photographed under Olympus (CX31).

For scanning electron microscopy (SEM), antennae were excised by fine scissors and immersed in 70% ethanol overnight at 4 °C. Specimens were then dehydrated in 80%, 90% and 100% ethanol, mounted on SEM stubs and sputter coated with gold–palladium (3:2) in a JEOL ion sputter JFC-1100. The specimens were visualized using a scanning electron microscope (LEO 435 VP, UK).

The number, distribution, and length of each morphological sensillum type were analyzed from light and SEM micrographs of the 14th segment from the base (Proximal segment) and the 14th segment before the tip (distal segment) of the antenna.

Results

Antenna morphology

The antenna of female *S. littoralis* was filiform, composed of a scape, a pedicel, and a flagellum with 65–80 segments (Fig. 1). The antenna was about 4 mm in length. The scape is 280 µm in length and 240 µm in diameter at the base. The pedicel is 150 µm in length and 200 µm in diameter at the base. The last segment of the flagellum was ending with a small narrow and tapering crown (20 µm in length and 12 µm in diameter at the base diameter), sometimes it was 1–3 branches, each branch ended with 3–6 apical aporous sensilla styloconica. The sensory field full of sensilla of various types was found on the ventral surface and lateral edges of each antennal segment, except the membrane between segments of flagella which do not have any sensilla. The dorsal edges of antennal segments were covered with scales with few scattered individual sensilla.

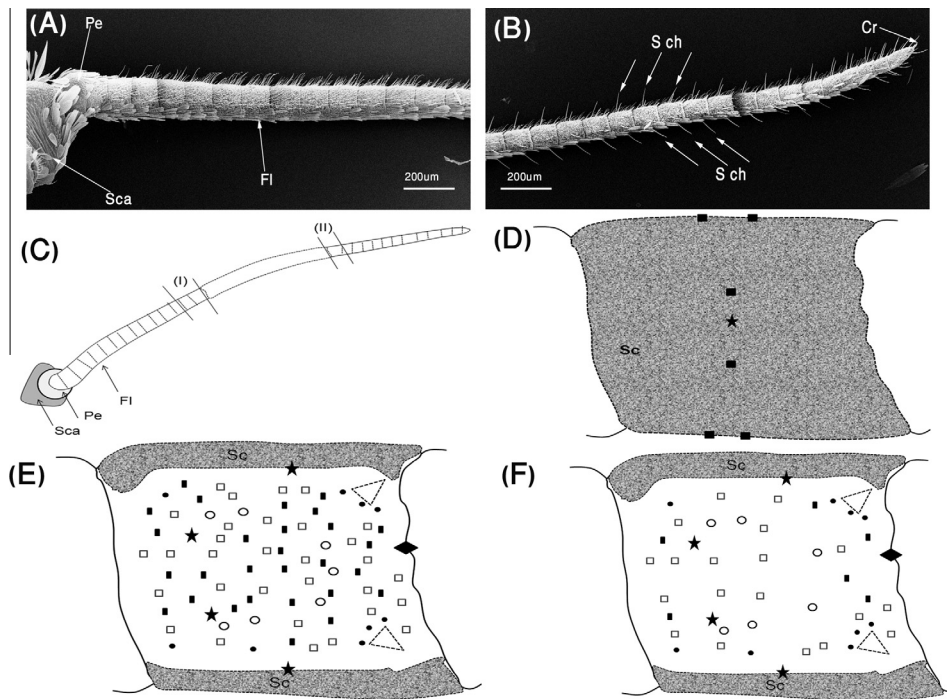


Figure 1 Overview and schematic diagrams of the antenna of female *S. littoralis* with various scattered sensory sensilla. (A) SEM showing the proximal portion of the antenna with the sensilla distributed throughout the ventral and lateral surfaces. Pe, pedicel; Sca, scape; Fl, flagellum. (B) SEM showing the distal portion of the antenna, arrows point to the longer sensilla chaetica (S ch), and apical crown (Cr). scale bar in (A) and (B) = 200 μ m. (C) Schematic drawing of the antenna showing the two portions; I proximal segments, and II the distal segments. (D) Schematic diagram of the dorsal and view of one antennal segment showing the packed field of scales (Sc) and sensillum chaeticum (S ch, star), sensilla squamiformia (S sq, closed squares). (E) Schematic diagram of the ventral and lateral views of one antennal segment showing the distribution of the antennal sensilla in detail. Note that the white area represents the sensory field of one proximal antennal segment showing the distribution of short sensilla trichodea (sS tr, opened squares) and basiconica (S bs, closed rectangles). Stars represent positions of four sensilla chaetica (S ch), solid circles represent long sensilla trichodea (lS tr), open circles represent sensilla coeloconica (S coe), triangles represent the positions of sensilla auriculica and peg in pit sensillum, and Sc represents scales. (F) Schematic diagram of the ventral and lateral views of one distal antennal segment showing the distribution of different sensilla, note the decreasing of the number of sensilla trichodea (S ch) and basiconica (S bs) in comparison with proximal antennal segment.

Antennal sensillum types and distribution

Eight types of sensilla were observed on the sensory field of each antennal segment: trichodea, basiconica, chaetica, auriculica, coeloconica, styloconica, uniporous peg, and squamiformia sensilla. The density of sensilla decreases from proximal to distal ends but all types of sensilla were found in all segments. The dominant types of sensilla were trichoid and basiconic sensilla, especially on proximal segments (Figs. 2 and 3).

Sensilla trichodea (S tr)

Sensilla trichodea were divided into two subtypes; long and short sensilla trichodea. Short sensilla trichodea (sS tr) were the dominant sensillum type (44 ± 7 per segment proximally, 24 ± 6 per segment distally), averaging 40 μ m in length and 2 μ m in diameter at the base and tapering to 0.5 μ m diameter at the tip (Fig. 1B and 2B, C, E). They were distributed in parallel bands over the ventral surface and lateral edges of the antennal segment. 10 ± 2 long sensilla trichodea (lS tr) were present on the distal lateral edges of each segment (5 ± 1 on each lateral edge) and averaged 50 μ m in length, 1.5 μ m in diameter at the base, and tapered to 0.5 μ m (Fig. 2A–C). Both

short and long sensilla trichodea were multiporous sensilla which dedicate the olfactory function of these sensilla (Fig. 2E). They have a sharp tip and arc-like morphology. They do not have a basal socket (Fig. 2A–C and E). There was a difference in the number of sensilla trichodea between proximal and distal segments, whereas, the distal segment has fewer number of short trichoid sensilla (Figs. 2 and 3A–C).

Sensilla basiconica (S bs)

Sensilla basiconica were the second most common sensillum type (36 ± 3 per segment proximally, and 6 ± 2 per segment distally), on average 13 μ m long, 1.4 μ m in diameter at the base, and tapering to 0.75 μ m at the tip (Fig. 2D and E). They were found on parallel bands over the ventral surface and lateral edges of the antennal segments overlapped with the short sensilla trichodea. They were multiporous sensilla which dedicate the olfactory function of these sensilla (Fig. 2D and E). They have a blunt tip and curved rod-like morphology. They do not have a basal socket (Fig. 2A–E). There was a difference in the number of sensilla basiconica between proximal and distal segments, whereas, the distal segment has a lower number of sensilla basiconica (Figs. 2C and 3B).

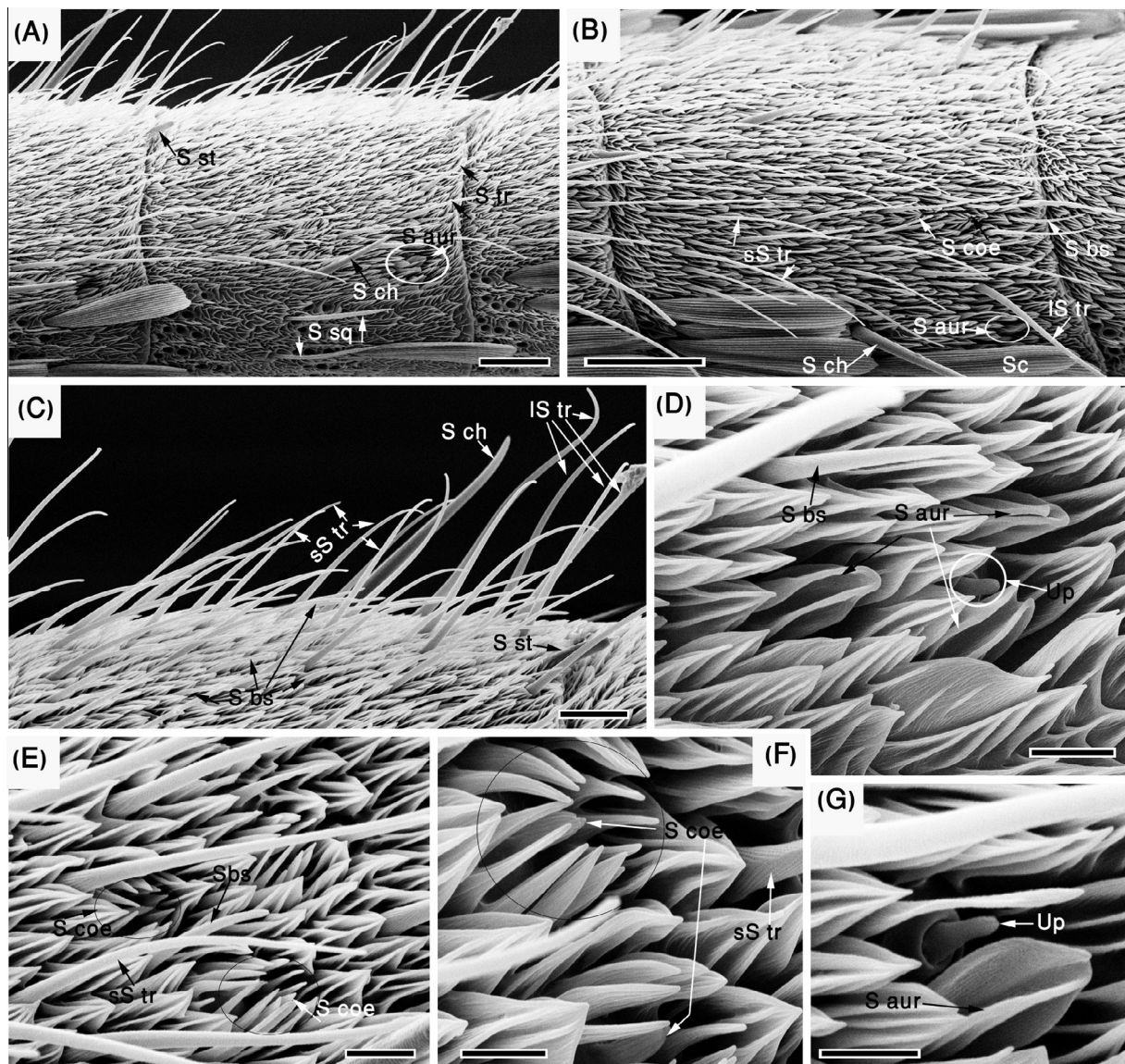


Figure 2 Scanning electron micrographs (SEM) of a proximal antennal segment (14th segment after the pedicel) of female *S. littoralis* and its associated sensilla. (A) and (B) SEM showing ventro-lateral view of proximal segments, scale bar = 20 μm . (C) Ventral view of antennal segment showing distribution and characteristics of long and short sensilla trichodea (IS tr, and sS tr), one distal sensillum styliconicum (S st), numerous short curved sensilla basiconica (S bs). Scale bar = 10 μm . (D) The lateral distal position of the three sensilla auriculica (S aur) in invaginations of the antennal surface, and one uniporous peg sensillum (Up) ending with a pore (circle). Note, one basiconic sensillum (S bs) whereas its surface is perforated with numerous pores. (E) Ventral view of the antennal segment showing a field of sensilla coeloconica (S coe) distributed in between a field of short sensilla trichodea and basiconic sensilla, note the absence of a basal socket on the basal insertion of sensilla trichodea (square). (F) Higher magnification of two sensilla coeloconica (S coe) showing it as a grooved peg settled in a pit surrounded by 12 cuticular spines (circle). Note, the basal portion of one sensillum trichodeum (S tr) perforated with numerous pores. (G) Higher magnification of one sensillum auriculicum and a uniporous peg sensillum (Up) found in a cleft beside the field of sensilla auriculica (S aur), note the basal mortar like socket around the base of the peg sensillum which ends with a terminal pore. Scale bars in (D–G) = 5 μm .

Sensilla coeloconica (S coe)

6 ± 2 sensilla coeloconica (S coe) were found on each segment, mostly located on the ventral surface of the antenna, and averaged 4 μm in length and 1.5 μm thick at the base (Figs. 2F and 3H). This sensillum type appears as a grooved peg, settled in a pit surrounded by 12 cuticular spines, which are pointing inward and form a circle around the peg. There was no differ-

ence between proximal and distal segments in the number or distribution of sensilla coeloconica.

Sensilla auriculica (S aur)

Six sensilla auriculica (rabbit ear sensilla); each three were situated on lateral edges close to the distal margin of each segment (5 μm long and 4 μm wide at the base) in protected loca-

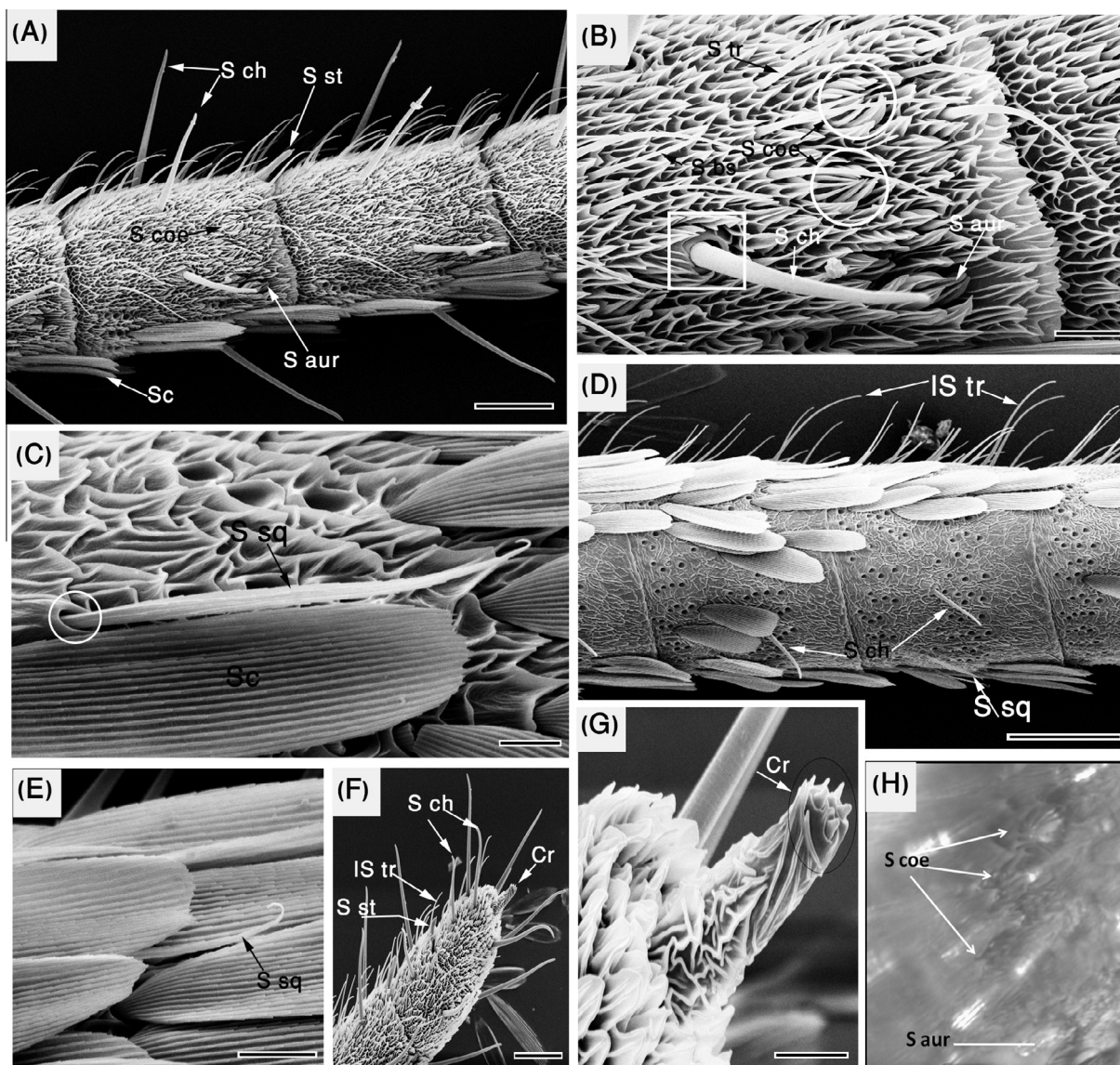


Figure 3 Scanning electron micrographs (SEM) of the distal antennal segments (14th and 15th segments before the antennal tip) of female *S. littoralis* and its associated sensilla. (A) SEM showing distal segments of antenna with sensilla chaetica (S ch) (arrows), coeloconica (S coe), and lateral distal auricillica (S aur) in a cleft. Scale bar = 20 μ m. (B) SEM showing the ventral view of a distal antennal segment with sensilla coeloconica (S coe) (circles), a group of three sensilla auricillica (S aur), sensilla chaetica (S ch), and low numbers of sensilla trichodea (S tr) and basiconica (S bs). Note the basal membranous socket (square) and the distal blunt tip of sensilla chaetica. Scale bar = 5 μ m. (C) Dorsal view of the antennal segment showing sensillum squamiformium (S sq) and scales (Sc). Note the longitudinal warts and the basal socket membrane. Scale bar = 5 μ m. (D) Dorsal view of the antennal segments showing the distribution of the dorsal sensilla chaetica (S ch), sensilla squamiformia (S sq), and lateral long sensilla trichodea (IS tr). Scale bar = 50 μ m. (E) Dorsal view of the antennal segment showing a field of scales (Sc) and one sensillum squamiformium (S sq). Scale bar = 5 μ m. (F) Higher magnification of the last antennal segment of antenna with higher number of sensilla chaetica (S ch, arrows), long sensilla trichodea (IS tr), one sensillum styloconicum (S st), and the distal crown (Cr). Scale bar = 5 μ m. (G) Antennal crown at the antennal tip showing six aporous sensilla styloconica at the distal surface. Scale bar = 5 μ m. (H) Light micrograph of the ventral view of distal antennal sensilla showing three sensilla coeloconica (S coe) and one sensillum auricillicum (S aur). Scale bar = 10 μ m.

tions among the surrounding longer sensory hairs, sunken in shallow depressions only slightly above the level of the microtrichia (Fig. 2D and G). They are not clearly visible from the antennal surface surrounded with microtrichia (mt). The lateral surface of this sensillum is deeply, concaved (Fig. 2D and G). They were multiporous sensilla (Fig. 2G).

Uniporous peg sensilla (Up)

Only two uniporous peg sensilla (one on each distal edge of the antennal segment) were sunken in deep pits. They were small in size (5 μ m long and 2.5 μ m in basal diameter) surrounded with a mortar like basal socket membrane and ended with a pore at

its terminal tip. They were mainly found on the ventro-lateral surface and close to the sensilla auricillica at the distal margin of each segment (Fig. 2D and G).

Sensilla chaetica (S ch)

Sensilla chaetica are similar to sensilla trichodea except that these bristles and hairs are set in a socket and can be identified by their thick walls. They are found on distinct locations at each antennal segment. They were two sensilla chaetica at the lateral edges of the antennal segment (proximally, 40 μm long, and 1.6 μm in diameter at the base, and 50 μm long, and 2 μm in diameter at the base; distally) and two at the ventral surface of the segment (proximally, 25 μm long, and 1.8 μm in diameter at the base; 30 μm long, 2 μm in diameter at the base, distally). In addition there are 1–2 sensilla chaetica at the dorsal surface of each antennal segment between the scales (25 μm long, and 1.5 μm in diameter at the base) (Fig. 3A, B and E). They were uniporous sensilla which dedicate the gustatory function (Fig. 3B). They have a blunt tip and curved rod-like morphology. They were protruding from a mortar-like cavity with a basal articulating membrane (Fig. 3B). There was no differences between proximal and distal segments in number or distribution of sensilla chaetica but the distal segments have the longer sensilla chaetica than the proximal ones (Figs. 2 and 3A). Moreover, the last antennal segment had the higher number of sensilla chaetica (7–8) than the other antennal segments (Fig. 3F).

Sensilla styloconica (Sst)

Sensilla styloconica are peg-like, set on top of a conical cuticular style attached to a membranous socket (9 μm long, and 3.5 μm in the basal diameter) (Figs. 2B and 3A). Only one sensillum styloconicum (Sst) was found on the distal margin of the ventral surface of each antennal segment along the entire antennal flagellum, parallel to antennal surface with tip pointing to the distal ends (Figs. 2B and 3A). Moreover, there are a group of 4–6 apical aporous sensilla styloconica arranged at the top of the distal styliform crown of the last antennal segment.

Sensilla squamiformia (Ssq)

They were 5 ± 2 sensilla squamiformia (Ssq), each two distributed at the lateral margins of the antennal segment and one at the middle dorsal margin of the antennal segment, all were covered with scales, growing out of basal membranous sockets (Fig. 3C–D). The surface of sensilla squamiformia looks like scales, but narrower, with obvious longitudinal warts (35 μm long, 2 μm in the basal diameter). No pores have been detected on the surface of these sensilla.

Discussion

Insects rely on multitude, distinct organs for different senses. Some reports have clearly indicated large numbers of chemosensilla located on antennae, mouthparts, tarsi and ovipositor in insects (Stadler, 1984; Whitehead, 1981). The multiplicity of receptors allows detection of a vast number of

chemicals and determines insect behavior (Su et al., 2009). The effectiveness of stimulus trapping is related in part to the number and distribution of chemosensilla for contact with solutions (Maes and Vedder, 1978) or exposure to odors (Steinbrecht, 1973). Functions of sensilla in insects were proposed based on their structure and physiological/behavioral responses.

There are eight types of sensilla on the antennal flagellum of the female moth, *S. littoralis*: trichodea, basiconica, auricillica, coeloconica, peg-in pit, chaetica, styloconica, and squamiformia. Morphological evidence indicates that of these eight types, five appear to be olfactory, one appears to be gustatory, one appears to be thermo-hygrosensory, and one appears to be mechanosensory. The types and topology of antennal sensilla were very different from those in other insects such as Hymenoptera and Coleoptera, but are very similar to those found in Lepidoptera (Shields and Hildebrand, 2001). These observations indicate that not only the sibling species have similar morphology and behavior, but also similar sensillum types and distribution.

In antennae of female *S. littoralis*, short trichodea and basiconica sensilla make up the bulk of the sensory field of the antennal flagellum. There were also several scattered long sensilla trichodea scattered at the edges of each antennal segment. In general, sensilla trichodea are setiform hairs of variable lengths. Their diameters are generally proportional to their lengths. Basiconic sensilla can be distinguished from trichoid sensilla in that they (1) are shorter; (2) have a thinner cuticular wall that is pitted by a higher density of pores (Keil and Steinbrecht, 1984; Shields and Hildebrand, 2001). The fine structure of the trichoid sensilla of female *S. littoralis* resembles that of trichoid sensilla of female and male *Bombyx mori* (Steinbrecht and Gnatzy, 1984), females *Manduca sexta* (Shields and Hildebrand, 2001), males of *M. sexta* (Keil, 1989; Lee and Strausfeld, 1990). In this study, it has been found that the antennal trichoid sensilla of female *S. littoralis* were divided into two subtypes (long and short sensilla trichodea) as in *Noctua pronuba* (Faucheux, 1990). Binyameen et al. (2012) reported the olfactory function of the long and short trichoid sensilla of antenna of female *S. littoralis*. It has been investigated that long trichoid sensilla responded to female-produced sex pheromone components. In contrast, the short-trichoid sensilla elicited spontaneous activity, but did not respond to plant odors or female sex pheromone.

Similarly, studies of many other lepidopteran species have shown that these sensilla can be divided into more subtypes according to their size and pore density (Faucheux, 1999). For example, three subtypes of sensilla trichodea were found on *Synanthedon scitula* and *Ostrinia nubilalis* (Hübner) (Frank et al., 2010; Hallberg et al., 1994). However, Xiangqun et al. (2014) found only one type of sensilla trichodea at the antennae of *Parnara* and *Pelopidas* species (Lepidoptera, Hesperidae). Furthermore, the morphological characteristics of the basiconic sensilla of female *S. littoralis* resemble that of basiconic sensilla of female and male *B. mori* (Steinbrecht and Gnatzy, 1984), female *M. sexta* (Shields and Hildebrand, 2001), males of *M. sexta* (Keil, 1989; Lee and Strausfeld, 1990).

Short trichoid and basiconic sensilla are the main two types of sensilla present at the distal segments and were most abundant along the ventral surface and lateral margins of each antennal segment. They were found in U-shaped bands merged

toward the distal margins. This pattern is similar to the pattern of distribution of the specific trichoid sensilla of male and female *M. sexta* (Lee and Strausfeld, 1990; Shields and Hildebrand, 1999, 2001). Moreover, the accumulated studies have shown that multiporous sensilla trichodea are associated with olfactory reception of sex pheromones (Hansson et al., 1995; Ebbinghaus et al., 1997; Ma and Du, 2000). However, sensilla with a similar structure to short sensilla trichodea have been found to function as contact chemo- and mechanoreceptors (Zacharuk, 1985).

Coeloconic sensilla of female *S. littoralis* are grooved peg in pit surrounded with cuticular finger-like processes, most abundant only on the ventral surface of the antennal segments. This structure suggests the olfactory function of these sensilla. Binyameen et al. (2012) investigated the olfactory function of sensilla coeloconica of female *S. littoralis* to plant volatiles. Moreover, there was no difference in the number or distribution of sensilla coeloconica between the proximal and distal segments except the last segment which do not have any of these sensilla. This constant distribution in proximal and distal portions of the antenna suggests that these sensilla might be having a vital role in host plant detection for oviposition.

The fine structure of coeloconic sensilla of female *S. littoralis* closely resembles that of sensilla in both sexes of *Adoxophyes orana* (Den Otter et al., 1978), male *M. sexta* (Lee and Strausfeld, 1990), female *M. sexta* (Shields and Hildebrand, 2001), *Parnara* and *Pelopidas* species (Xiangqun et al., 2014). Similar coeloconic sensilla have also been described in other insects, such as *Simulium* species (Mercer and McIver, 1973), *Aedes aegypti* (McIver, 1974), *Locusta migratoria* (Altner et al., 1981). Furthermore, the multiporous sensilla coeloconica of female *S. littoralis* resemble those observed in many other Lepidoptera. Pophof (1997) reported that in *B. mori* L., they were sensitive to plant volatiles and are possibly involved in the selection of oviposition sites.

Sensilla auricillica (rabbit ear sensilla) were multiporous sensilla, sunken in shallow depressions only slightly above the level of the microtrichia. Sensilla auricillica have been described in different moth species, including both sexes of adult *A. orana* (Den Otter et al., 1978), *Agrotis segetum* (Hallberg, 1981), *N. pronuba* (Faucheux, 1990), *O. nubilalis* (Hallberg et al., 1994), and *M. sexta* (Shields and Hildebrand, 2001). However, these sensilla on the antenna of butterfly were described in *Pieris rapae* L. (Faucheux, 1999), and *Hesperidae* species (Xiangqun et al., 2014). The similarity of this sensillum type to morphologically or physiologically characterized sensilla auricillica of other insects suggests that this sensillum of female *S. littoralis* is olfactory sensillum. In a previous study, it has been investigated that sensilla auricillica of female *S. littoralis* were olfactory sensilla harbor olfactory sensory neurons (OSNs) tuned with plant volatiles (Binyameen et al., 2012). Several studies on other moth species considered multiporous sensilla auricillica as olfactory receptors for plant volatiles (Boekx et al., 1965; Kaissling, 1971). Others suggest they respond to sex pheromone compounds (Ebbinghaus et al., 1997; Anderson et al., 2000; Faucheux, 2006). Moreover, there was no difference in the number or distribution of sensilla auricillica between the proximal and distal segments except the last segment; without these sensilla. This constant distribution in proximal and distal portions of antenna suggests that these sensilla might have a vital role in host plant detection for oviposition. In addition, their position in

shallow depressions on the antennal segment makes them secure enough in the distal portion of antenna from damage or friction with the wind.

In female antenna of *S. littoralis*, only two pegs have been found in pit sensilla; one on each distal edge of the antennal segment. They were sunken in deep pits surrounded with a mortar like basal socket membrane and ended with a pore at its terminal tip. These sensilla were described for the first time in *N. pronuba* (Faucheux, 1990); they were found in 4 other noctuid species (Faucheux, 1993) and also in 13 species belonging to 8 different noctuid subfamilies (Faucheux, 1997, 1999). So far, they have been observed only in the family Noctuidae. These uniporous pegs are also considered to be uniporous short sensilla styloconica. Binyameen et al. (2012) investigated the olfactory function of these sensilla but they did not respond to any plant volatiles or sex pheromone components that were used in this study. Therefore, these sensilla might be sensitive to carbon dioxide or humidity as a hygroreceptor. Further electrophysiological studies are needed to reveal its specific function.

The sensilla chaetica were found distributed around each antennal segment of female *S. littoralis* except the last segment which had a higher number of sensilla chaetica. Several investigations showed a trend to the multiplication of the contact chemosensory units at the tip of the antenna. Sensilla chaetica of the antenna of *S. littoralis* fit the description of Zacharuk (1985) of sensilla which have been suggested to have contact/chemoreceptor functions, whereas, they were uniporous with basal membranous sockets and ending with blunt tips. Uniporous sensilla are a generalized group of sensilla which are structurally and functionally most similar to the thick-walled category of Slifer (1970). All have in common an opening at one point in the cuticle through which chemical communication can occur between the dendrites and the external environment. Several studies noted that these uniporous sensilla are contact chemoreceptors (Altner and Prillinger, 1980; Hallberg et al., 1994). In a previous study, we have investigated the gustatory function of these sensilla to phagostimulative and deterrent compounds with electrophysiology (Seada et al., unpublished data). Sensilla chaetica found in this study are similar in structure to those reported for *Chilades pandava* and *Heliophorus phoenicopyrphus* (Jiang et al., 2000; Xu and Wang, 2013), and *Parnara* and *Pelopidas* species (Xiangqun et al., 2014), *Cydia nigricana*, and *Zamagiria dixolophella* (Wall, 1978; Gomez et al., 2003). There were no differences between proximal and distal segments in the number or distribution of sensilla chaetica, but the distal segments have the longer sensilla chaetica than the proximal ones. Moreover, the last antennal segment has a higher number of sensilla chaetica than other antennal segments. The increasing of the lengths of these sensilla at the distal portion of antenna of female *S. littoralis*, in addition of higher number of them at the last antennal segment makes them easily contact and access different substrates. This suggests that these gustatory sensilla are important for females in final assessment and accepting or rejecting the host plant for feeding or oviposition.

The styloconicum sensillum of female *S. littoralis* was found singly at the distal margin of each antennal segment. It stands parallel to the antennal surface and is surrounded mainly by trichoid and basiconic sensilla. In addition of the group of the apical aporous sensilla styloconica that were present on the styliform crown. These sensilla might have a

humidity and temperature sensitive function. Therefore it could be speculated that sensilla styloconica were hygroreceptor sensillum (review in Faucheux, 1999), important in host localization. But their exact function requires further studies into ultrastructure and electrophysiology. Shields and Hildebrand (2001) investigated that the styloform complex sensillum at the antennae of female *M. sexta* is single-walled, aporous, and resembles a peg. A similar styloconic sensilla has been described in several insects, such as ermine moths (Cuperus, 1985), silkmoths (Steinbrecht et al., 1989), turnip moths (Hallberg, 1981), European cornborers (Hallberg et al., 1994), and male *M. sexta* (Lee and Strausfeld, 1990).

In this study, it has been observed that sensilla squamiformia were surrounded by a thick cuticle and basal socket membrane, and lack wall pores. Therefore, their morphology and distribution suggest a mechanoreceptive function. These aporous sensilla are inferred to have a mechanoreceptive function (Schneider, 1964). Sensilla squamiformia are commonly present in lepidopteran insects (Faucheux, 1999).

These long sensilla on the dorsal surface and lateral margins of antennal segments between scales may enable the insect to determine the positions of the antenna with respect to its surroundings through contact mechanoreception with the substrate, or they may function as wind velocity receptors (Dyer and Seabrook, 1978). However, in pine cone weevil *Pissodes validirostris* sensilla scolopalia were believed to be typical sound sensors (Zhao et al., 2012). The sensilla squamiformia of female *S. littoralis* are similar in shape and distribution to those reported in different moth species, *S. scitula* (Harris), *Coleophora* sp. and *Z. dixolophella* Dyar (Gomez et al., 2003; Frank et al., 2010; Faucheux, 2011) and different from Coleoptera (Gao et al., 2013; Xiangqun et al., 2014).

Novel sensillum types of insects were being continuously discovered, but the collective studies on the function of these sensilla were not sufficient. The function of many sensillum types awaits further studies. As a summary, the distal antennal segments of female *S. littoralis* had significantly less antennal sensilla basiconic and trichoid than proximal ones. However, the other five types of sensilla are the same in distribution and number. Moreover, distal sensilla chaetica were longer than the proximal ones. The small number of olfactory sensilla trichodea and basiconica at the distal segments of antenna and concentration of gustatory sensilla chaetica might lead to the speculation that the main role of the proximal part of the antenna is olfaction and that of the distal part is gustation. Typically, Binyameen et al. (2012) investigated that a spatial variation existed in sensitivity of proximal and distal sensilla of female *S. littoralis*. He recorded that the olfactory sensory neurons presented on the more proximal segment were more sensitive to plant compounds and sex pheromone components than those on the more distal segment. This result was distinct from that of Ochieng et al. (1998), whereas, EAG response of the desert locust, *Schistocerca gregaria* to plant volatile compounds was elicited mainly by the sensilla beyond the proximal seven segments. This indicated that the olfactory perception in this insect is mainly accomplished by the distal part of the antenna apart from the seven proximal segments (Chen et al., 2003).

In previous studies there were substantial differences in sensillum types, structure and functions among species. To the best of my knowledge, the differences in number or distribution of antennal sensilla of female *S. littoralis* along antenna

have not been investigated before. However, differences in antennal topology, sensillum types, sizes, diameters and numbers have been investigated between male and female of the same insect species. Chapman (1982) attributed the dimorphism of insect antennal sensilla number either to the different feeding habits of sexes, or to the attraction of the male by the female pheromone.

Finally, further behavioral and physiological studies are needed to distinguish if there is any link between this antennal topology, sensilla morphology and distribution through antennal length in host selection for feeding or oviposition.

Acknowledgment

The author is deeply grateful to Prof. Dr. Peter Anderson (Unit of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden) for technical assistance and the possibility to carry out experimental investigations.

References

- Akenteva, N.A., Yu Chaika, S., 2007. Antennas and mouth appendages of larvae of *Rhagium inquisitor* L. (Coleoptera: Cerambycidae). Byul. Oip. Otd. Biol. 112 (4), 62–67.
- Altner, H., Prillinger, L., 1980. Ultrastructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. Int. Rev. Cytol. 67, 69–139.
- Altner, H., Routil, Ch., Loftus, R., 1981. The structure of bimodal chemo-, thermo-, and hygroreceptive sensilla on the antenna of *Locusta*. Cell Tissue Res. 215, 289–308.
- Anderson, P., Hallberg, E., Subchev, M., 2000. Morphology of antennal sensilla auricillica and their detection of plant volatiles in the Herald moth, *Scoliopteryx libatrix* L. (Lepidoptera: Noctuidae). Arthropod Struct. Dev. 29, 33–41.
- Binyameen, M., Anderson, P., Ignell, R., Seada, M.A., Hansson, B.S., Schlyter, F., 2012. Spatial organization of antennal olfactory sensory neurons in the female *Spodoptera littoralis* moth: differences in sensitivity and temporal characteristics. Chem. Senses 37, 613–629.
- Boekh, J., Kaissling, K.E., Schneider, D., 1965. Insect olfactory receptors. Cold Spring Harb. Symp. Quant. Biol. 30, 263–280.
- Byers, J.A., 1995. Host tree chemistry affecting colonization in bark beetles. In: Card, R.T., Bell, W.J. (Eds.), Chemical Ecology of Insects. Academic Press, New York, pp. 154–213.
- Chapman, R.F., 1982. Chemoreception: the significance of receptor numbers. Adv. Insect Physiol. 16, 247–333.
- Chen, H.H., Zhao, Y.X., Kang, L., 2003. Antennal sensilla of grasshoppers (Orthoptera: Acrididae) in relation to food preferences and habits. J. Biosci. 28, 743–752.
- Cuperus, P.L., 1985. Ultrastructure of antennal sense organs of small ermine moths, *Yponomeuta* spp. (Lepidoptera: Yponomeutidae). Int. J. Insect Morphol. Embryol. 14, 179–191.
- Den Otter, C.J., Schuil, H.A., Sander Van Oosten, A., 1978. Reception of host-plant odours and female sex pheromone in *Adoxophyes orana* (Lepidoptera: Tortricidae): electrophysiology and morphology. Entomol. Exp. Appl. 24, 370–378.
- Dyer, L.J., Seabrook, W.D., 1978. Some aspects of oviposition site selection in *Monochamus notatus* and *M. scutellatus* (Coleoptera: Cerambycidae). J. Chem. Ecol. 4, 199–210.
- Ebbinghaus, D., Losel, P.M., Lindemann, M., Scherckenbeck, J., Zebitz, C.P.W., 1997. Detection of major and minor sex pheromone components by the male codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). J. Insect Physiol. 44 (1), 49–58.

- Faucheux, M.J., 1990. External structure of sensilla on the male and female flagellum of *Noctua pronuba* L. (Lepidoptera: Noctuidae). *Ann. Soc. Ent. Fr. (N.S.)* 26, 173–184.
- Faucheux, M.J., 1993. Uniporous pegs associated with sensilla auticillica on the antennae of Noctuidae (Lepidoptera). *Nota Lepidop.* 16, 13–17.
- Faucheux, M.J., 1997. Structures sensorielles antennaires des Noctuelles (Lepidoptera: Noctuidae) et leurs implications phylogénétiques. *Bull. Soc. Sci. Nat. Ouest Fr. (N.S.)* 19, 29–39.
- Faucheux, M.J., 1999. Biodiversity and unity of sensory organs in lepidopteran insects. *Soc. Sci. Nat. Ouest Fr.*, Nantes, 296.
- Faucheux, M.J., 2006. Antennal sensilla of male *Lophocorona pediasia* Common 1973 and their phylogenetic implications (Lepidoptera: Lophocoronidae). *Ann. Soc. Ent. Fr* 42 (1), 113–118.
- Faucheux, M.J., 2011. Antennal sensilla in adult males of five species of *Coleophora* sp. (Lepidoptera: Coleophoridae). Considerations on their structure and function. *Nota Lepidop.* 34 (1), 61–69.
- Frank, D.L., Leskey, T.C., Bergh, J.C., 2010. Morphological characterization of antennal sensilla of the Dogwood Borer (Lepidoptera: Sesiiidae). *Ann. Ent. Soc. Am.* 103 (6), 993–1002.
- Gao, Y., Chen, Z.M., Sun, X.L., 2013. Antennal sensilla of the tea weevil *Myllocerinus aurolineatus*. *Plant Prot.* 39 (3), 45–50.
- Gomez, V.R.C., Nieto, G., Valdes, J., Castrejon, F., Rojas, J.C., 2003. The antennal sensilla of *Zamagiria dixolophella* Dyar (Lepidoptera: Pyralidae). *Ann. Ent. Soc. Am.* 96 (5), 672–678.
- Hallberg, E., 1981. Fine-structural characteristics of the antennal sensilla of *Agrotis segetum* (Insecta: Lepidoptera). *Cell Tissue Res.* 218, 209–218.
- Hallberg, E., Hansson, B.S., Steinbrecht, R.A., 1994. Morphological characteristics of antennal sensilla in the European cornborer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Tissue Cell* 26 (4), 489–502.
- Hansson, B.S., Blackwell, A., Hallberg, E., Lofqvist, J., 1995. Physiological and morphological characteristics of the sex pheromone detecting system in male corn stemborers, *Chilo partellus* (Lepidoptera: Pyralidae). *J. Insect Physiol.* 41 (2), 171–178.
- Jiang, G.F., He, D.C., Yan, Z.G., 2000. Scanning electron microscopy observations of antennal sensilla of male *Teinopalpus aureus* Mell. *Guan. Sci.* 7 (2), 144–146, 149.
- Kaissling, K.E., 1971. Insect olfaction. In: Beidler, L.M. (Ed.), *Handbook of sensory physiology, Chemical Senses: Olfaction*, vol. IV. Springer-Verlag, Berlin, pp. 351–431.
- Keil, T.A., Steinbrecht, R.A., 1984. Mechanosensitive and olfactory sensilla of insects. In: King, R.C., Akai, H. (Eds.), *Insect ultrastructure*. 2. Plenum, New York, pp. 477–516.
- Keil, T.A., 1989. Fine structure of the pheromone-sensitive sensilla on the antenna of the hawkmoth, *Manduca sexta*. *Tissue Cell* 21, 139–151.
- Lee, J.K., Strausfeld, N.J., 1990. Structure, distribution and number of surface sensilla and their receptor cells on the olfactory appendage of the male moth, *Manduca sexta*. *J. Neurocytol.* 19, 519–538.
- Ma, R.Y., Du, J.W., 2000. Insect antennal sensilla. *Entomol. Knowl.* 37 (3), 179–183.
- Maes, F.W., Vedder, C.O., 1978. A morphological and electrophysiological inventory of labellar taste hairs of the blowfly *Calliphora vicina*. *J. Insect Physiol.* 24, 667–672.
- McIver, S.B., 1974. Fine structure of antennal grooved pegs of the mosquito, *Aedes aegypti*. *Cell Tissue Res.* 153, 327–337.
- McIver, S.B., 1975. Structure of cuticular mechanoreceptors of arthropods. *Ann. Rev. Entomol.* 20 (38), 1–97.
- Mercer, K.L., McIver, S.B., 1973. Studies on the antennal sensilla of selected blackflies (Diptera: Simuliidae). *Can. J. Zool.* 51, 729–734.
- Mitchell, B.K., Itagaki, H., Rivet, M.P., 1999. Peripheral and central structure involved in insect gustation. *Microsc. Res. Tech.* 47, 401–415.
- Ochieng, S.A., Hallberg, E., Hansson, B.S., 1998. Fine structure and distribution of antennal sensilla of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). *Cell Tissue Res.* 291, 525–536.
- Pophof, B., 1997. Olfactory responses recorded from sensilla coelocanica of the silkworm *Bombyx mori*. *Physiol. Entomol.* 22, 239–248.
- Salama, H.S., Khalifa, A., Azmy, N., Sharaby, A., 1984. Gustation in the lepidopterous moth *Spodoptera littoralis* (Boisd.). *Zool. J. Physiol.* 88, 165–178.
- Schneider, D., 1964. Insect antennae. *Annu. Rev. Entomol.* 9, 103–122.
- Schneider, D., Steinbrecht, R.A., 1968. Checklist of insect olfactory sensilla. In: *Symp. Zool. Soc. L.: Invertebrate receptors*, vol. 23, pp. 279–297.
- Shields, V.D.C., Hildebrand, J.G., 1999. Fine structure of antennal sensilla of the female sphinx moth, *Manduca sexta* (Lepidoptera: Sphingidae). II. Auriculate, coelocanica, and styliform complex sensilla. *Can. J. Zool.* 77, 302–313.
- Shields, V.D.C., Hildebrand, J.G., 2001. Recent advances in insect olfaction, specifically regarding the morphology and sensory physiology of antennal sensilla of the female sphinx moth *Manduca sexta*. *Microsc. Res. Tech.* 55 (5), 307–329.
- Slifer, E.H., 1961. The fine structure of insect sense organs. *Int. Rev. Cytol.* 11, 125–169.
- Slifer, E.H., 1970. The structure of arthropod chemoreceptors. *Ann. Rev. Entomol.* 15, 121–142.
- Snodgrass, R.E., 1935. *Principles of Insect Morphology*. Mo Graw Hill, New York.
- Stadler, E., 1984. Contact chemoreception. In: Bell, W.J., Cardé, R.T. (Eds.), *Chemical Ecology of Insects*. Chapman and Hall, London, pp. 3–35.
- Steinbrecht, R.A., 1973. Der Feinbau olfaktorischer Sensillen des Seidenspinners Rezeptorfortsätze und reizleitender Apparat. *Z. Zellforsch. Mikrosk. Anat.* 139, 533–565.
- Steinbrecht, R.A., Gnatzy, W., 1984. Pheromone receptors in *Bombyx mori* and *Antheraea pernyi*. I. Reconstruction of the cellular organization of the sensilla trichodea. *Cell Tissue Res.* 235, 25–34.
- Steinbrecht, R.A., Lee, J.K., Altner, H., Zimmermann, B., 1989. Volume and surface of receptor and auxiliary cells in hygro-/thermoreceptive sensilla of moths (*Bombyx mori*, *Antheraea pernyi*, and *A. polyphemus*). *Cell Tissue Res.* 255, 59–67.
- Su, C.Y., Menz, K., Carlson, J.R., 2009. Olfactory perception: receptors, cells, and circuits. *Cell* 139 (1), 45–59.
- Wall, C., 1978. Morphology and histology of the antenna of *Cydia nigricana* (F.) (Lepidoptera: Tortricidae). *Int. J. Insect Morph. Embryol.* 7 (3), 237–250.
- Whitehead, A.T., 1981. Ultrastructure of sensilla of the female mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *Int. J. Insect Morph. Embryol.* 10, 19–28.
- Wigglesworth, V.B., 1972. *The Principles of Insect Physiology*. Chapman and Hall Publications, pp. 827.
- Xiangqun, Y., Ke1, G., Feng, Y., Yalin, Z., 2014. Ultrastructure of antennal sensilla of four skipper butterflies in *Parnara* sp. and *Pelopidas* sp. (Lepidoptera, Hesperidae). *ZooKeys* 399, 17–27.
- Xu, M., Wang, M. (2013) Scanning electron microscopy observation of antennal sensilla of *Heliophorus phoenicopyrphus* (Holland). <<http://www.paper.edu.cn/releasepaper/content/201303-415>> .
- Zacharuk, R.Y., 1962. Exuvial sheaths of sensory neurones in the larva of *Ctenicera destructor* (Brown). *J. Morphol.* 111, 35–47.
- Zacharuk, R.Y., 1985. Antennae and sensilla. In: Kerkut, G.A., Gilbert, L.I. (Eds.), *Comparative Insect Physiology, Biochemistry and Pharmacology*, vol. 6. Pergamon Press, Oxford, pp. 1–69.
- Zhao, H., Wang, Z., Gao, Y., Ge, S., 2012. Antennal morphology and sensillum distribution in *Pissides validirostris* (Coleoptera: Curculionidae). *Indian J. Agric. Res.* 46 (1), 70–74.