

Morphology, Ultrastructure and Possible Functions of Antennal Sensilla of *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae)

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Subject Editor: Nannan Liu

Received 5 June 2016; Accepted 26 July 2016

Abstract

To better understand the olfactory receptive mechanisms involved in host selection and courtship behavior of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae), one of the most important pests of wheat, scanning and transmission electron microscopy were used to examine the external morphology and ultrastructure of the antennal sensilla. The moniliform antennae exhibit obvious sexual dimorphism: antennae of the males are markedly longer than those of the females. Furthermore, each male flagellomere consists of two globular nodes, whereas each female flagellomere is cylindrical. Seven types of sensilla were identified in both sexes. Two types of s. chaetica have a lumen without dendrites and thick walls, suggesting that they are mechanoreceptors. S. trichodea and s. circumfila are typical chemoreceptors, possessing thin multiporous walls encircling a lumen with multiple dendrites. There are significantly more s. trichodea in female than in male, which may be related to host plant localization. In contrast, male s. circumfila are highly elongated compared to those of females, perhaps for pheromone detection. Peg-shaped s. coeloconica are innervated with unbranched dendrites extending from the base to the distal tip. Type 1 s. coeloconica, which have deep longitudinal grooves and finger-like projections on the surface, may serve as olfactory or humidity receptors, whereas type 2 s. coeloconica, smooth with a terminal pore, may be contact chemoreceptors. Also, this is the first report of Böhm' bristles at proximal scape on antennae of Cecidomyiid species potentially functioning as mechanoreceptors.

Key words: *Sitodiplosis mosellana*, antennal sensilla, host selection, pheromone detection, electron microscopy

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is one of the most important pests of wheat and is distributed in most wheat-growing regions of the world, including Asia, Europe, and North America (Doane and Olfert 2008, Gaafar and Volkmar 2010, Miao et al. 2013, Jacquemin et al. 2014). This species has one generation per year and overwinters as diapausing co-cooned larvae. Adults emerge from pupae in the soil in spring and, after mating, flying females search for and lay eggs on wheat heads prior to flowering. Larvae feed on the developing kernels, leading to shriveling, and pre-sprouting damage. Yield loss can be very severe in years of high infestation. Infestation also facilitates fungal colonization (Mongrain et al. 2000), affecting quality of harvested grain.

Olfaction has a central role in locating oviposition sites and mates in *S. mosellana*. It has been demonstrated that female adults are attracted by volatiles from preanthesis wheat spikes, which are preferred for oviposition (Birkett et al. 2004). Consistently, volatile compounds from non-preferred wheat spikes reduce

oviposition (Ganehirachchi and Harris 2007, Gharalari et al. 2011). Furthermore, male adults exhibit strong behavioral response to 2,7-nonanediyol dibutyrate, the pheromone emitted by conspecific females (Gries et al. 2000, Oakley et al. 2005, Bruce et al. 2007). So far, very little is known of the olfactory system of *S. mosellana*.

Most insect olfactory sensilla are located at the antennae (Ahmed et al. 2013, Yuan et al. 2013, Carle et al. 2014). Antennal sensilla from a number of dipterans have been characterized (Blackwell et al. 1992, Chen and Fadamiro 2008, Setzu et al. 2011, Liu et al. 2013) including several important cecidomyiid species (Slifer and Sekhon 1971, Solinas and Nuzzaci 1987, Crook and Mordue 1999, Hillbur et al. 2001, Zhang and Yang 2008, Boddum et al. 2010). However, little information is available on *S. mosellana* (Yuan 2004). A detailed study of the ultrastructure of antennal sensilla is essential to better understand the olfactory perception.

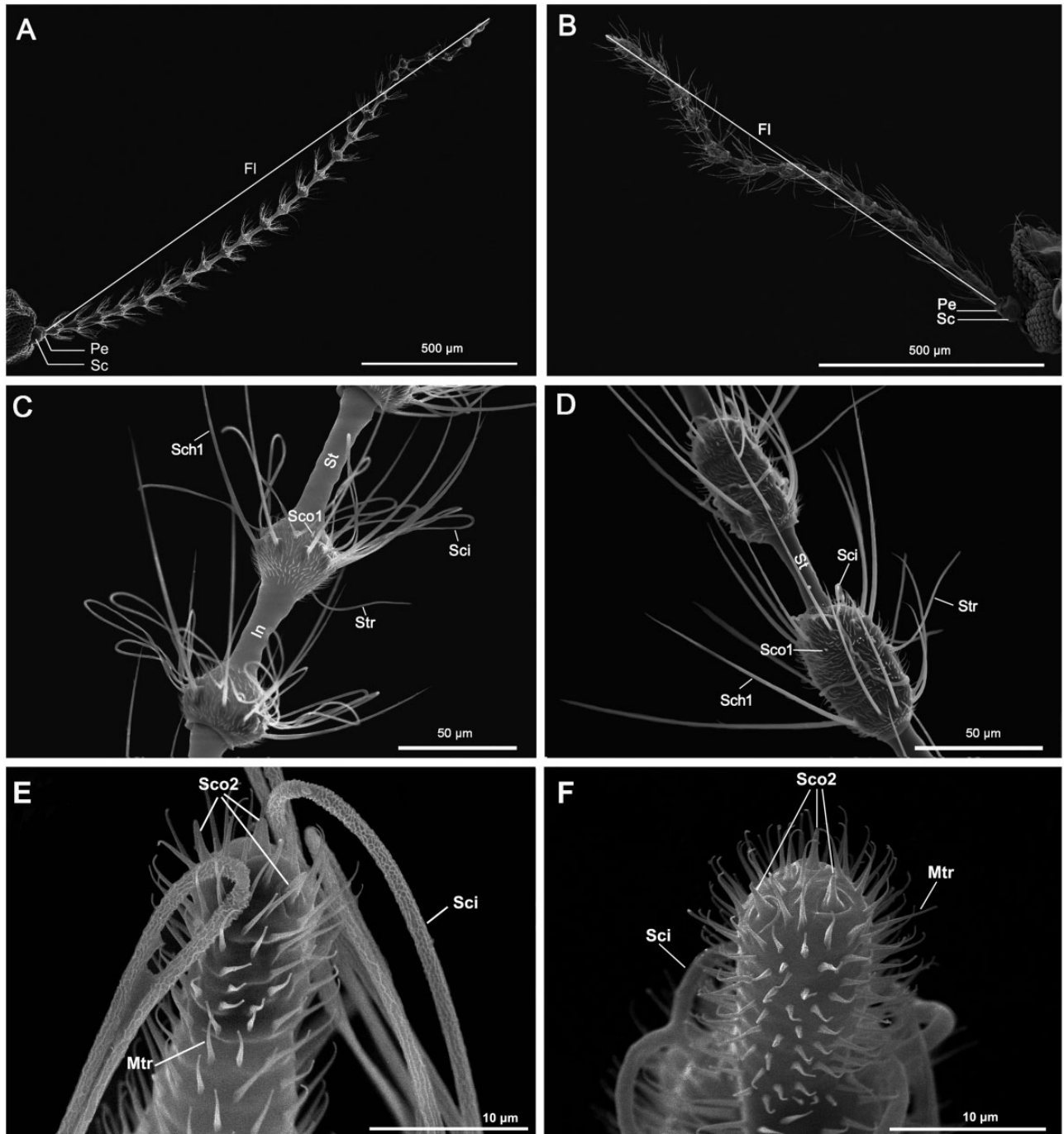


Fig. 1. SEM micrographs of male (A, C, and E) and female (B, D, and F) *S. mosellana* antennae. (A and B) A whole antenna consisted of scape (Sc), pedicel (Pe), and 12 flagellomeres (Fl). (C) Each male flagellomere consisted of two globular nodes separated by an internode (In) and flagellomeres were connected by slender stalks (St). (D) Female flagellomeres were cylindrical and connected by slender stalks (St). (E and F) Three type 2 s. coeloconica (Sco2) occurred on the antennal apex of both sexes. Shown were five sensillum types on antennal flagellum: type 1 s. chaetica (Sch1), s. trichodea (Str), s. circumfila (Sci), type 1 s. coeloconica (Sco1) and Sco2. Microtrichia (Mtr) covered the surface of antennae except internode and stalk regions.

In this study, we investigated the distribution, morphology, and ultrastructure of antennal sensilla in male and female *S. mosellana* using scanning (SEM) and transmission electron microscopy (TEM), and compared our results with those of other insects, especially cecidomyiidae. Possible functions were discussed with reference to their morphology, ultrastructure, and distribution. Research has improved our understanding of host selection and courtship behavior in *S. mosellana*.

Materials and Methods

Insects

In late February 2013, soil samples containing over-wintering larvae of *S. mosellana* were collected from a wheat field at Fuping County (34° 75' N, 109° 18' E), Shaanxi Province, China. Larvae were maintained at 23 °C, 80 ± 10% RH and a photoperiod of 16 h light:8 h dark. After emerging, adults were collected for SEM and TEM analyses.

Table 1. Length and width of each segment of female and male *S. mosellana* antennae

Segments	Length (μm)		Width (μm)	
	Female	Male	Female	Male
Scape	30.46 \pm 1.07a	33.95 \pm 1.21a	51.73 \pm 1.71a	49.92 \pm 1.02a
Pedicle	30.12 \pm 1.33a	29.42 \pm 1.08a	50.25 \pm 1.12a	48.34 \pm 1.23a
Flagellum	1091.07 \pm 23.33b	1946.50 \pm 34.03a	28.12 \pm 0.54b	34.23 \pm 0.50a
Stalk	30.98 \pm 1.24b	43.76 \pm 2.32a	12.72 \pm 0.58a	12.00 \pm 0.69a
Internode	–	39.24 \pm 0.80	–	11.98 \pm 0.56

Data are mean \pm SE of at least six antennae per sex. Means in the same rows followed by different letters are significantly different between sexes by the independent samples *t*-test ($P < 0.05$).

– indicates absent.

Table 2. Length of each flagellomere in female and male *S. mosellana* antennae

No. of flagellomeres	Length (μm)	
	Female	Male
1st	132.44 \pm 1.12a	164.17 \pm 4.56a
2nd	104.81 \pm 1.31b	162.80 \pm 3.93a
3rd	98.99 \pm 1.37b	158.71 \pm 5.32a
4th	92.26 \pm 2.44bc	160.00 \pm 3.77a
5th	89.65 \pm 2.00c	163.29 \pm 2.10a
6th	89.63 \pm 1.43c	162.71 \pm 3.29a
7th	84.06 \pm 1.94cd	163.00 \pm 4.20a
8th	84.19 \pm 2.21cd	163.00 \pm 4.20a
9th	80.77 \pm 2.66cd	153.61 \pm 4.15a
10th	74.35 \pm 2.26d	146.71 \pm 3.42b
11th	74.71 \pm 1.47d	143.29 \pm 5.63b
12th	76.64 \pm 2.17d	125.12 \pm 6.64c

Data are mean \pm SE of at least six antennae per sex. Means in the same columns followed by different letters are significantly different by Tukey's multiple range test ($P < 0.05$).

Scanning Electron Microscopy

To observe the external morphology and distribution of antennal sensilla, freshly emerged adults were chilled in a refrigerator for 10 min. The anaesthetized adults were decapitated under a stereomicroscope. After air drying at room temperature for 48 h, the

antennae were mounted separately by gender on aluminum stubs with tape and were sputter-coated with gold for 2 min (model MSP-1S ion sputter, Shikku VD, Japan). At least six antennae of each sex were examined using an SEM (model S-3400N, Hitachi, Japan) with accelerating voltage of 5–15 kV. Images were recorded digitally and stored in the computer.

Transmission Electron Microscopy

Newly emerged adults were fixed in 2.5% glutaraldehyde in PBS buffer (0.1 M, pH 7.2) at 4 °C for 24 h. After rinsing in the same buffer for six times (5, 10, 15, 20, 25, and 30 min), the heads were excised and fixed in 1% osmium tetroxide in phosphate buffer solution for 2 h, and then carefully washed as stated above. The samples were then gradually dehydrated in ethanol of ascending concentrations (30, 50, 70, 80, and 90% for 15 min each; and 100% for 30 min two times), infiltrated with LR-White resin (London Resin Company, Reading, United Kingdom; 50% in absolute ethanol overnight, 100% one time for 2 h and one time for 1 h, respectively), embedded in absolute LR-White resin, and polymerized for 48 h at 60 °C. Ultrathin (70–100 nm) sections both in transverse and longitudinal planes were cut with an ultramicrotome (model EM UC7, Leica, Germany), subsequently stained with uranyl acetate and lead citrate, and examined with a TEM (model JEM-1230, JEOL, Japan) at 80 kV.

Table 3. The size of antennal sensilla of female and male *S. mosellana*^A

Sensillum	Length (μm)		Diameter at base (μm)		Socket diameter (μm)	
	Female	Male	Female	Male	Female	Male
Sch1	81.30 \pm 1.44b	99.06 \pm 1.95a	1.87 \pm 0.04a	1.96 \pm 0.04a	1.47–2.60	1.50–2.60 ^B
Sch2	32.26 \pm 0.91b	47.71 \pm 1.37a	1.21 \pm 0.02b	1.36 \pm 0.03a	1.00–2.20	1.24–2.20
Str	55.58 \pm 0.75b	52.76 \pm 1.79a	1.61 \pm 0.03a	1.56 \pm 0.04a	2.51 \pm 0.48a	2.64 \pm 0.10a
Sci	– ^C	59.84 \pm 1.02	1.54 \pm 0.07b	1.73 \pm 0.04a	2.48 \pm 0.05b	3.34 \pm 0.04a
Sco1	4.11 \pm 0.17a	3.88 \pm 0.10a	1.06 \pm 0.02a	1.04 \pm 0.03a	2.48 \pm 0.12a	2.52 \pm 0.08a
Sco2	2.32 \pm 0.07a	2.13 \pm 0.05b	1.13 \pm 0.04a	1.05 \pm 0.03a	1.73 \pm 0.08a	1.50 \pm 0.05b
BB ^D	6.00–10.50	6.55–8.50	0.65–1.10	0.60–1.00	1.45–2.10	1.60–2.00

Sch1, type 1 sensilla chaetica; Sch2, type 2 sensilla chaetica; Str, sensilla trichodea; Sci, sensilla circumfla; Sco1, type 1 sensilla coeloconica; Sco2, type 2 sensilla coeloconica; BB, Bohm's bristles.

^AData are mean \pm SE of at least 10 sensilla per type. Means in the same rows followed by different letters are significantly different between sexes by the independent samples *t*-test ($P < 0.05$).

^BRange of socket dimension. Average diameter was not calculated owing to their oval shape of the Sch sockets.

^CUndetermined.

^DBecause of technical difficulty, only ranges of sensilla BB dimension data were collected.

Table 4. Number and distribution pattern of antennal sensilla of female and male *S. mosellana*

Antennae	Gender	BB	Sch1	Sch2	Str	Sci	Sco1	Sco2
Scape	F	2 ^A	–	14.00 ± 1.36	–	–	–	–
	M	2–3	–	10.00 ± 0.73	–	–	–	–
Pedicel	F	–	–	15.67 ± 0.61	–	–	–	–
	M	–	–	15.00 ± 1.34	–	–	–	–
f1	F	–	31.67 ± 1.89	–	–	20.67 ± 1.23	0.67 ± 0.42	–
	MN1	–	16.00 ± 1.93	–	–	11.33 ± 0.84	–	–
	MN2	–	13.00 ± 0.68	–	–	10.00 ± 0.73	1.00 ± 0.48	–
f2	F	–	25.33 ± 2.11	–	–	21.33 ± 1.84	1.33 ± 0.67	–
	MN1	–	13.00 ± 0.68	–	–	10.33 ± 0.61	–	–
	MN2	–	12.00 ± 1.15	–	–	10.67 ± 0.67	1.33 ± 0.67	0.33 ± 0.33
f3	F	–	24.67 ± 1.91	–	0.33 ± 0.33	21.33 ± 2.17	1.00 ± 0.45	0.67 ± 0.42
	MN1	–	14.33 ± 1.19	–	–	11.33 ± 0.84	–	–
	MN2	–	13.67 ± 1.74	–	–	10.00 ± 0.52	1.67 ± 0.80	0.50 ± 0.34
f4	F	–	23.00 ± 1.91	–	1.33 ± 0.84	20.67 ± 2.11	0.67 ± 0.42	–
	MN1	–	14.33 ± 1.17	–	–	12.00 ± 0.52	1.33 ± 0.67	–
	MN2	–	13.00 ± 1.53	–	0.17 ± 0.17	11.00 ± 0.68	1.67 ± 0.33	0.67 ± 0.33
f5	F	–	21.67 ± 2.09	–	2.33 ± 0.80	21.33 ± 0.99	1.00 ± 0.45	–
	MN1	–	16.33 ± 1.20	–	–	13.33 ± 0.99	1.00 ± 0.45	–
	MN2	–	13.67 ± 1.31	–	–	10.33 ± 0.95	3.00 ± 0.68	0.83 ± 0.40
f6	F	–	21.00 ± 1.53	–	3.67 ± 1.41	20.67 ± 2.62	2.67 ± 0.84	–
	M	–	17.33 ± 1.52	–	–	12.33 ± 0.80	1.83 ± 0.17	–
f7	F	–	12.67 ± 0.99	–	0.50 ± 0.22	10.67 ± 0.42	2.83 ± 0.54	–
	MN1	–	18.33 ± 1.58	–	7.33 ± 2.23	22.67 ± 2.46	1.33 ± 0.42	–
	MN2	–	16.57 ± 0.84	–	–	12.00 ± 0.52	2.67 ± 0.42	0.83 ± 0.40
f8	F	–	14.00 ± 0.73	–	0.67 ± 0.21	10.00 ± 0.52	2.50 ± 0.50	1.67 ± 0.33
	MN1	–	19.33 ± 1.12	–	–	10.33 ± 0.61	2.33 ± 0.21	–
	MN2	–	15.17 ± 1.22	–	–	11.33 ± 0.42	3.00 ± 0.37	–
f9	F	–	14.17 ± 0.91	–	0.83 ± 0.17	11.33 ± 0.42	3.00 ± 0.37	–
	MN1	–	18.00 ± 1.15	–	16.67 ± 1.98	25.00 ± 1.98	2.00 ± 0.73	–
	MN2	–	15.33 ± 0.99	–	–	11.50 ± 0.34	2.33 ± 0.56	–
f10	F	–	15.17 ± 1.33	–	1.67 ± 0.42	11.50 ± 0.34	1.50 ± 0.56	1.00 ± 0.37
	MN1	–	15.67 ± 0.61	–	17.33 ± 1.84	23.67 ± 1.82	2.67 ± 0.42	–
	MN2	–	16.17 ± 0.75	–	0.33 ± 0.21	10.33 ± 0.42	1.50 ± 0.34	–
f11	F	–	13.33 ± 1.17	–	1.17 ± 0.17	9.00 ± 0.89	1.50 ± 0.34	–
	MN1	–	13.67 ± 0.33	–	20.00 ± 1.46	23.33 ± 1.52	1.33 ± 0.42	–
	MN2	–	17.17 ± 1.76	–	0.33 ± 0.21	9.50 ± 0.67	1.33 ± 0.17	–
f12	F	–	14.17 ± 1.17	–	2.00 ± 0.26	9.67 ± 0.21	1.83 ± 0.21	–
	MN1	–	13.00 ± 1.13	–	22.67 ± 2.40	22.33 ± 0.95	–	3 ^A
	MN2	–	19.67 ± 1.74	–	–	9.33 ± 0.42	2.33 ± 0.21	–
Total	F	2 [#]	245.33 ± 13.80b	29.67 ± 1.31a	101.33 ± 9.99a	264.33 ± 8.86a	16.33 ± 1.82b	3.67 ± 0.42b
	M	2–3	355.67 ± 18.19a	24.67 ± 1.23b	10.17 ± 0.60b	256.83 ± 8.86a	38.50 ± 3.86a	8.83 ± 0.54a

F, female; M, male; MN1, node 1 of male; MN2, node 2 of male; f1–f12; flagellomere 1–12; Sch1, type 1 sensilla chaetica; Sch2, type 2 sensilla chaetica; Str, sensilla trichodea; Sci, sensilla circumfila; Sco1, type 1 sensilla coeloconica; Sco2, type 2 sensilla coeloconica; BB, Böhm's bristles.

Data are mean ± SE of six antennae per sex. Means in the same columns followed by different letters are significantly different between sexes by the independent samples *t*-test ($P < 0.05$).

– indicates sensilla are absent.

^ANumber of sensilla is constant.

Data Analysis

Sensilla on each segment of antennae were identified, measured and counted. Classification of the sensillum types was based mainly on morphological characters described by Blackwell et al. (1992), Boddum et al. (2010), Keil (1999) and Zacharuk (1985). Lengths and widths of antennae, lengths and the basal diameters of sensilla, and diameters of sensillum sockets were measured (Hitachi's SEM Data Manager). All scales were marked on the images with Photoshop CS6 (Adobe).

Differences in the size of antennae and in size and number of sensilla between sexes were determined using the independent samples *t*-test. Difference in length among flagellomeres within a sex was analyzed using a one-way ANOVA followed by Tukey's multiple

range test ($P = 0.05$). Data analyses were performed using SPSS 20 software (SPSS, Inc., Chicago, IL, United States).

Results

General Description of the Antennae

Antennae of both male and female *S. mosellana* were moniliform, consisting of the scape, the pedicel and the flagellum that contains 12 flagellomeres (Fig. 1A and B). They were located between the compound eyes. The cup-shaped scape appeared to be the widest segment. The pedicel, shaped as an inverted cup, was slightly smaller than the scape (Figs. 1A, B and 3A; Table 1). Each male flagellomere possessed two globular nodes, separated by a slender internode.

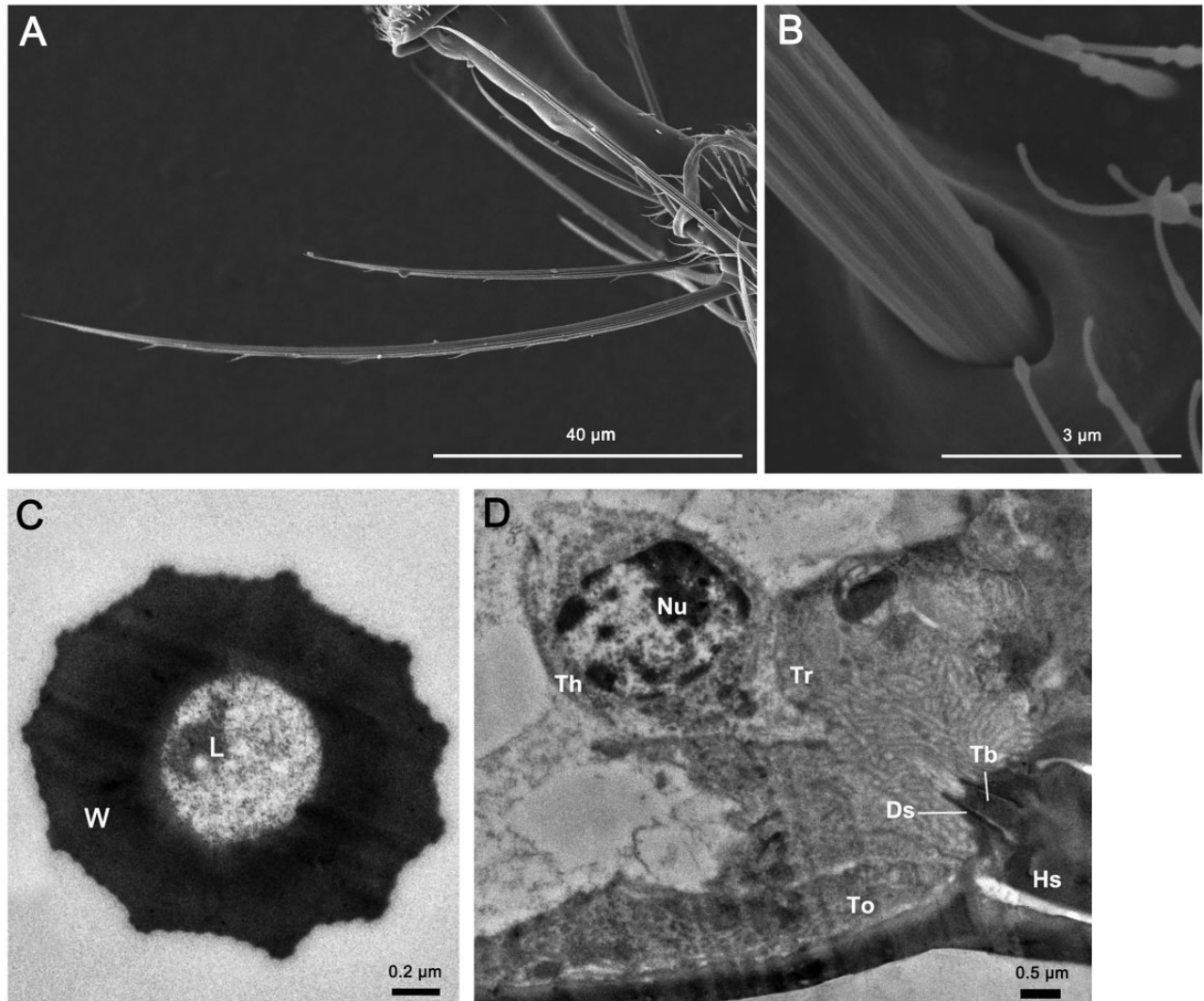


Fig. 2. SEM (A and B) and TEM (C and D) micrographs of type 1 sensilla chaetica. (A) Entire sensillum. (B) A flexible socket and longitudinal ridges on the surface of the hair shaft. (C) Cross-section showing an inner lumen (L) without dendrites and thick grooved wall (W). (D) Longitudinal section at the base of hair shaft (Hs) showing one sensory neuron (Nu) enveloped by the thecogen (Th), trichogen (Tr), and tormogen cell (To), and a tubular body (Tb) enclosed by a dendrite sheath (Ds).

They were joined together by slender stalks (Fig. 1C). In females, however, flagellomeres were cylindrical and separated by stalks short than those in the male (Fig. 1D; Table 1). As a result, the male flagellum was significantly longer than that of females (Table 1). In both sexes, flagellomeres gradually reduced in size from the base to the apex (Table 2).

Numerous sensilla of different types were observed on the flagellum, whereas fewer sensilla were seen on the scape and the pedicel (Figs. 1 and 3). Microtrichia, a fine hair-like non-socketed cuticular structure, uniformly covered the surface of antennae except inter-node and stalk regions (Fig. 1C–F).

Sensilla on Antennae

Based on external morphology and internal structure, five major sensillum types were identified on antennae of *S. mosellana*: sensilla chaetica (Sch), Böhm'bristles (BB), s. circumfila (Sci), s. trichodea (Str), s. coeloconica (Sco). *S. chaetica*, and *s. coeloconica* were further classified into two subtypes: Sch1 and Sch2, Sco1, and Sco2,

respectively. The size, number, and distribution of these sensilla were shown in Tables 3 and 4.

Sensilla Chaetica

Sensilla chaetica (Sch1) were the longest type of sensilla found on antennae of both sexes. Derived from a flexible circular socket, these sensilla gradually tapered to a pointed tip and were slightly curved (Fig. 2A). High magnification microscopy revealed large and minor longitudinal ridges on the surface. Large ridges extended the hair shaft down into the socket, while the minor ridges stopped at some distance above it (Fig. 2B). Small lateral branches were also observed in mid- and distal regions of Sch1 (Fig. 2A). These sensilla had a thick-walled cuticle and a lumen that was devoid of dendrites (Fig. 2C). Each sensillum was innervated by a single sensory neuron enveloped by the thecogen, trichogen, and tormogen cells. Dendrite of the neuron ended with a tubular body that was surrounded by a dendritic sheath at the base of the sensillum (Fig. 2D).

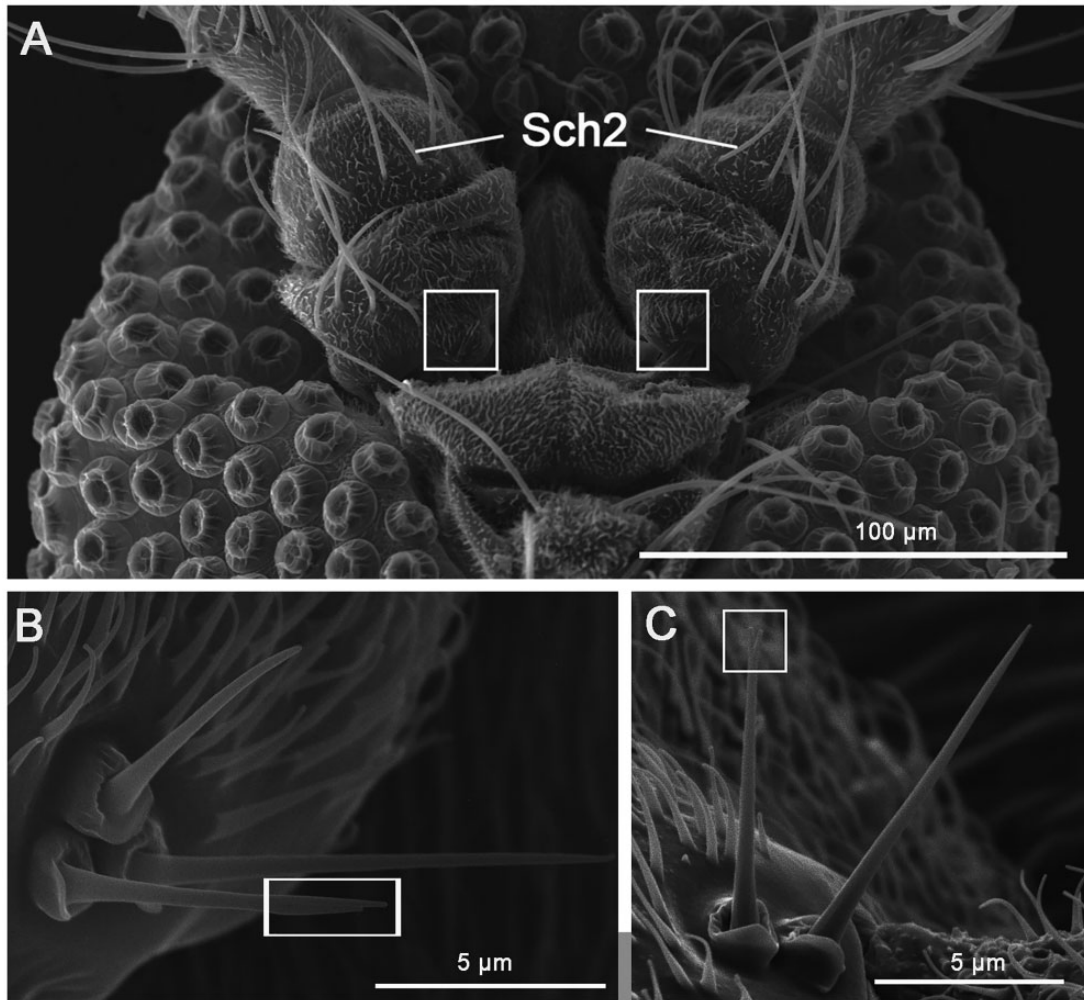


Fig. 3. SEM micrographs of type 2 sensilla chaetica (Sch2) and Böhm's bristles. (A) The frontal view of the male head showing distributions of Sch2 and Böhm's bristles (in white frames). (B) Three Böhm's bristles were located at the proximal scape in the male and one of them shaped as a fork at the tip (framed). (C) Two Böhm's bristles were located at proximal scape in the female and one of them also shaped as a fork at the tip (framed).

Sch1 were arranged in two circles surrounding each flagellomere near its two ends in females, and in one circle around basal region of each node in males (Fig. 1C and D; Table 3). Numbers of Sch1 in female gradually decreased from flagellomere 1 to 12, but were more evenly distributed among flagellomeres in male. Male Sch1 were significantly longer in size and larger in number than those in females (Tables 3 and 4).

Sch2 were shorter and fewer than Sch1 (Tables 3 and 4), distributed circularly on the scape and pedicel of both sexes (Fig. 3A). Similar to Sch1, Sch2 were larger in size in males than in females, but differing from Sch1, they were significantly more abundant in females than in males. Other than size, they were structurally and morphologically identical.

Böhm's Bristles

Generally, two Böhm's bristles (BB) were recorded on each antenna of both sexes, and occasionally three in male. One of them was found to have a fork-like shape at the tip. They displayed a spine-like structure with a blunt tip, a smooth surface and a non-flexible socket. Furthermore, they were only visible in the frontal view of the head (Fig. 3). This sensillum type, located at the proximal scape (Fig. 3), was relatively short, 6.00–10.50 μm long and 0.60–1.10 μm

wide at base (Table 3). Its minute structure and hidden position made it challenging for sample preparation and location, the ultra-structure was thus not further pursued.

Sensilla Trichoidea

Sensilla trichoidea (Str) had a special horseshoe-shaped socket with small tooth-like protrusions on top. They became thinner as they extended towards apex and exhibited distinctive sigmoid curvature (Fig. 4A and B). Surface of Str was smooth at the base, but had a series of longitudinal ridges in the middle and transverse ridges at distal end (Fig. 4A and C). This sensillum type had a thin-walled cuticle containing of a large number of pores (Fig. 4D and E). Two dendrites with conspicuous varicosities were observed in lumen (Fig. 4D).

Str occurred on flagellomeres 3–12 in females and 4–12 in males (Table 4). They were usually located at the medial region of female flagellomeres and the proximal region of male globular nodes (below Sch1; Fig. 1C and D). Contrasting to Sch1, the number of female Str gradually increased from the third flagellomere to distal one and was in significantly higher density than that in males (Table 4).

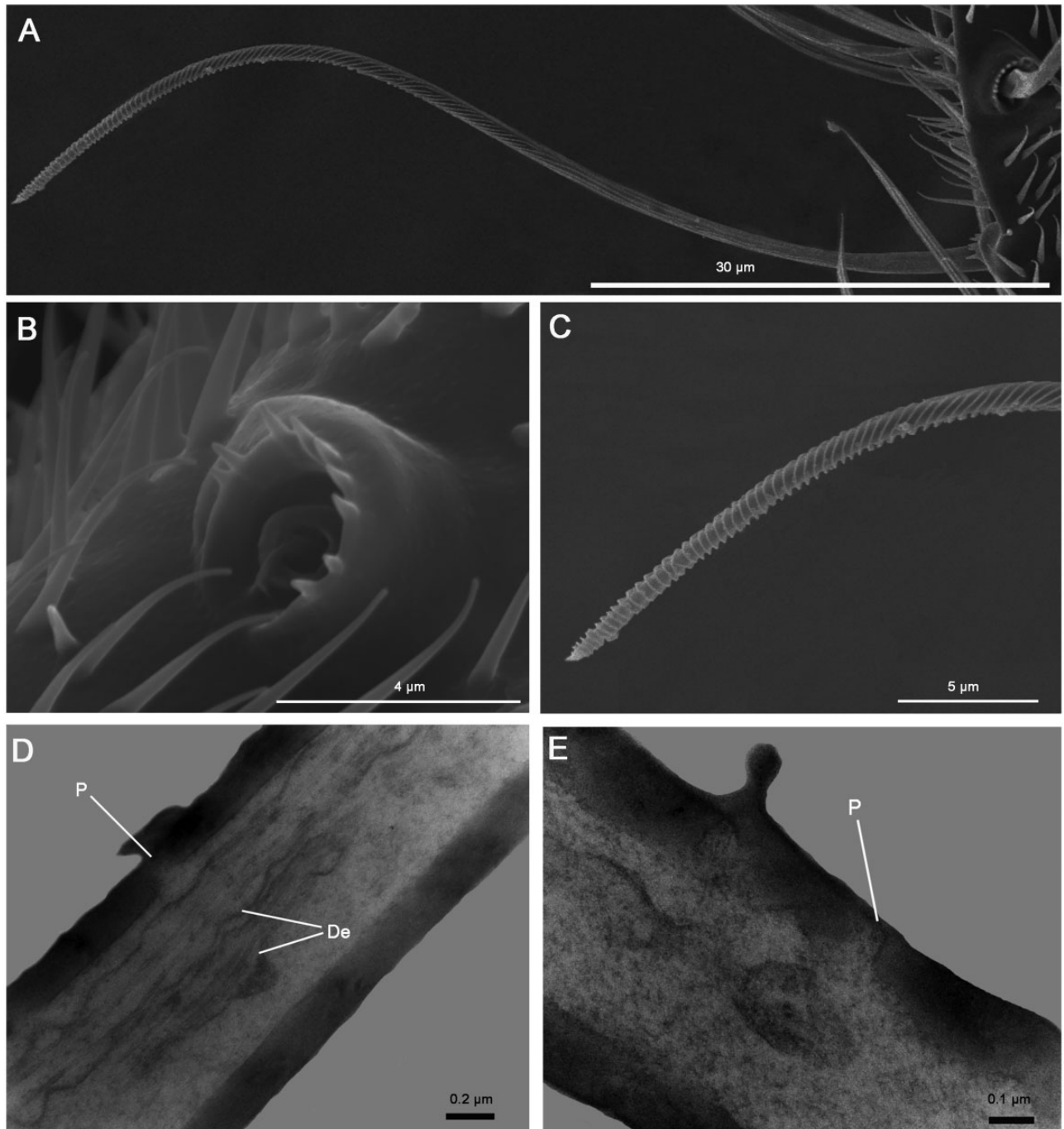


Fig. 4. SEM (A–C) and TEM (D and E) micrographs of sensilla trichodea. (A) Entire sensillum. (B) A horseshoe-shaped socket with small teeth on their opening. (C) Distal region of the sensillum showing transverse ridges. (D and E) Longitudinal section showing thin porous (P) wall and two dendrites (De).

Sensilla Circumfila

Sensilla circumfila (Sci) displayed apparent sexual dimorphism. Male Sci consisted of 9–14 sensilla per node (Table 4). Each sensillum bifurcated approximately 2 µm above the antennal surface and fused with the neighboring sensilla 59.84 µm above the bifurcation (Fig. 5A; Table 3). They formed a continuous elongated loop along the circumference of each node above Sch1 (Fig. 1C). In females, however, Sci consisted of 20–26 forked sensilla per flagellomere, forming two transverse loops close to the antennal surface and joined with two axial connections (Fig. 1D; Table 4). In addition, surface of Sci above bifurcations had irregular ridges in males (Fig.

5A), but was smooth or slightly wrinkled in females (Fig. 6A). Also, the basal diameter of the sensillum and the diameter of the socket were significantly smaller in females than in males (Table 3).

TEM showed that the thin cuticular walls of these sensilla displayed no pores before bifurcations, whereas the branches were highly porous (Figs. 5B–F, 6B and D). In males, each sensillum was innervated by one sensory neuron surrounded at the base by a thecogen cell, a trichogen cell, and a tormogen cell (Fig. 5B). Two dendritic branches were visible at the base of the sensillum (Fig. 5B) and two to seven in the sensillum branches (Fig. 5C and D). Cross- and vertical-section of the area in which two adjacent sensilla fused

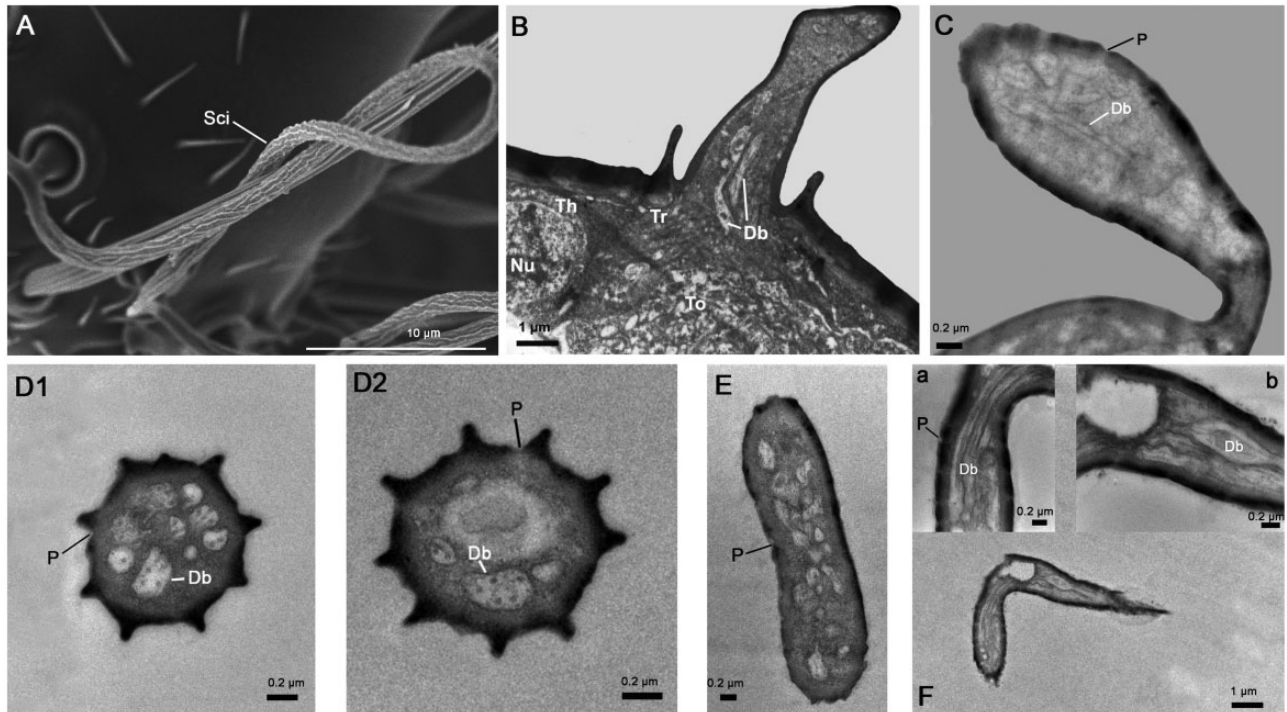


Fig. 5. SEM (A) and TEM (B–F) micrographs of sensilla circumfila in males. (A) The *s. circumfila* (Sci) was bifurcate, protruded from the antennal surface and had irregular ridges on its surface. (B) Longitudinal section showing one sensory neuron (Nu) surrounded by a thecal cell (Th), a trichogen cell (Tr), and a tormogen cell (To), and two dendritic branches (Db) at the junction between the base and the sensillum stalk. (C) Longitudinal and (D) cross-section of the sensillum branch showing dendritic branches (Db) and pores (P) on the wall. (E) Cross- and (F) longitudinal-section of the region of two sensilla fusion showing confluent lymph cavity and pores (P) on the wall.

revealed that dendritic branches and lymph cavity were confluent between sensilla (Fig. 5E and F). In females, one dendrite was visible at the base of each sensillum, but more dendritic branches were observed in the sensillum branch compared with males (Figs. 5D and 6D). Dendritic branches and lymph cavity were also confluent at the region where three sensilla fused (Fig. 6C).

Sensilla Coeloconica

Peg-shaped sensilla coeloconica (Sco) were differentiated into two subtypes according to external morphology. The surface of Sco1 was longitudinally deep grooved and appeared to be made up of closely apposed cuticular finger-like projections. We observed 11 such fingers, which were almost the same in width. These fingers ran along the length of the hair shaft and did not taper from base to tip (Fig. 7A). These sensilla were often located at the regions of distal end to Sci of each flagellomere (Fig. 1C and D). On average, 39 and 16 per antennae were observed for the males and females, respectively (Table 4). They were about 4 μm long and 1 μm wide at the base. The diameter of the socket was about 2.5 μm (Table 3). Each of these thin-walled pegs was innervated by unbranched dendrites that extended from the base to the tip and were enclosed by a conspicuous dendrite sheath (Fig. 7B).

A smooth surface and a rounded tip appeared to be the characteristics of Sco2 (Fig. 8A). Except for three sensilla that were always located at the tip of antennae (Fig. 1E and F), the rest were distributed sporadically on the flagellum. They were about 2.2 μm long and 1.1 μm wide at the base (Table 3), very similar to the surrounding microtrichia, which were of comparable length. However, they could be distinguished by the sunken sockets they were associated with (Figs. 1E, F and 8A). In microtrichia, the sockets were missing.

The cuticular walls of the smooth pegs were thick relative to its diameter and a terminal pore was present (Fig. 8B). Similar to Sco1, their dendrites extended from the base to the tip and were enclosed by a dendrite sheath (Fig. 8B).

Discussion

The moniliform antennae of *S. mosellana* exhibit obvious sexual dimorphism. Each female flagellomere is cylindrical and connected with a stalk, whereas the male flagellomere consists of two globular nodes separated by an internode and flagellomeres by a stalk. This is similar to other cecidomyiid species including *Contarinia sorghicola* (Slifer and Sekhon 1971), *Mycodiplosis erysiphe* (Solinas and Nuzzaci 1987), *Contarinia pisi* (Hillbur et al. 2001), and *Aphidoletes aphidimyza* (Zhang and Yang 2008), but slightly different from *Dasineura tetensi* (Crook and Mordue 1999), *Contarinia nasturtii*, and *Mayetiola destructor* (Boddum et al. 2010, Schneeberg et al. 2013), whose flagellomere is cylindrical and connected without a stalk in female, or contains only a node in male. Furthermore, the male antennae are markedly longer than female's, consistent with previous observations in the above gall midges. The adaptive significance of the longer male antennae might be the more surface area for sense organs helps to detect sex pheromones (Schneider 1964, Dweck 2009). The normal sensilla of gall midge antennae include *s. chaetica*, *s. trichodea*, *s. coeloconica*, and *s. circumfila* (Boddum et al. 2010, Hall et al. 2012). They are often located on the flagellum (Slifer and Sekhon 1971, Crook and Mordue 1999). In this investigation, we found all four types on the flagellum. Additionally, we discovered Böhm's bristles on the scape. *S. chaetica* and *s. coeloconica* were also differentiated into two types, respectively.

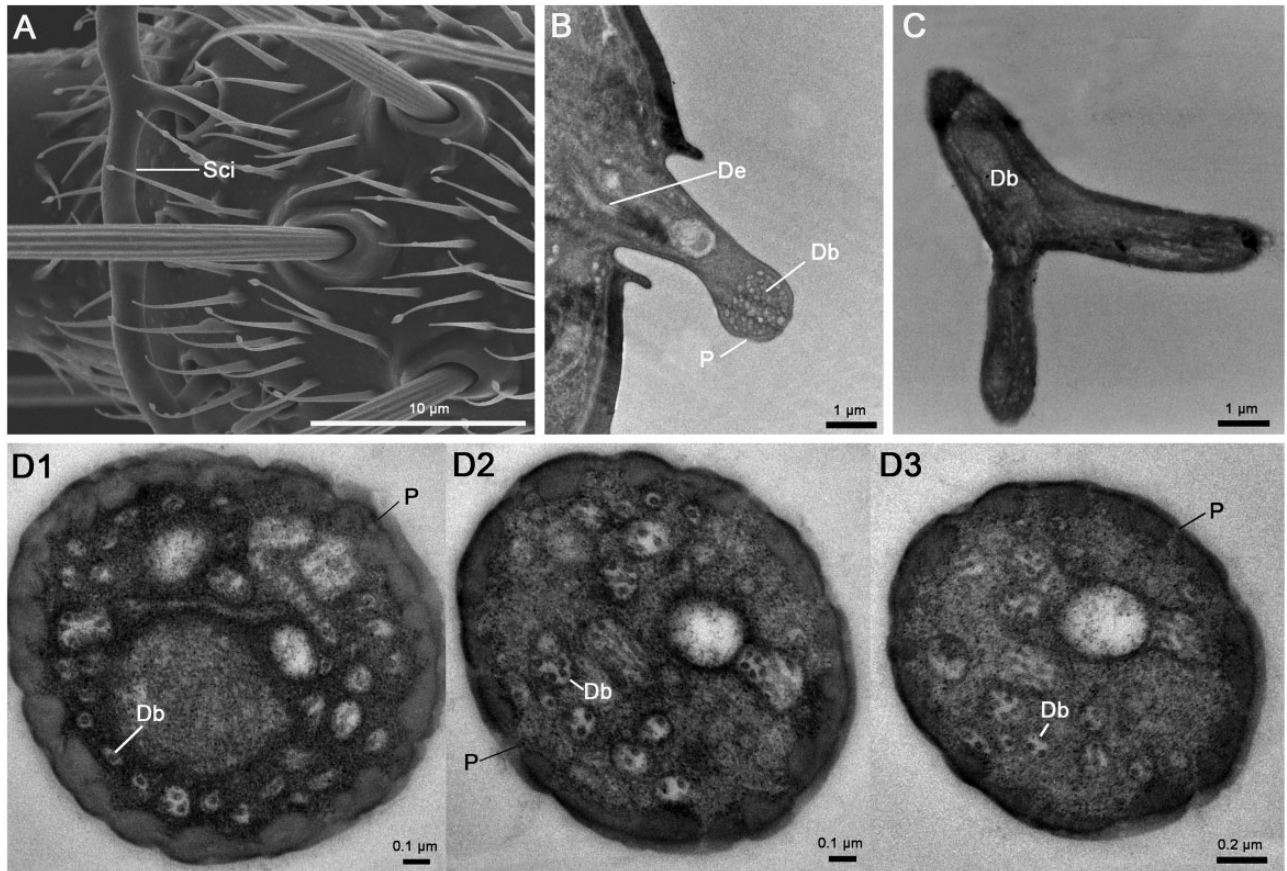


Fig. 6. SEM (A) and TEM (B–D) micrographs of sensilla circumfila in females. (A) The s. circumfila (Sci) was located near the antennal surface and smooth at the stalk and the branch. (B) Longitudinal section showing one dendrite (De) at the base, numerous dendritic branches (Db) and pores on the wall (P) at sensillum branch. (C) Cross-section of the region where three sensilla fused showing confluent lymph cavity. (D) Cross-section of the sensillum branch showing plentiful dendritic branches (Db) and pores on the wall (P).

Both types of s. chaetica observed in this study are the most prominent in their location. They have a longitudinally grooved cuticular apparatus with a flexible socket, thick walls, and a single sensory neuron whose dendrite terminates in a tubular body. These traits are highly correlated with s. chaetica in many insects including other cecidomyiid species as stated above, and strongly indicate that these sensilla are mechanoreceptors (McIver 1975, Saïd et al. 2003, Ren et al. 2012). They may protect the finer underlying structure on the antennae or perceive difference in substrate texture, movement, and wind direction (Crook and Mordue 1999, Ren et al. 2012, Yuan et al. 2013). The male s. chaetica are longer compared with females, suggesting that the male may also utilize these sensilla to orientate to sound as is common in male mosquitoes (Slifer and Sekhon 1962, McIver and Hudson 1972).

To our knowledge, no Böhm' bristle structure from cecidomyiid species has been reported. Perhaps previous researchers mainly focused on the sensilla of flagellum rather than on scape and pedicel. Böhm' bristles in *S. mosellana* shared similarity with Böhm' bristles described in a beetle *Dastarcus helophoroides* (Ren et al. 2012), five psocid species including *Liposcelis bostrychophila*, *L. entomophila*, *L. tricolor*, *L. decolor*, and *L. paeta* (Hu et al. 2009), and sawfly *Acantholyda posticalis* (Yuan et al. 2013). In addition, they appeared close to the bristle sensillae on the scape and pedicel of screw worm fly *Cochliomyia hominivorax* (Fernandes et al. 2004). The fact that Böhm' bristles in *S. mosellana* is located at proximal scape suggests that they may be mechanoreceptors, which record

antennal position and movement (Schneider 1964, Zacharuk 1985, Merivee et al. 1998).

Distribution, morphology, and fine structure of s. trichodea of *S. mosellana* share high similarity with s. trichodea found in other gall midges. The presence of thin multiporous walls encircling an inner lumen with two dendrites suggests that they are chemoreceptors (Altner and Prillinger 1980, Zacharuk et al. 1980). It has been demonstrated that male s. trichodea responded to the sex pheromone in *M. destructor* where s. trichodea are more numerous in males, whilst in species where s. trichodea are more in females, they may be involved in host location (Boddum et al. 2010, Hall et al. 2012). In *S. mosellana*, female s. trichodea are significantly more compared with males, thus we speculate that they may play a key role in localization of host plants.

The s. circumfila are a unique type of sensilla in gall midges (Hall et al. 2012). Presumably, s. circumfila may be evolved from s. basiconia (Boddum et al. 2010), which are found in many insects but absent in all cecidomyiid species studied so far. Like other gall midges, s. circumfila in *S. mosellana* have thin multiporous walls and a lumen filled with plentiful dendritic branches, indicating an olfactory function (Solinas and Nuzzaci 1987, Crook and Mordue 1999, Hillbur et al. 2001, Boddum et al. 2010). The s. circumfila of *S. mosellana* are sexually dimorphic. In males, they are highly elongated, whereas in female they are located close to the antennal surface. Similar dimorphism have also been observed for *C. sorghicola* (Slifer and Sekhon 1971), *M. erysiphe* (Solinas and Nuzzaci 1987),

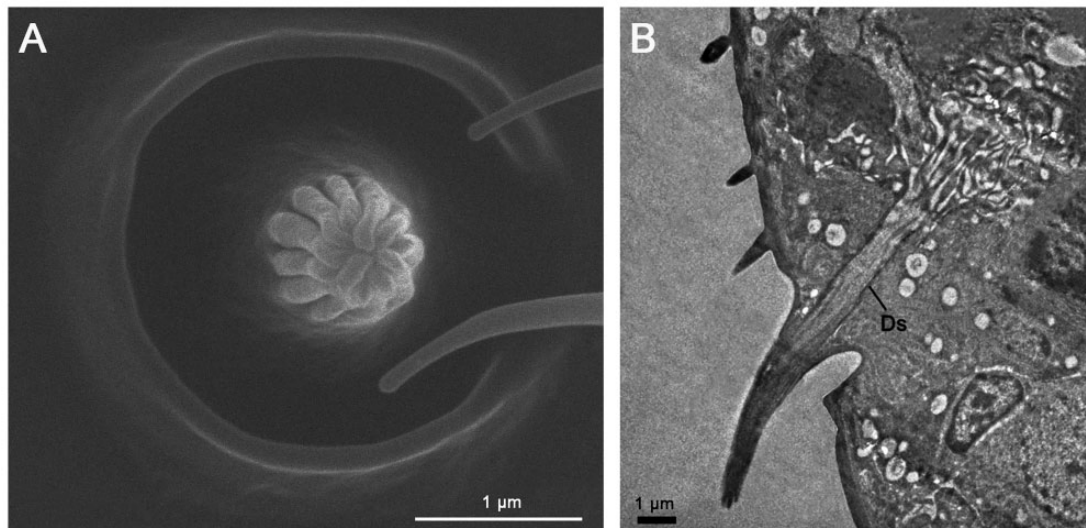


Fig. 7. SEM (A) and TEM (B) micrographs of type 1 sensilla coeloconica. (A) Whole sensillum showing the nummular sunken socket and eleven finger-like projections. (B) Longitudinal section showing the thin wall and four to five dendrites that extended from the base to the distal tip and were enclosed by a dendrite sheath (Ds).

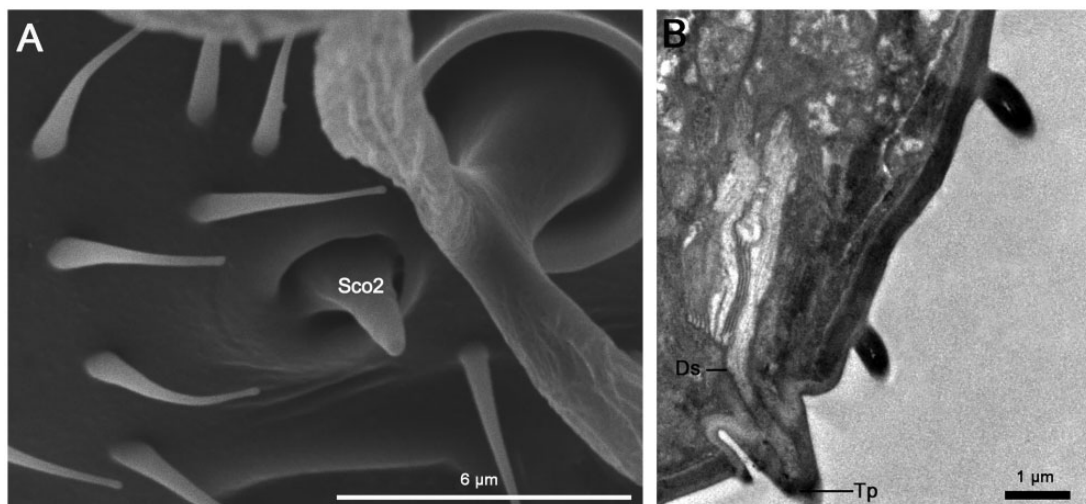


Fig. 8. SEM (A) and TEM (B) micrographs of type 2 sensilla coeloconica (Sco2). (A) Entire sensillum showing the nummular sunken socket, smooth surface and rounded tip. (B) Longitudinal section showing the thick wall, a terminal pore (Tp) and dendrites which extended from the base to the distal tip and were enclosed by a dendrite sheath (Ds).

C. pisi (Hillbur et al. 2001), *A. aphidimyza* (Zhang and Yang 2008), and *C. nasturtii* (Boddum et al. 2010). In contrast, in *D. tetensi* and *M. destructor*, s. circumfila are attached to antennal surface in both sexes (Crook and Mordue 1999, Boddum et al. 2010). It has been suggested that s. circumfila are involved in pheromone detection in species where male s. circumfila are enlarged as shown in *C. nasturtii* using single sensillum recording (Boddum et al. 2010). It is thus possible that they also perform that function in male *S. mosellana*.

Distribution and shape of type 1 s. coeloconica of *S. mosellana* are similar to s. coeloconica in gall midges *C. pisi*, *C. nasturtii*, and *M. destructor* (Slifer and Sekhon 1971, Hillbur et al. 2001, Boddum et al. 2010). Morphologically, they resembled the grooved-peg sensilla reported for mosquito *Aedes aegypti* (Cribb and Jones 1995), and s. coeloconica reported for other Diptera species such as *Drosophila melanogaster* (Shanbhag et al. 1999), *Pseudacteon*

tricuspis (Chen and Fadamiro 2008), *Pales pavidus* (Liu et al. 2013), and *Protophormia terraenovae* (Setzu et al. 2011). Grooved pegs generally contain unbranched dendrites extending from the base to the distal tip, as demonstrated in this study. Majority of them also possess very small cuticular pores at the bottom of the groove, connecting the inside of sensilla via cuticular pore channels, which are often very difficult to see (Cribb and Jones 1995). Thus, we concluded that this sensillum type might be olfactory or humidity receptors, as demonstrated in previous studies (Zacharuk et al. 1980, Altner and Loftus 2003).

Type 2 s. coeloconica appear to have a similar external structure to those found in gall midges *D. tetensi* (Crook and Mordue 1999) and male mosquito *C. impunctatus* and *C. nubeculosus* (Blackwell et al. 1992). In the former studies, they were described as terminal sensory peg, as only found at the tip of the antennae. In this study,

most Sco2 were located on the antennal apex. They have a terminal pore and dendrites extending from the base to the distal tip. Sensilla with similar location and structures to Sco2 were considered to be contact chemoreceptors used to touch host plants or potential mates (Walker and Gordh 1989, Blackwell et al. 1992, Yuan et al. 2013).

In conclusion, this study has identified and characterized for the first time both morphology and ultrastructure of different sensillum types on the antennae of *S. mosellana*, and probable function of each sensillum has been suggested. Results have improved our understanding of host selection and courtship behavior of *S. mosellana* and also provide important information for future electrophysiological investigation.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31-371-933). We would like to thank Jinghong Hao for his assistance with sensilla classification. We also thank Rongrong Xie and Wu Dai for their suggestions and comments on this manuscript.

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