Morphology of the sensilla from the antennae and ovipositor of the adults of a non-feeding specialist cossid moth, *Comadia redtenbacheri*

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Abstract

Olfaction plays a crucial role in the survival of insects. Through olfaction, insects navigate their environment and locate food and partners. The morphology of the external olfactory system is assumed to be the result of adaptation to the insect environment; thus, studying the external morphology of sensilla may provide information on the habits of a species. There is scarce information about the changes that might have occurred in the morphology of sensilla in specialist insects that do not feed as adults. The agave red worm, *Comadia redtenbacheri* (Hammerschmidt) (Lepidoptera Cossidae), is a non-feeding specialist moth of economic and cultural importance in Mexico. In this study, we analysed sensilla from female and male antennae and female ovipositor using scanning electron microscopy. Six types of sensilla were found in the male and female antennae: trichodea (four subtypes), basiconica (five subtypes), coeloconica (three subtypes), chaetica, squamiformia, and Böhm's bristles (three subtypes). Female antennae had a higher abundance of trichodea III and IV, basiconica I and V, coeloconica I, and chaetica, whereas male antennae had higher numbers of trichodea I and II, and coeloconica II. Some of these subtypes are unique to this species. The ovipositor exhibited five subtypes of sensilla trichodea and two subtypes of sensilla basiconica, which differed in shape from those found on the antennae. The biological importance of sensilla is also discussed.

Key words: agave red worm, chinicuil, adult sensilla, chemoreception, mechanoreception.

Introduction

Insects rely extensively on their sense of smell to perform a number of vital tasks, such as locating food sources, finding partners, and discriminating hosts for oviposition. Molecules emitted by food sources, partners, or hosts are detected through a complex array of olfactory binding proteins (OBPs), olfactory receptors (ORs), and olfactory receptor neurons (ORNs) housed inside specialized structures called sensilla. The subset of expressed OBPs, ORs, ORNs, as well as the type and number of sensilla may vary among species, and in some cases, may be correlated with particular habits. This is clearly illustrated in the speciation process in *Drosophila*: faced with differences in host types and abundances in their new habitats, the olfactory system of some species became composed of a large OR family, able to detect a large number of odorants, while in others, their olfactory system became adapted to detect only a few (Ramdya and Benton, 2010; Ramasamy *et al.*, 2016). Predictably, the olfactory systems in the species that detect few volatiles show rapid changes in OBP genes (Vieira *et al.*, 2007), a reduction in OR genes (Gardiner *et al.*, 2008), and some morphological changes and losses of sensilla. For example, *Drosophila mojavensis* Patterson et Crow, a desert-dwelling fly that feeds solely on cacti, has several populations that are isolated from each other and feed on different species. They possess a sensory equipment that is specifically tuned to the most relevant odorants produced by the cacti, while the sensitivity towards the volatiles that are absent has decreased due to changes in ORN sensitivity and abundance of sensillar subtypes (Crowley-Gall *et al.*, 2016). In *Drosophila sechellia* Tsacas et Baechli, which oviposits exclusively in *Morinda citrifolia* L., the sensilla that housed ORNs tuned to volatiles absent in the host have been lost. In contrast, sensilla housing ORNs sensitive to compounds found in the host became more abundant, and the large sensilla basiconica became 60% shorter than those found in the generalist *Drosophila melanogaster* Meigen. This has resulted in an olfactory system that is more adept at detecting host fruits (Stensmyr *et al.*, 2003; Dekker *et al.*, 2006). In other insects, changes in the peripheral olfactory system result in remarkable changes in feeding behaviour. For example, the noctuid *Calyptra thalictri* (Borkhausen) pierces fruits to feed on the juice, although some males occasionally pierce the skin of vertebrates to take blood. An analysis of *C. thalictri* antennae revealed that blood-feeding males have fewer sensilla coeloconica, which are sensitive to vertebrate compounds (Hill *et al.*, 2010). This seems to show that the decrease in the number of sensilla, and consequently of ORNs, diminished the repulsion of vertebrates and allowed the moths to feed more often on them. The peripheral olfactory system probably shows a high degree of adaptation to particular habitat volatiles, and the external morphology of the olfactory organs may provide clues regarding the habits of an insect.

If the olfactory system is well adapted and reflects the

habits of a species, an interesting question would be what changes may have taken place in the morphology of the olfactory system of non-feeding insects that specialize in a few hosts. One such insect is the agave red worm, *Comadia redtenbacheri* (Hammerschmidt) (Lepidoptera Cossidae), native to the arid and semi-arid regions of the southern USA and Mexico. The larvae of this species are an important source of food and income for people living in these zones, because they collect and sell them annually. Despite its importance, relatively little is known regarding this species. It is known that adults live for a few days and do not feed; their mouthparts are entirely absent (Castro-Torres and Llanderal-Cázares, 2016) and oviposit exclusively on *Agave* plants (Llanderal-Cázares *et al.*, 2017). Males detect female sex pheromones, and females use plant volatiles during host searching (unpublished data), but whether morphological changes have occurred at the sensillar level because of their non-feeding habits remains unknown. Specialist insects orient themselves through the detection of only a few compounds that are characteristic of their hosts, which allows rapid, unequivocal identification of the target; consequently, their olfactory system contains fewer, but much more sensitive receptors than those of generalists (Bernays, 2001). We hypothesize that, since *C. redtenbacheri* does not need to find food sources, the peripheral olfactory system might have undergone changes at the sensillar level, with some types increasing or decreasing their density. The objective of this study was to identify the different types of sensilla that are present in the antenna and ovipositor, and to compare the sensilla with those reported from other related species in the superfamilies Cossoidea and Zygaenoidea. This study aims to serve as a basis for further studies on the olfaction, ecology, and taxonomy of this species.

Materials and methods

Insects

Last-instar larvae were collected from agave plants at several locations in Hidalgo, Mexico. The larvae were placed in plastic trays with a mixture of vermiculite and garden soil in a proportion 1:1 to allow pupation (Miranda-Perkins *et al.*, 2013). Pupae were collected and placed individually in 500 mL plastic jars. Upon emergence, adults were killed in a lethal chamber with a few drops of ethyl acetate.

Scanning electron microscopy (SEM)

The antennae were dissected in 50% ethanol and dehydrated in an ascending ethanol series to 100%. Ovipositors were dissected in 4% paraformaldehyde in phosphate-buffered saline (PBS), fixed for 12 hours, rinsed with PBS four times, and dehydrated in an ascending series of ethanol. Subsequently, the antennae and ovipositors were critical-point dried, mounted on SEM stubs, and sputter-coated with gold. Voltages of 5-15 keV were used depending on the amount of static charge observed. The samples were observed using a JSM-6390 SEM Microscope (Jeol, Japan).

Image and data analysis

Sensilla from flagellomeres 5, 10, 25, 50, and the terminal segment of the antennae of four males and four females, and the distal region of three ovipositors were analysed. The images obtained were adjusted as follows: contrast and sharpness were modified using Adobe Photoshop CC 2015®; to increase the depth of field, a series of images were acquired and combined with Helicon Focus 7.0.2®, where necessary. Measurements were taken using the open code program Fiji (version 2.0.0.0-rc-30/1.49s) (Schindelin *et al.*, 2012).

The mean, standard deviation (SD), and number of sensilla measured (n) are presented. Statistical analyses were calculated in the software R (version 3.5.1).

Results

Antenna

Male antennae are bipectinate to the tip, with the rami progressively becoming longer from the proximal end to the medial area, and then decreasing again towards the distal end. The flagellomeres are slightly asymmetrical, with a somewhat flattened and curved rami (figure 1A, C). Female flagellomeres lack rami but are slightly serrated and asymmetrical (figure 1B, D), diminishing in size towards the distal end of the antenna. Six types of sensilla were found in male and female antennae: trichodea, basiconica, coeloconica, chaetica, squamiformia, and Böhm's bristles; sensilla styloconica and auricillica were absent.

T r i c h o d e a

This type of sensilla is slender, broader at the base, and ends with a sharp tip; the surface is smooth, with pores arranged in a helicoid pattern. Sensilla were found mainly on the ventral side of the antenna in both sexes. Four subtypes were distinguished based on their size and distribution; two were present exclusively in males, while the rest were found in both males and females.

Trichodea subtype I (figure 2A). This subtype is hair-like, long, and characteristically s-shaped or recurved, with a mean length of 35.2 µm (SD = 6.5, n = 64). It is present exclusively in males and is one of the most numerous subtypes. This subtype occurs exclusively on the ventral side of the rami. A couple of sensilla presented a bifid tip; however, because they were rarely found and fit the description of this subtype, we decided to group them here.

Trichodea subtype II (figure 2B). This subtype is hair-like, strongly curved backward, but much shorter than subtype I, with a mean length of 18.9 µm (SD = 4.6, n = 12). It is also exclusively found in males on the ventral side of the rami, although some sensilla can also be found on the lateral and dorsolateral sides.

Trichodea subtype III (figure 2C). This subtype is long and straight or slightly curved towards the cuticle, never s-shaped, and with a length of 36.1 µm (SD = 5.6, n = 44). These sensilla are present in both males and females, mainly on the ventral side of the rami in males and on the lateral and ventral sides of the flagellomere in females, but a few can be found in the dorsolateral region of the rami.
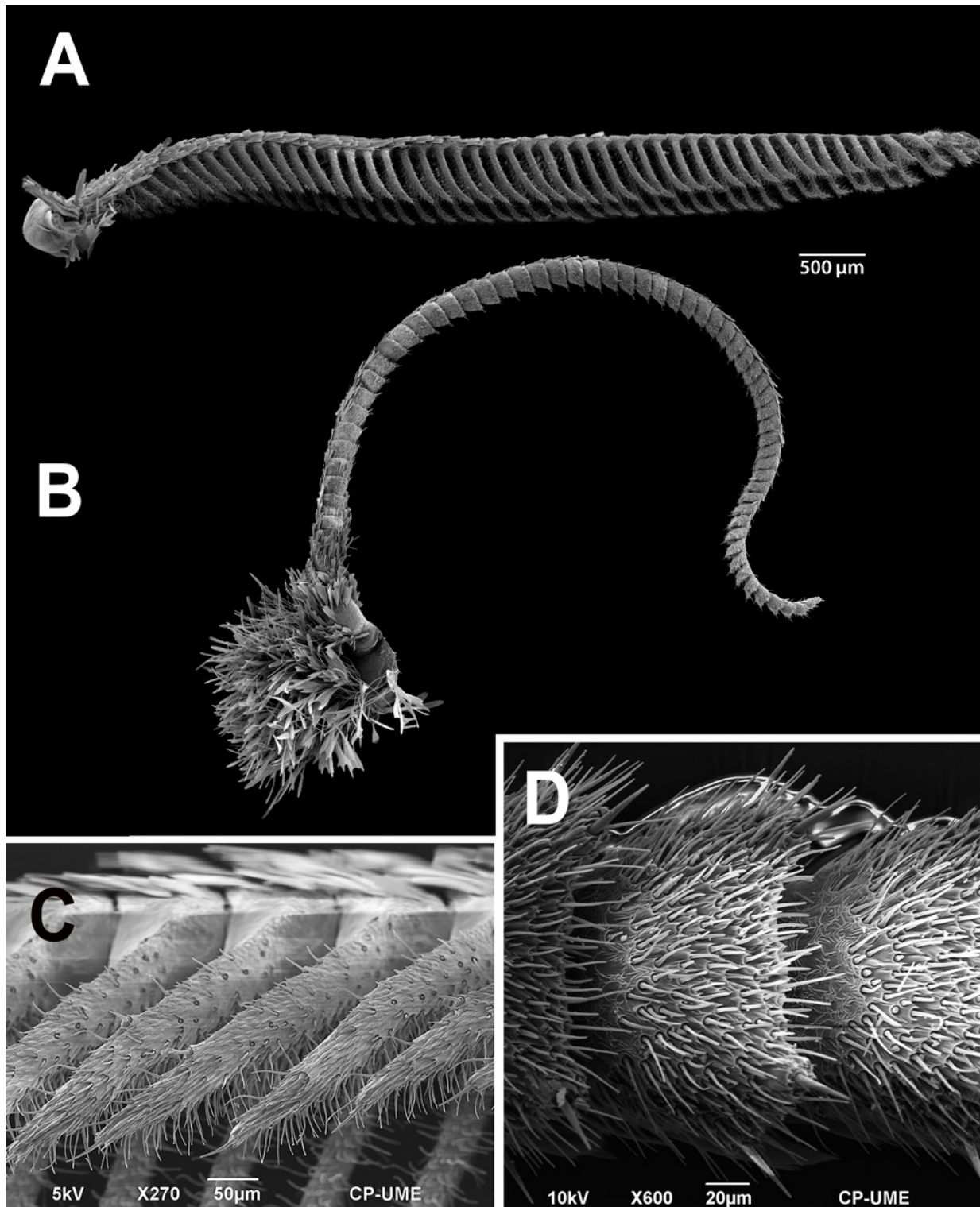
Figure 1. General view of the antennae. (**A**) low magnification of the male antenna. (**B**) low magnification of the female antenna. (**C**) close up of the flagellomeres in the male antenna (lateral). (**D**) close up of the flagellomeres in the female antenna (ventral). A-B, with permission from *SHILAP Revista de Lepidopterologia*, 45: 373-383 (2017).

Trichodea subtype IV (figure 2D). This subtype is straight and short, approximately 17.4 µm long (SD = 3.8; n = 14). They were very similar to subtype III, but shorter and distributed on the ventral side of the antennae of both males and females. This subtype is not as numerous as previous subtypes.

B a s i c o n i c a

The sensilla are smooth, with pores in a herringbone pattern or in rows, but never in a helicoid arrangement. They are found especially on the ventral and lateral sides of the rami and flagellomeres of both males and females, and a few on the dorsum. The five subtypes were distinguished based on their sizes and shapes.
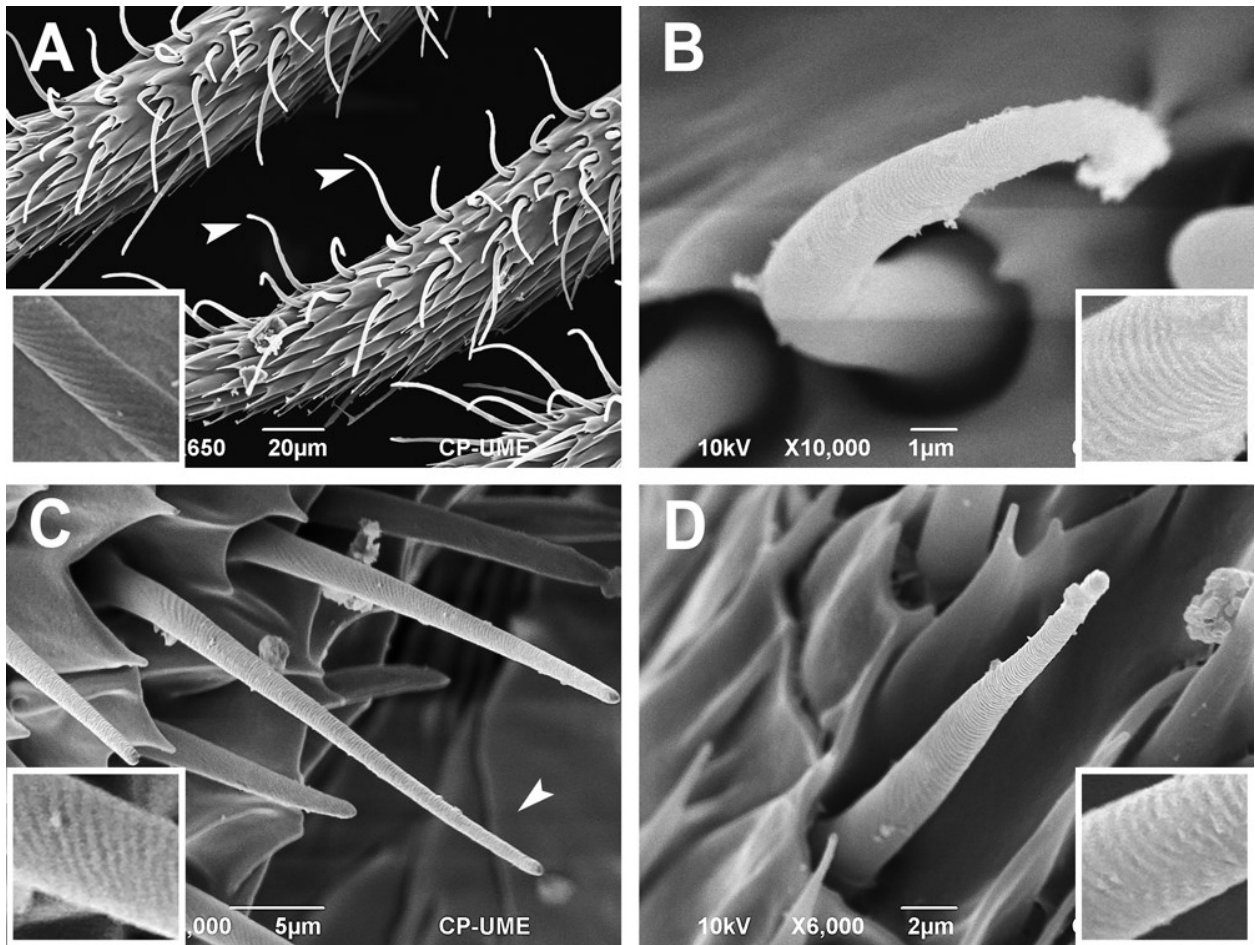
Figure 2. Sensilla trichodea. (**A**) subtype I (arrowheads). (**B**) subtype II. (**C**) subtype III (arrowhead). (**D**) subtype IV. The insets show the pores of each type of sensillum. A-B taken from male antennae, C-D from female antennae.

Basiconica subtype I (figure 3A). This subtype is the longest among all subtypes. They are long, smooth, with a slight terminal club and approximately 13.7 μm in length (SD = 2.5, n = 27). These may be straight or slightly curved. The pores on the surface form a herringbone pattern. This is the most common subtype in males and females, present on both the dorsal and ventral sides of the rami and on the lateral and ventral parts of the flagellomere in females.

Basiconica subtype II (figure 3B). These sensilla are somewhat flattened and spindle-shaped, ending with a sharp tip, and approximately 11.8 μm long (SD = 1.9, n = 14). The pores run along the length and do not form a herringbone pattern, as in the other subtypes. They are present almost exclusively on the ventral side of both males and females. This subtype is slightly similar to an auricillicum, but since it is not as flat or wide, it was classified as basiconicum.

Basiconica subtype III (figure 3C). These sensilla are very short, approximately 4.7 μm long (SD = 0.95, n = 13), with blunt tips and pores forming a herringbone pattern. This subtype is found mainly in the last segments of males and females.

Basiconica subtype IV (figure 3D). These sensilla are short (6.4 μm, SD = 1.5, n = 10), strongly curved, lying almost parallel to the cuticle, with pointed tips. They are not numerous and appear to be present mainly on the ventral and lateral sides.

Basiconica subtype V (figure 3E). These are also short sensilla, approximately 7.8 μm in length (SD = 1.4, n = 7), with sharp tips. They have pore-forming lines running along the length of the sensilla. This subtype is the least abundant subtype of basiconica.

Coeloconica

These sensilla are peg-like projections deeply recessed in a pit. They are present exclusively on the lateral and dorsal sides of the rami in males and on the lateral and ventral parts of flagellomeres in females. Three subtypes were identified in males and females.

Coeloconica subtype I (figure 4A). This subtype has from 3-10 microtrichia around the pit and the peg is composed of approximately 15 digitiform projections tightly together, 2.5 μm in length (SD = 0.5; n = 10). In males, they are located on the lateral surface of the rami, whereas in females, they are on the lateral and ventral sides of the flagellomeres.

Coeloconica subtype II (figure 4B). This subtype is very similar to the previous in shape and length (2.7 μm, SD = 0.26, n = 11). However, no microtrichia surrounding the pit were observed.

Coeloconica subtype III (figure 4C). The morphology

of this subtype is completely different from the other two: the central peg is bulbous at the base, the tip has six digitiform projections that are apparently fused together, and there is a large pore at the tip, although it may be the result of the fusion of the projections; the wall is smooth with no apparent pores. The peg is inside a socket, which in turn is inside a pit, similar to those of the previous two subtypes. This subtype is very sparsely distributed on the lateral and ventral sides of flagellomeres in males and females, although females bear more of them on the last three flagellomeres.

Chaetica

These sensilla are long and stout (figure 4D). They are one of the longest in the antenna, with a length of approximately 46.8 µm (SD = 6.8, n = 19). The wall showed a herringbone pattern, but without pores, and the tip was somewhat rounded with a pore at the tip; they were inserted in a deep, thick socket. Two sensilla are present exclusively on the dorsum of the flagellomeres in males, and four or five on the dorsolateral, lateral, and ventrolateral sides of the flagellomeres in females, except for the distal-most segment. Up to 15 sensilla may be present in both sexes.
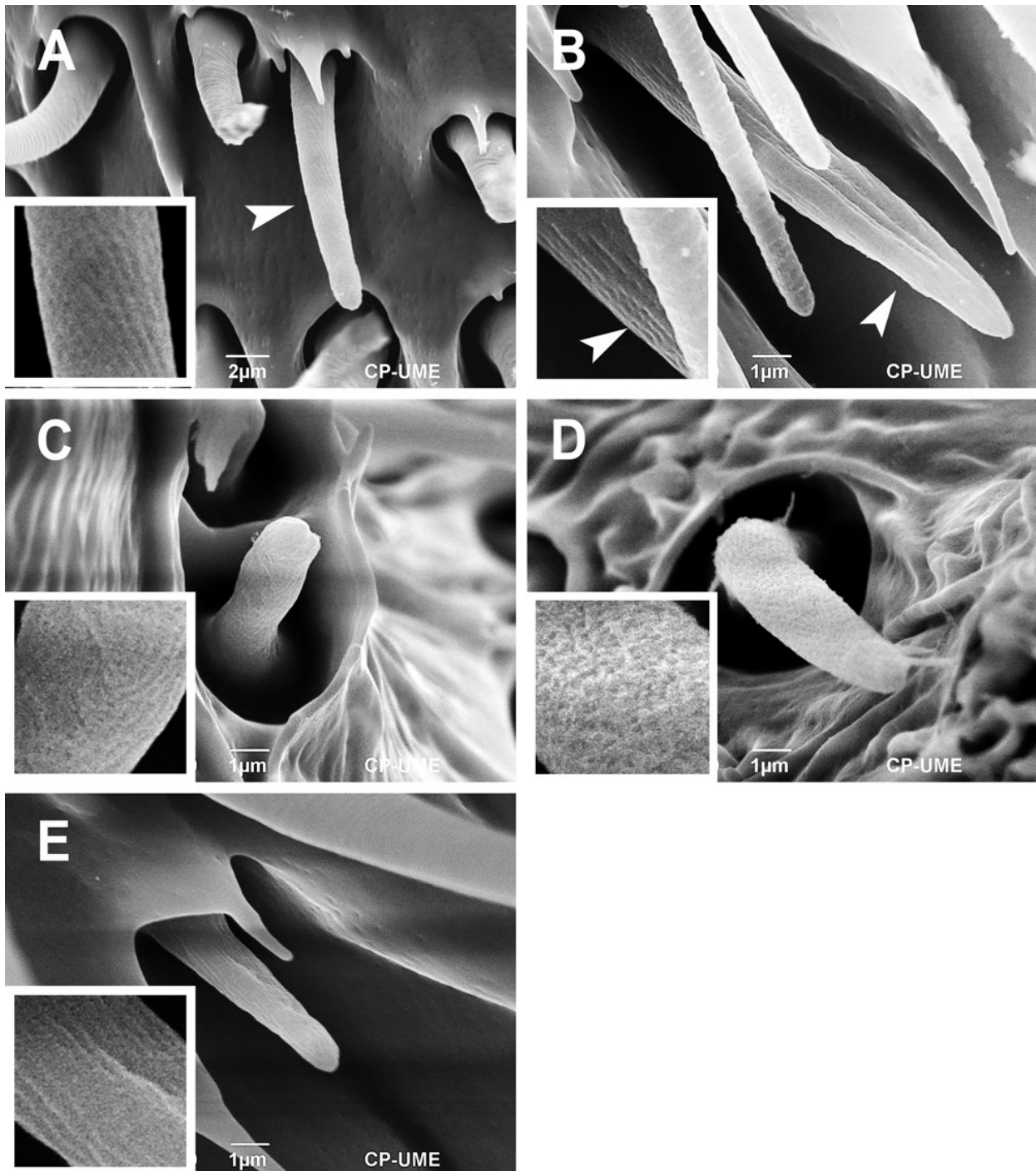

Figure 3. Sensilla basiconica. (**A**) subtype I. (**B**) subtype II. (**C**) subtype III. (**D**) subtype IV. (**E**) subtype V. The insets show the pores of each type of sensillum. A and C-E taken from male antennae, B from female antennae.
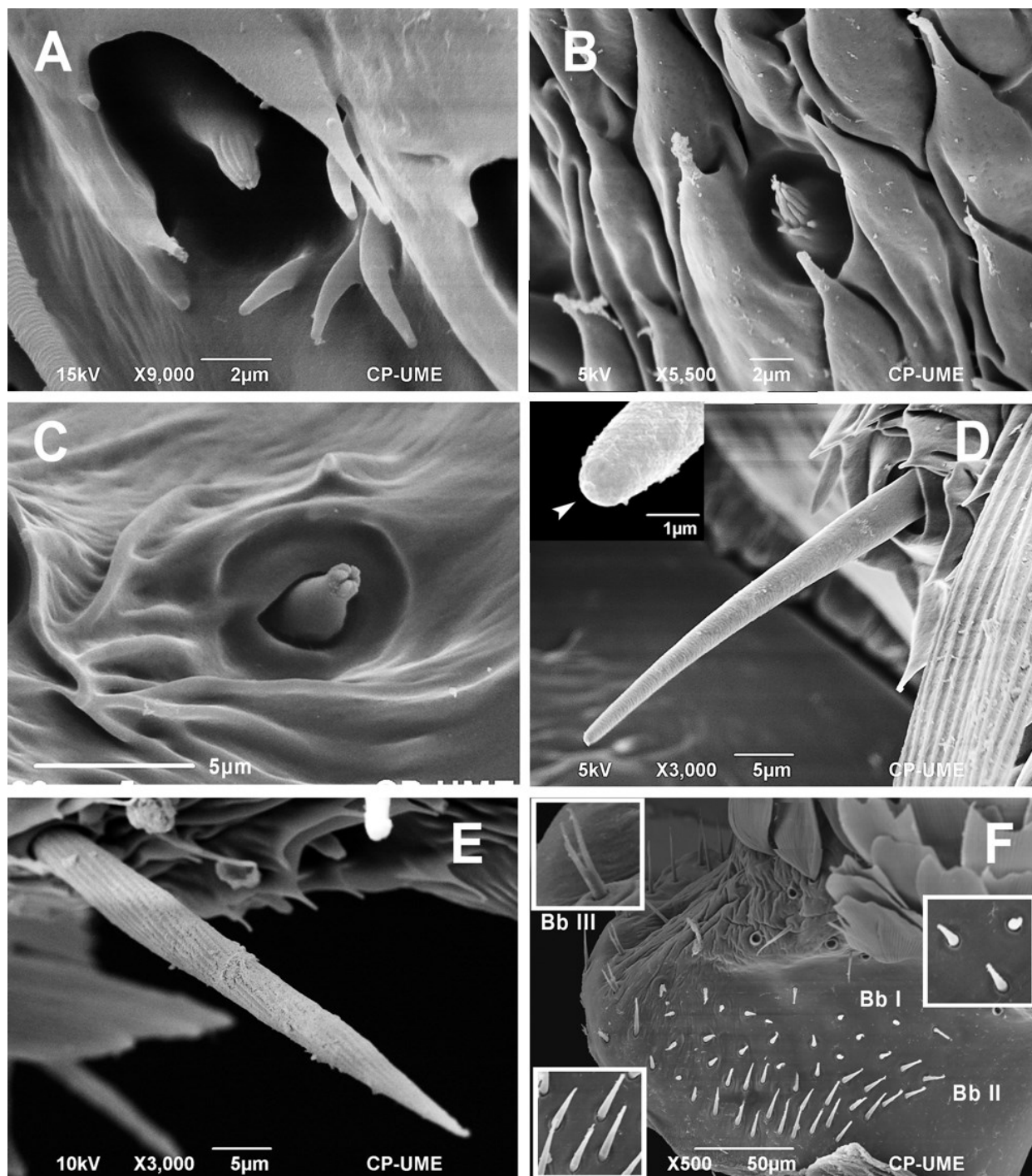
Figure 4. Sensilla coeloconica, chaetica, and squamiformia. (**A**) coeloconicum subtype I. (**B**) coeloconicum subtype II. (**C**) coeloconicum subtype III. (**D**) chaeticum. Inset: close-up showing the apical pore. (**E**) squamiformium, (**F**) Böhm's bristles. Subtype I (Bb I), subtype II (Bb II) and subtype III (Bb III). Inset, close up of the Böhm's bristles subtype I (left), subtype II (bottom right), and subtype III (top right). A-F taken from male antennae.

Squamiformia

The sensilla of this type are long, approximately 52.1 µm (SD = 18.3, n = 10), but slender than sensilla chaetica (figure 4E). The wall has striae running along the length of the seta, the tip is sharp, and no pores are apparent in either the tip or the wall; they are inserted in a narrow socket. Males and females presented two pairs on either side of the flagellomeres, exclusively on the dorsal side.

Böhm's bristles

These sensilla are only found on the scape and pedicel in both males and females. They are short, with smooth walls, and inserted into a slightly elevated socket; they end with a sharp tip. Their surfaces are smooth, and there is no indication that they contained pores. The following three subtypes were identified.

Böhm's bristle subtype I (figure 4F). These sensilla are short, approximately 5.2 µm long (SD = 1.6, n = 10), and

176

were found in clusters on the scape and pedicel.

Böhm's bristle subtype II (figure 4F)*.* The sensilla of this subtype are longer, approximately 14.4 µm in length (SD = 2.1, n = 17). They are very similar to the previous subtype and are located in a cluster below subtype I.

Böhm's bristle subtype III (figure 4F)*.* These are bifid sensilla, approximately 23 µm in length (SD = 4.2, n = 12). They are found in a cluster adjacent to sensilla subtypes I and II.

Ovipositor

The ovipositor is long and telescopic, with the sensilla located almost exclusively on the papillae analis (figure 5A). Only sensilla trichodea and basiconica are found, the former being the more abundant.

Trichodea

There are five subtypes, all of which are aporous and similar to each other. The tip is sharp, the wall shows a herringbone pattern, and the socket is slightly raised but
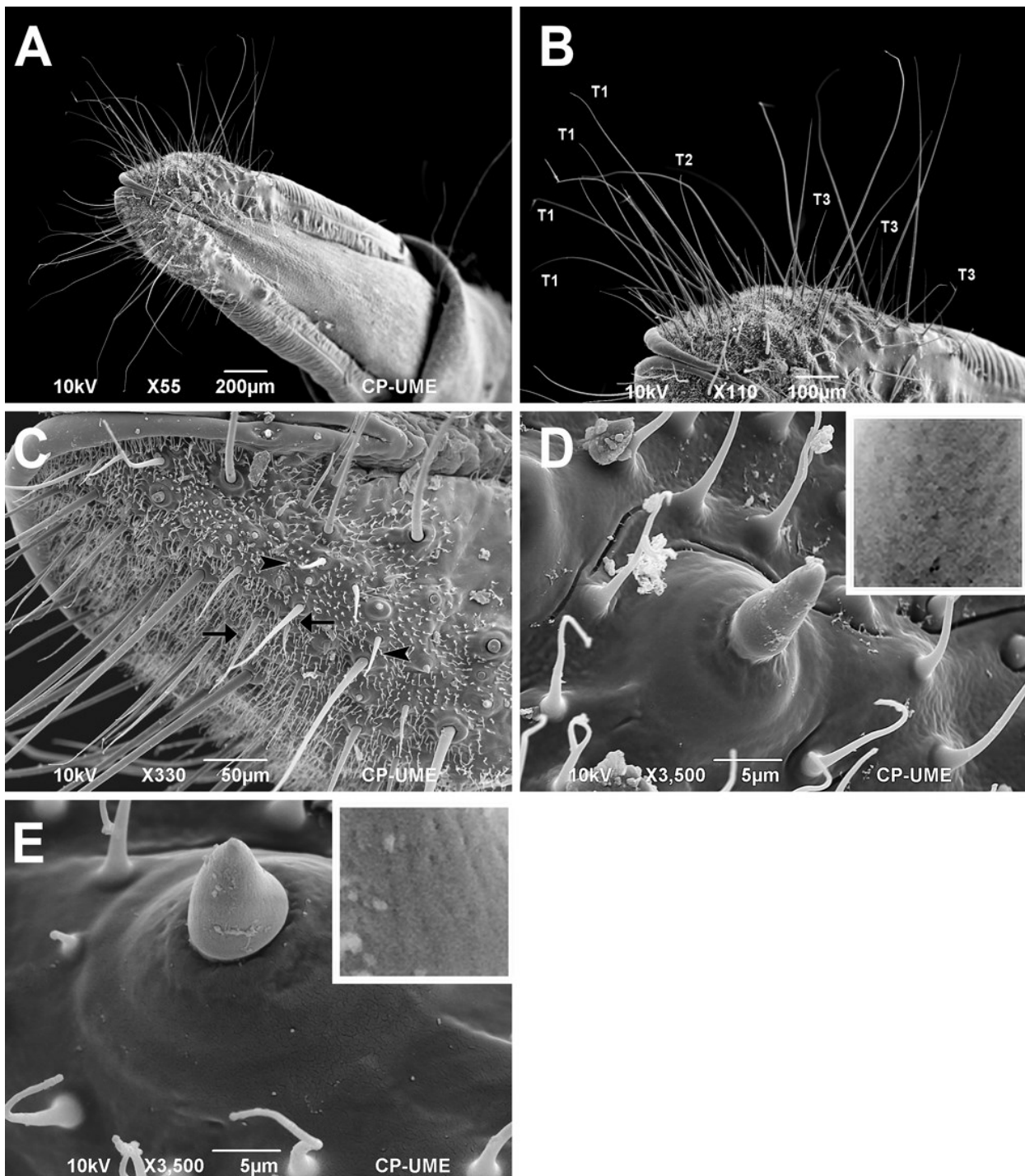

Figure 5. Ovipositor. (**A**) general view, ventral. (**B**) trichodea subtypes I-III. (**C**) trichodea subtype IV (arrows) and V (arrowheads). (**D**) basiconicum subtype I (Note the multiple microtrichia around the sensillum). (**E**) basiconicum subtype II. The insets show the pores of the sensilla basiconica.

never as thick as in a sensillum chaeticum. The only visible difference among them is the length, which varies from 30 to 657 μm. The mean length of each subtype is approximately half of that of the previous subtype.

Trichodea subtype I (figure 5B). This subtype is the longest. Its length varied from 604 to 657 μm, and the average length is 623.5 μm (SD = 22.1, n = 10).

Trichodea subtype II (figure 5C). These were the second longest sensilla. The length was between 319 and 489 μm, with an average of 382.9 μm (SD = 57.1, n = 10).

Trichodea subtype III (figure 5C). This sensillum is quite similar to the previous subtypes, with a length of 174.8 μm (SD = 28.2, n = 10).

Trichodea subtype IV (figure 5C). Short sensilla, somewhat cone-shaped with a long, sharp tip and length of 79.8 μm (SD = 11.4, n = 10).

Trichodea subtype V (figure 5C). This is the shortest subtype, measuring between 30 and 48 μm, and an average of 43.5 μm (SD = 7.3, n = 10).

Basiconica

There are two subtypes, the most abundant of which is subtype I. Both arise directly from a bump in the cuticle. Multiple pores are present on the walls but not at the tip.

Basiconica subtype I (figure 5D). These sensilla are peg-like with a length of 12.6 μm and 2.3 times longer than wide, with the pores uniformly distributed on the walls.

Basiconica subtype II (figure 5E). In addition, the peg-like, but shorter than the previous subtype, with a length of 6.6 μm and about 1.2 times longer than wide. The pores seemed to be more irregularly spaced than in the previous subtype.

Discussion

Antenna

Six novel sensilla subtypes were identified in *C. redtenbacheri*: trichodea II, basiconica III and IV, coeloconica III, and Böhm's bristles I and III. Compared with other Cossoidea and Zygaenoidea, not only does *C. redtenbacheri* have more subtypes, but also some of them are found exclusively in this species (table 1). This is surprising given that *C. redtenbacheri* adults do not feed, a reduction in sensilla might have been expected. Insects normally discriminate between hosts using the differences between the volatiles produced by those hosts and the background odours. If this difference is large enough, the number or type of volatiles that are necessary for host discrimination will be small, but if the difference is small, a larger number of compounds may be needed (Haverkamp *et al.*, 2018), which might have an effect on the type of ORNs or sensilla present. For example, the specialist psyllid *Trioza apicalis* Forster shows a reduction in the number of antennal sensilla, and it has been speculated that this may be an adaptation to a host that is conspicuous because it produces large amounts of volatiles (Kristoffersen *et al.*, 2006). However, it is known that a larger number of sensilla increases the capacity of the antenna to detect compounds (Spaethe *et al.*, 2007). Agave plants, the only host of *C. redtenbacheri*, naturally release fewer compounds, probably at lower rates than other plants (unpublished data). Therefore, insects might require different types or subtypes of sensilla to detect more types of compounds to find the plant, even if they no longer need to find food sources. It is necessary to determine how many ORs are expressed and what odorants are detected by the sensilla in this species to gain a deeper understanding of the reason.

The sensilla coeloconica III are very different from other sensilla found in Lepidoptera. The lack of pores on the wall and its minute length shows that it may not have a chemoreceptive function, but a thermo- or hygroreceptory function (Hallberg *et al.*, 2003). Its presence was not sexually dimorphic, indicating that its function is relevant to both sexes, which may strengthen the thermo- or hygroreceptor hypothesis. This subtype was not found in other closely related species of Cossidae, such as *Eogystia* (=*Holcocerus*) *hippophaecolus* (Hua, Chou, Fang et Chen)

Table 1. Antennal sensilla from species of Cossidae, Sesiidae, Castniidae, Limacodidae, and Phaudidae. The subtypes were homologized with *Comadia redtenbacheri* based on the descriptions from the authors; the names inside the cells show their original classification. The underlined subtypes were exclusively found in *C. redtenbacheri*.

Species	Sensilla subtypes																
	Trichodea				Basiconica					Coeloconica			Ch	Sq	Böhm's bristles			St	Au	As
Comadia redtenbacheri	Tr I	<u>Tr II</u>	Tr III	Tr IV	B I	B II	<u>B III</u>	<u>B IV</u>	B V	C I	C II	<u>C III</u>	Ch I	Sq	<u>Bh I</u>	Bh II	<u>Bh III</u>	–	–	–
Streltzoviella insularis [A]	–	–	Tr	–	B I	B II	–	–	–	–	C	–	Ch	Sq	–	Bh	–	–	–	–
Eogystia hippophaecolus male [B]	–	–	Tr I	–	B I	B II	–	–	–	–	C	–	Tr II	–	–	Bh	–	–	–	–
Eogystia hippophaecolus [C]	–	–	Tr II	–	B I	B II	–	–	–	–	C	–	Tr I	Ch	–	Bh	–	–	–	–
Yakuzda vicarius [D]	–	–	Tr	Tr II	B I	–	–	–	–	–	C	–	Ch	–	ND	ND	ND	–	–	–
Eogystia sibirica [E]	Tr II	–	Tr I	–	B I	–	–	–	B II	C I	C II	–	Ch	–	–	Bh	–	St	–	–
Synanthedon scitula [F]	Large tr		Medi tr	Small tr	–	–	–	–	B	C	–	–	Ch	Sq	–	Bh	–	St	Au	As
Telchin licus [G]	–	–	Tr I	Tr II	B	–	–	–	–	C	–	–	Ch	Sq	ND	ND	ND	–	Au	–
Monema flavescens [H]	Tr	–	–	–	B IV	B II	*Unclear homologies*			–	C	–	Ch	–	–	Bh	–	St	–	–
Phauda flammans [I]	Tr II	–	Tr I	–	B I	–	–	–	–	–	C	–	–	Ch	–	Bh	–	St	–	–

As - Apical sensors; Au - Auricillica; B - Basiconica; Bh - Böhm's bristles; Ch - Chaetica; C - Coeloconica; ND - Not described; Sq - Squamiformia; St - Styloconica; Tr - Trichodea.
[A] Liu *et al.*, 2018a; [B] Hu *et al.*, 2018; [C] Wang *et al.*, 2015; [D] Yang *et al.*, 2011; [E] Liu *et al.*, 2014; [F] Frank *et al.*, 2010; [G] Triana *et al.*, 2020; [H] Yang *et al.*, 2017; [I] Liu *et al.*, 2018b.

(Wang *et al.*, 2015; Hu *et al.*, 2018), *Eogystia* (=*Isoceras*) *sibirica* (Alpheraky) (Liu *et al.*, 2014), Yakudza (=*Holcocerus*) *vicarius* (Walker) (Yang *et al.*, 2011), and *Streltzoviella insularis* (Staudinger) (Liu *et al.*, 2018a). A slightly similar sensillum was found in the dogwood borer, *Synanthedon scitula* (Harris) (Sesiidae). The sensillum is recessed in the socket, seems to be aporous, and is sparsely distributed on the antennae of both sexes. However, it lacks the apical projections seen in *C. redtenbacheri*, and is cylindrical (Frank *et al.*, 2010). It would be necessary to determine the classes of ORNs present and their site of arborization within the antennal lobe or suboesophageal ganglion and perform electrophysiological experiments to elucidate their function.

No sensilla auricillica were found in this species. This type seems to have been lost in other cossids such as *E. hippophaecolus* (Wang *et al.*, 2015; Hu *et al.*, 2018), *E. sibirica* (Liu *et al.*, 2014), *Y. vicarius* (Yang *et al.*, 2011), and *S. insularis* (Liu *et al.*, 2018a), as well as in *Monema flavescens* Walker (Limacodidae) (Yang *et al.*, 2017) and *Phauda flammans* Walker (Phaudidae) (Liu *et al.*, 2018b). The absence of sensilla auricillica is a parallelism that Cossidae apparently shares with the phaudid *P. flammans* and the limacodid *M. flavescens* (table 1), both members of Zygaenoidea. However, the castniid *Telchin licus* (Drury) (Triana *et al.*, 2020) and the sesiid *S. scitula* (Frank *et al.*, 2010), both associated with Cossoidea *sensu* Van Nieukerken *et al.* (2011), have multiple sensilla on each segment. The presence of sensilla auricillica might be a synapomorphy at the superfamily level, although we were unable to locate other studies dealing with the antennae from other zygaenoids and cossoids to confirm this.

Sensilla styloconica are also absent in this species, and cossids *E. hippophaecolus* (Wang *et al.*, 2015; Hu *et al.*, 2018), *Y. vicarius* (Yang *et al.*, 2011), and *S. insularis* (Liu *et al.*, 2018a), but not in *E. sibirica* (Liu *et al.*, 2014), *M. flavescens* (Yang *et al.*, 2017) or *P. flammans* (Liu *et al.*, 2018b). This is not completely unexpected because its presence varies among members of the same family and genus (Faucheux *et al.*, 2006). The absence of this type is a character shared with some cossids, but not with *E. sibirica*, as it was noted above, nor with the sesiid *S. scitula* or the zygaenoids *P. flammans* and *M. flavescens*.

Of the four subtypes of sensilla trichodea, two were male-specific, indicating that these may detect molecules that are relevant to males only. The long trichodea have been found to be pheromone-specific in several members of Noctuidae (Hillier *et al.*, 2006; Hillier and Vickers, 2007). They contain ORNs that are narrowly tuned to pheromone components, and, in general, these neurons do not respond to naturally found concentrations of plant compounds, which isolates the responses to pheromone components from those elicited by other compounds (Zhang *et al.*, 2015). The other two subtypes were found in both sexes but were more abundant in females; therefore, they probably detect plant compounds, as in other species (Hillier *et al.*, 2006). It has been known for a long time that plant volatiles have a synergistic effect on the attraction of males towards calling females and on female reproductive physiology (reviewed by Landolt and Phillips, 1997; Reddy and Guerrero, 2004; Xu and Turlings,

2018). Therefore, males of this species may still use plant-produced volatiles to find mates, while females use the chemicals to find their hosts.

The sensilla chaetica found are morphologically very similar to those found in many other lepidopterans, including those from the Cossoidea - Sesioidea superfamilies (Frank *et al.*, 2010; Liu *et al.*, 2014; 2018b; Wang *et al.*, 2015; Hu *et al.*, 2018; Triana *et al.*, 2020; also, see table 1). The presence of a pore at the tip suggests they may be used to detect compounds of low volatility, such as sugars and salts (Amat *et al.*, 2022), while the flexible socket probably points to a mechanoreceptive function (Hallberg *et al.*, 2003).

The sensilla squamiformia found in the present work were more or less rounded, aporous, and with a flexible socket. This type seems to be ubiquitous among the Lepidoptera, and as such it has been found in many members of Cossoidea and related groups, such as *S. insularis*, *T. licus* and many others (Liu *et al.*, 2018a; Triana *et al.*, 2020). This type is classified as a subtype of sensillum chaeticum by some authors, for example, in *E. hippophaecolus* and *P. flammans* (see table 1 and references therein); however, we decided to classify it as squamiformium because its appearance is similar to a scale and it has a flexible socket, as described by Hallberg *et al.* (2003). They were not found in the cossids *E. hippophaecolus*, *E. sibirica*, *Y. vicarius*, and in the limacodid *M. flavescens* (see references in table 1); however, due to their similarity to scales, it may be that they were simply overlooked. The role of this type seems to be mechanoreceptive, given their thick walls, flexible sockets, and lack of pores (Hallberg *et al.*, 2003; Seada, 2015).

Böhm's bristles are located on the scape and pedicel of insects, where they serve as proprioceptors and indicate the insect the position of its antennae during flight (Krishnan *et al.*, 2012). They occur in most, if not all, insects. These sensilla have not been described in some Lepidopterans (see table 1); probably because they were overlooked.

Ovipositor

Two subtypes of multiporous sensilla basiconica, probably with olfactory functions, were identified based on their size and pore distribution. This is similar to what has been found in the cossids *S. insularis* (Liu *et al.*, 2018a) and *E. hippophaecolus* (Wang *et al.*, 2015), where one subtype of basiconica, similar to the subtype I, was found. The sensilla basiconica have also been found in other species, such as in the pyralid *Homoeosoma nebulella* Denis et Schiffermuller (Faucheux, 1991) and the tineid *Monopis crocicapitella* (Clemens) (Faucheux, 1988). The presence of more subtypes of basiconica than in other related species parallels the larger number of subtypes found on the antennae. It is difficult to compare with other Cossoidea and Zygaenoidea because sensilla from ovipositors have been described in very few species. In *Manduca sexta* L., chemosensory sensilla harbor ORNs sensitive to different classes of compounds, such as green leaf volatiles (Klinner *et al.*, 2016). Recently, Li *et al.* (2020) showed that the ovipositor of *Helicoverpa assulta* (Guenee)

expressed high amounts of the OR gene HassOR31; when ectopically expressed with its co-receptor in *Xenopus* oocytes, it responded to 12 plant volatiles, including green leaf volatiles and terpenes. Although the ORs in these sensilla seem to be broadly tuned and some of the responses probably overlap with those from the antenna, it is likely that the information they relay to the nervous system is interpreted differently, owing to differences in neuronal connections (Haverkamp *et al.*, 2018). From this, we can infer that the sensilla from the ovipositor provide a unique representation of the host and are likely to be used as one of the last steps of host assessment. In the agave red worm, we observed that females kept in cloth bags repeatedly probed the surface with the ovipositor for a long time and refused to oviposit. After a long period, if they have no choice, females finally oviposit on rough surfaces or between the wings of dead congeners (personal observation).

No gustatory sensilla were found on the ovipositor, similar to that found in *S. insularis* (Liu *et al.*, 2018a). However, in *E. hippophaecolus*, there is a large slit at the tip of the longest sensilla chaetica (Wang *et al.*, 2015). There are two possible explanations for this finding. It could be that in the agave red worm, the olfactory and mechanoreceptive sensilla are more important; thus, no gustation is needed, or the pores are difficult to observe. According to the images obtained by Wang *et al.* (2015), it seems that the slit collapses easily, which may render its observation difficult.

Conclusions

It is necessary to expand the description of antennae and ovipositors from Cossoidea and related groups to draw more definitive conclusions about their taxonomic importance, but sensilla seem to be a good source of information to explore character states to help unravel phylogenetic relationships.

In summary, we found more subtypes of sensilla on the antennae of *C. redtenbacheri* males and females than those reported for related species. This suggests that this insect might require more types of ORNs to discriminate between its host plants. Further experiments such as single sensillum recording are required to evaluate this hypothesis. Additionally, some sensilla subtypes are unique to this species and are likely to be useful for taxonomic purposes.

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