Antennal Morphology and Ultrastructure of *Holotrichia parallela* (Coleoptera: Scarabaeidae)[1]

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Abstract Antennal morphology and type and distribution of antennal sensilla of *Holotrichia parallela* (Motschulsky) (Coleoptera: Scarabaeidae) were determined using scanning electron microscopy and transmission electron microscopy. *Holotrichia parallela* antennae were a typical lamellate type, including a basal scape, a pedicel, a flagellum, and a lamellar club composed of the three terminal flagellomeres. The antennae of males were slightly longer than those of females. Twenty-two subtypes of sensilla were identified on the outer and inner surfaces of the lamellae in both sexes: three sensilla trichodea (TRS), three sensilla chaetica (CHS), four sensilla placodea (PLAS), two sensilla coeloconica (COS), six sensilla auricilica (AUS), one Böhm's bristle (BB), and three sensilla basiconica (BAS). TRS, CHS, and BB were distributed primarily on the outer surfaces of the antennae as mechanoreceptor sensilla, while PLAS, COS, AUS, and BAS were concentrated on the inner surfaces of the lamellae as chemoreceptors. PLAS and BAS were more abundant in males than in females, but females had more COS and AUS. PLAS were distributed along the lamellar periphery, while COS, BAS, and AUS were distributed in the center of the lamellae.

Key Words *Holotrichia parallela*, antennal sensilla, ultrastructure, scanning electron microscopy, transmission electron microscopy

Holotrichia parallela (Motschulsky) (Coleoptera: Scarabaeidae) is an economically important pest of many agricultural crops in China (Wang et al. 2017). Adults feed on the leaves, flowers, and fruits of crops, while the larvae attack the roots and other underground parts of crop plants, resulting in reduced quality and yield and even plant death (Yi et al. 2018). Recent studies have focused on the functional characteristics of chemosensory proteins (Wang et al. 2017, Yi et al. 2018), as well as electrophysiology and behavior (Zhou et al. 2009). Very little is known about the structural and functional characteristics of *H. parallela* antennal sensilla. Our objective in the study reported herein was to describe the morphology, types, and ultrastructure of the antennal sensilla of *H. parallela* with the intent to propose their putative functions.

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Materials and Methods

Adult *H. parallela* were obtained from the Anhui Academy of Agricultural Sciences (Hefei City, P.R. China). They were held in plastic containers (100 × 50 cm) containing damp soil (10**%** moisture) at 25°C, 80**%** RH, and on a 12L:12D h photoperiod) and supplied with fresh Chinese elm, *Ulmus parvifolia* Jacquin, leaves until used for microscopic examination.

Scanning electron microscopy. For scanning electron microscopy (SEM), the antennae from males and females were carefully excised from the antennal bases with dissection scissors and immediately placed in 70% alcohol. In addition, the three lamellae forming each antennal club were separated according to sex and lamellar side (distal or proximal surface) (Romero-López et al. 2017). The lamellae were cleaned in 70% ethanol for 5 s using an ultrasonic wave cleaner. Then the lamellae were dehydrated through a graded alcohol series of 80%, 90%, 95%, and 100% for 20 min each (Al-Dosary 2009). The antennae were mounted on aluminum stubs with double-sided sticky tape, dried for 6 h in a vacuum evaporating chamber, successively coated with gold/palladium, and then observed with a FEI Quanta 200 SEM (Field Electron and Ion Co., Hillsboro, OR) at 15 kV.

Transmission electron microscopy. For transmission electron microscopy (TEM), the antennae of *H. parallela* were cut through the base of the scape, placed in 70% ethanol, and sonicated for 5 s using an ultrasonic wave cleaner. They were fixed in phosphate buffer with 4% paraformaldehyde, 2% glutaraldehyde, and 5% sucrose (0.1 M, pH 7.4) for 12 h. After washing with phosphate buffer five times, the antennae were postfixed in 2% OsO_4 in phosphate buffer (0.1 M, pH 7.4). The samples were dehydrated through a graded ethanol series and 100% acetone. The fixed antennae were embedded in the epoxy resin Epon-812 by epoxy propylene. The resin blocks were then sectioned using a LKB-III ultramicrotome (LKB-Produkter A.B., Broma, Sweden) and placed on copper grids coated with Formvar resin. The sections were observed with a Hitachi H-7500 TEM (Hitachi Ltd., Tokyo, Japan).

Analyses. Classification of sensilla types was based primarily on the morphological characters described by Schneider (1964) and Zacharuk (1980). We also referred to classifications reported in other studies of antennal sensilla of Coleoptera and Scarabaeidae (Kim and Leal 2000; Mutis et al. 2014; Ochieng et al. 2002; Romero-López et al. 2010, 2017). Images were processed with Adobe Photoshop CS4. The length of antennae of each sex and the dimensions and numbers of the different types of sensilla were measured. Data were subjected to a *t*-test and one-way analysis of variance with Tukey's HSD multiple comparisons using software SPSS 13.0 (Statistical Package for the Social Sciences, Chicago, IL).

Results and Discussion

Antennal morphology. *Holotrichia parallela* has a typical lamellate scarab antenna, composed of three segments—scape, pedicel, and flagellum—which is similar to other scarab beetles (Al-Dosary 2009, Mutis et al. 2014, Zauli et al. 2016). The flagellum consists of five flagellomeres (F1 to F5) and three terminal plates,

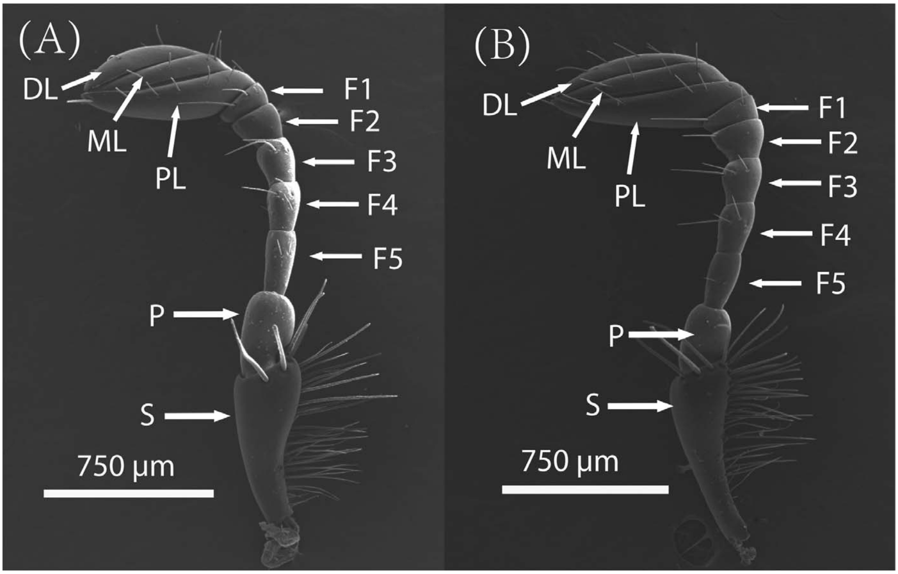

Fig. 1. Antennae of (A) male and (B) female *H. parallela*. Abbreviations: S, scape; P, pedicel; F, flagellomere; DL, distal lamella; ML, middle lamella; PL, proximal lamella.

which are the proximal lamella, middle lamella, and distal lamella (Fig. 1). The antennae of males were 3,063 μm in length and were not significantly longer ($t = 1.742$; df = 18; $P > 0.05$) than those of females, which measured 2,949 μm (Table 1). Differences in antenna shape were not observed, even in the length of antennal segments, indicating that both male and female antennae undergo frequent chemical communication (Romero-López et al. 2010).

Sensilla types. Twenty-two subtypes of sensilla were observed on the outer and inner surfaces of the lamellae in both sexes: three sensilla trichodea (TRS), three sensilla chaetica (CHS), four sensilla placodea (PLAS), two sensilla coeloconica (COS), six sensilla auricilica (AUS), one Böhm's bristle (BB), and three sensilla basiconica (BAS). TRS, CHS, and BB were widely distributed on the outer surfaces of the antennae as mechanoreceptors, while PLAS, AUS, COS, and BAS were all concentrated on the inner surfaces of the lamellae, which are considered the main sensilla involved in chemoreception (Mutis et al. 2014, Romero-López et al. 2010, Schneider 1964).

Sensilla trichodea. TRS are hairlike sensilla, elongated and tapered, which can be straight or curved with many cuticular projections on the surface. TRS subtype 1 (TRS1) and subtype 2 (TRS2) are distributed on the distal sides of the scape, with a tendency for increasing hair length toward the apex of the scape (Fig. 2C). TRS1 were significantly longer than TRS2 (350 versus 185 μm; $F = 307.7$; df = 2, 57; $P < 0.01$) (Table 2). Subtype 3 (TRS3) (as Ch.5 in *Osmoderma eremita* (Scopoli)) mainly appear on the outer surfaces of the lamellae (Fig. 2E, F) and may function to

Table 1. Mean (± SE) length (μm) of the antennal segments of *H. parallela* by sex ($n = 10$).

Sex	Scape	Pedicel	F1*	F2*	F3*	F4*	F5*	Lamella	Total**
♀	886 ± 18	290 ± 31	257 ± 39	235 ± 27	201 ± 23	161 ± 21	111 ± 21	808 ± 47	2,949 ± 191a
♂	940 ± 16	301 ± 24	261 ± 24	230 ± 10	206 ± 10	166 ± 15	116 ± 9	843 ± 33	3,063 ± 81a

* F1–F5, flagellomeres 1–5.

** Mean length between sex pairs followed by the same lowercase letter are not significantly different (*t*-test, $P = 0.05$).

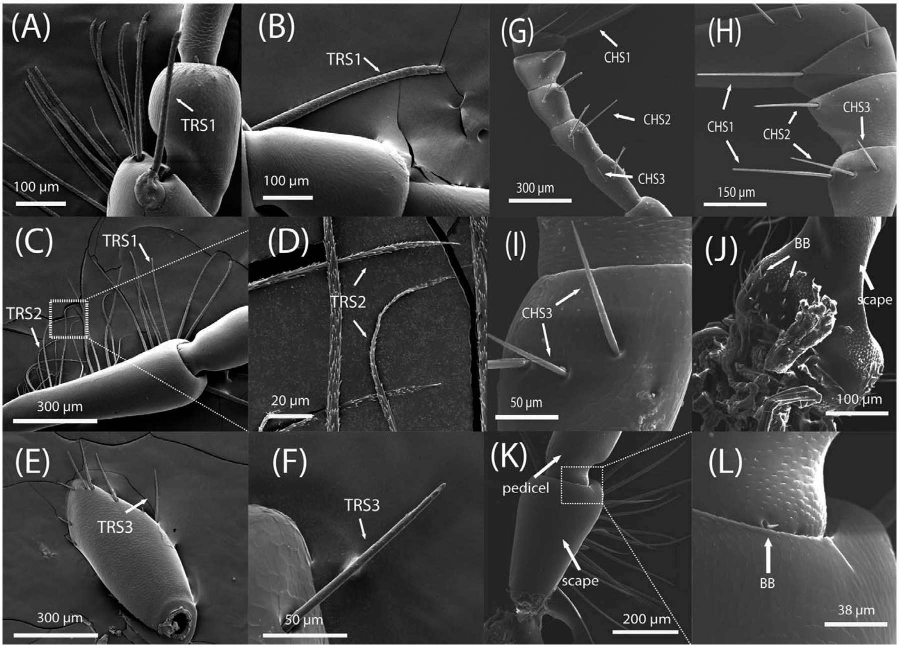

Fig. 2. Ultrastructural morphology of sensilla trichodea (TRS1, TRS2, TRS3) (images A-F), sensilla chaetica (CHS1, CHS2, CHS3) (images G-J), and Böhm's bristles (BB) on the outer surfaces of *H. parallela* antennae.

open lamellae, exposing their inner surface to olfactory cues (Zauli et al. 2016). TRS on insect antennae of many coleopteran families, such as Curculionidae, Cerambycidae, and Scolytidae, are known as pheromone receptors (Peng et al. 2018). However, TRS has putative mechanosensorial functions in Scarabaeidae, such as touch, sound, air currents, and gravity (Mutis et al. 2014, Ochieng et al. 2002).

Sensilla chaetica. CHS are divided into three subtypes—CHS1, CHS2, and CHS3—which are straight, spinelike bristles on flagellomeres (Fig. 2G, H). The basal diameter of CHS1 and CHS2 were similar (approximately 8 μm), but the length of CHS1 (296 μm) was much longer than CHS2 (170 μm) and CHS3 (59 μm) ($F = 341.8$; df $= 2, 57$; $P < 0.01$) (Table 2). The abundance and position of CHS (as Ch.3 in *O. eremita*) in the flagellomeres suggest that they have a role in the proprioception of the antenna position and movement (Mutis et al. 2014, Zauli et al. 2016). CHS are associated with direct mechanical contact but are also involved in regulation of body position (Ochieng et al. 2002).

Böhm's bristles. BB, described for the first time in Lepidoptera (Böhm 1911), display a thornlike shape with sharp tip. They were present at the base of both the scape and pedicel of *H. parallela* as expected and were 17 μm in length and 3 μm in diameter (Fig. 2J, L; Table 2). Their position suggests they might be mechanical receptors, which could sense antennal position and movement (Schneider 1964, Zauli et al. 2016).

Table 2. Characteristics and mean (± SE) dimensions of sensilla trichodea (TRS), sensilla chaetica (CHS), and Böhm's bristles (BB) on the antennal surface of *H. parallela* ($n = 20$).

Type of Sensilla	Length* (μm)	Basal Diameter (μm)	Number	Tip	Shape	Basal Socket	Location
TRS1	350.25 ± 24.89a	18.59 ± 2.98	14.1 ± 2.31	Tapered or blunt	Straight or curved	Wide	Scape
TRS2	185.37 ± 38.93b	6.29 ± 1.83	18.3 ± 3.25	Sharp	Straight or curved	Narrow	Scape
TRS3	130.31 ± 20.83c	7.05 ± 1.19	7.1 ± 1.21	Sharp or blunt	Straight	Wide	Lamellae
CHS1	286.72 ± 37.52a	8.70 ± 0.80	2.75 ± 0.64	Sharp	Straight	Narrow	Flagellum
CHS2	166.97 ± 24.20b	8.14 ± 0.76	2.0 ± 0.65	Sharp	Straight	Narrow	Flagellum
CHS3	58.62 ± 17.06c	5.71 ± 1.47	15.2 ± 2.19	Sharp	Straight	Narrow	Flagellum, lamellae
BB	16.90 ± 2.57	3.28 ± 0.40	>20	Sharp	Straight	Narrow	Scape, pedicel

* Mean length among different subtypes of the same sensilla were compared by one-way ANOVA ($P = 0.05$); means followed by different lowercase letters are significantly different (Tukey's HSD multiple comparisons).

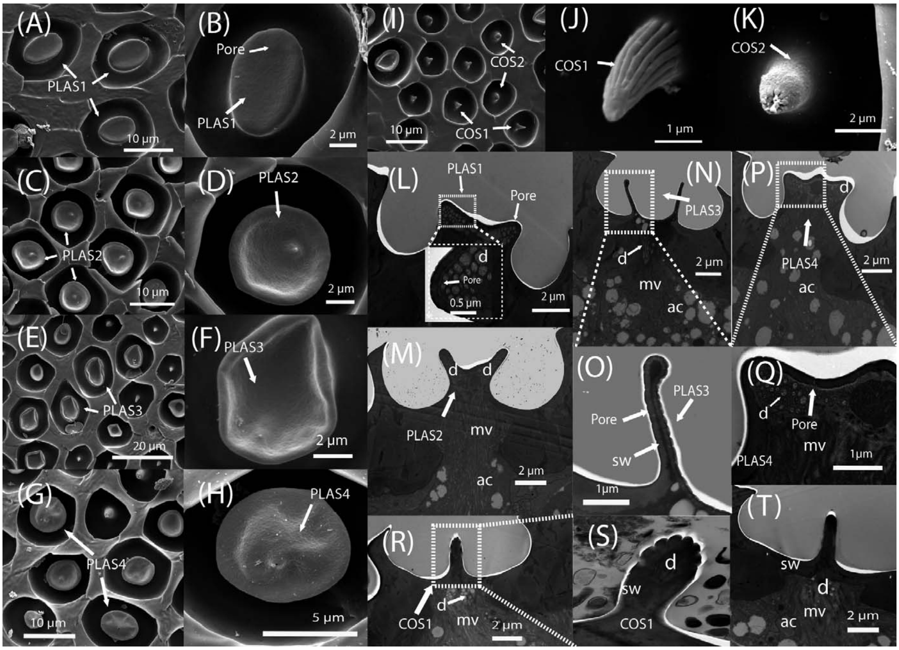

Fig. 3. Ultrastructural morphology of sensilla placodea (PLAS) (images A-H) and sensilla coeloconica (COS) (images I-T) on the inner surfaces of *H. parallela* antennal lamellae. Pore, dendrites (d), accessory cell (ac), microvilli (mv) and sensillum wall (sw) indicated on selected images.

Sensilla placodea. PLAS are spherical or elliptical, thin-walled plates, which are divided into four different subtypes. PLAS1 are flat or slightly concave, and PLAS4 are round upwardly convex-shaped discoidal plates (Fig. 3B, H). PLAS2 are sunken plates shaped like the letter "Y" (Fig. 3M), and PLAS3 are sunken into "bowl" shape (Fig. 3N). SEM showed multiple pores across the sensillum wall, and electrophysiological studies indicated that PLAS housed olfactory receptor neurons specific for sex pheromones (Kim and Leal 2000).

Sensilla coeloconica. Two subtypes of COS were found on the internal surfaces of all lamellae: COS1 and COS2. COS1 has a wall consisting of a pyramid-shaped palisade of cuticular fingers (Fig. 3J), meeting each other at the tip; whereas, COS2 has fingerlike structures on the distal portion (Fig. 3K). COS are short (2.5 μm for COS1 and 2.2 μm for COS2 [Table 3]), significantly different in length ($t = 2.336$; df $= 38$; $P < 0.05$), and are anchored in a pit. In some scarab beetles, COS have been suggested to possess an olfactory function (Kim and Leal 2000, Ochieng et al. 2002). Kim and Leal (2000) excluded the possibility of these being pheromone receptors, so they could be involved in the reception of plant volatiles (Kim and Leal 2000, Romero-López et al. 2004).

Table 3. Mean (± SE) dimensions of sensilla placodea (PLAS), sensilla coeloconica (COS)*, sensilla auricilica (AUS), and sensilla basiconica (BAS) on the inner surfaces of *H. parallela* antennal lamellae ($n = 20$).

Type of Sensilla	Length (μm)	Basal Diameter (μm)	Diameter of Pit
COS1	2.50 ± 0.41a	1.23 ± 0.13	8.65 ± 0.35
COS2	2.21 ± 0.40b	2.04 ± 0.26	8.02 ± 0.50
PLAS1	7.61 ± 0.35	—**	13.41 ± 0.58
PLAS2	6.59 ± 0.84	—	11.24 ± 0.82
PLAS3	7.39 ± 0.90	—	12.92 ± 0.75
PLAS4	6.32 ± 1.05	—	12.10 ± 0.99
AUS1	6.06 ± 0.39	—	12.08 ± 0.58
AUS2	4.45 ± 0.67	—	11.80 ± 1.03
AUS3	4.01 ± 0.24	—	10.57 ± 1.46
AUS4	6.82 ± 0.80	—	16.14 ± 1.71
AUS5	3.07 ± 0.36	—	10.84 ± 1.10
AUS6	3.24 ± 0.36	—	10.02 ± 0.97
BAS1	3.30 ± 0.40	1.86 ± 0.12	8.06 ± 0.78
BAS2	5.78 ± 0.81	2.35 ± 0.17	8.42 ± 0.76

* Mean length between different subtypes of COS were compared by *t*-test ($P = 0.05$); means followed by different lowercase letters are significantly different.
** Not applicable.

Sensilla auricilica. Six AUS subtypes are distinguished by shape. AUS1 are a "heart" or "peach" shape with a deeply sunken plate; AUS2 have a fusiform shape with a narrow base; AUS3 have a "rabbit-ear" shape, while AUS4 have a "human-ear" shape; AUS5 are characterized by a "rabbit-ear" shape; and AUS6 are "tongue"-shaped structures (Fig. 4A–L). The role of AUS, previously described as a special type of PLAS, has been suggested to involve sexual attraction (Ochieng et al. 2002, Romero-López et al. 2010). Similar to PLAS, AUS also possess thin-walled plates with multiple pores, implying that AUS can efficiently receive chemicals. However, the number of AUS in females is higher than that in males, increasing the probability of a role in the recognition of plant volatiles, or receiving sex pheromones, complementing the PLAS in females.

Sensilla basiconica. BAS1 and BAS2 are large and cone-shaped sensilla. However, BAS1 have a smooth surface, while BAS2 have a group of cuticular ridges that resemble fingers at the top (Fig. 4N, P). Other BAS subtypes are found

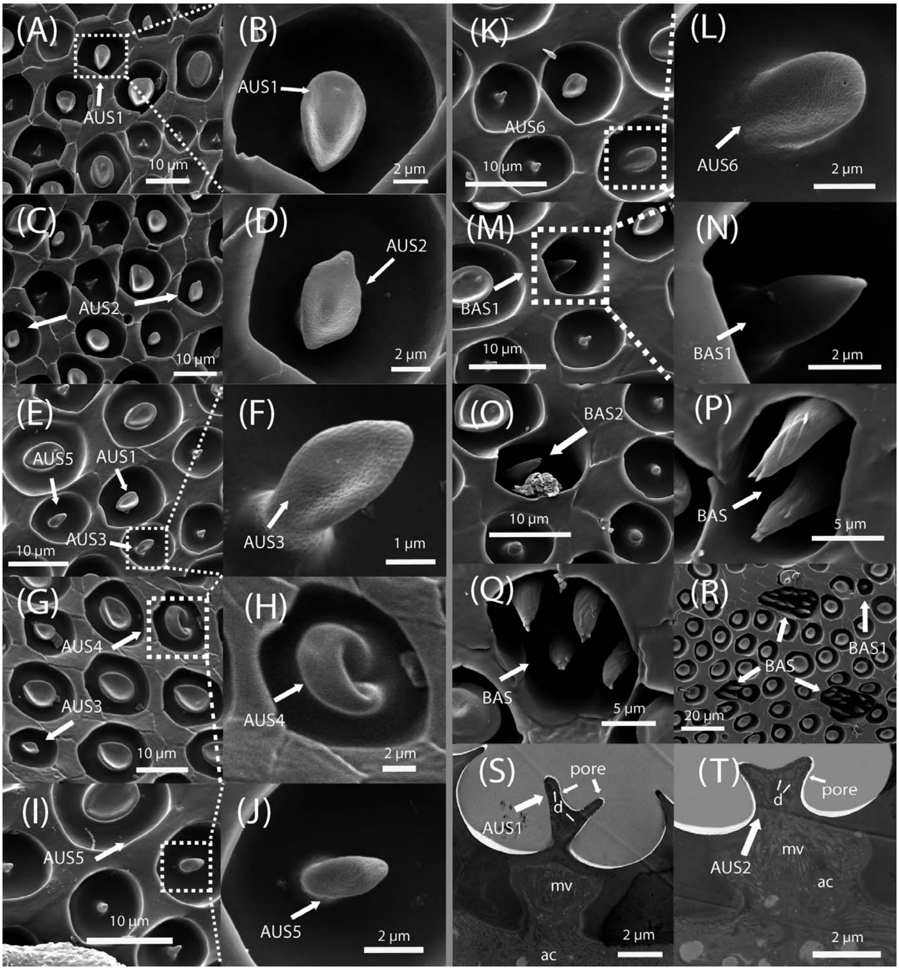

Fig. 4. Ultrastructural morphology of sensilla auricilica (AUS) (images A-L) and sensilla basiconica (BAS) (images M-T) on the inner surfaces of *H. parallela* antennal lamellae. Pore, dendrites (d), accessory cell (ac), microvilli (mv) and sensillum wall (sw) indicated in images S and T.

as aggregations of two or more BAS2 (as COS I–COS IV in *Phyllophaga ravida* (Blanchard)) (Romero-López et al. 2010).

Gender differences. Significant differences were found between the two sexes in the abundance and distribution of sensilla in lamellae (Table 4). PLAS ($t = 10.85$; df = 18; $P < 0.01$) and BAS ($t = 6.021$; df = 18; $P < 0.01$) were more abundant in males than in females, while females had more COS ($t = 3.063$; df = 18; $P < 0.01$) and AUS ($t = 7.221$; df = 18; $P <0 .01$). PLAS were the most abundant morphological type in both males and females, suggesting both sexes are attracted

Table 4. Mean (± SE) number of sensilla on the inner surfaces of the antennal lamellae* in both sexes of *H. parallela*.**

Sensilla†	Sex	DL	MLD	MLP	PL	Total
PLAS	♂	316 ± 38B	269 ± 12C	382 ± 15A	331 ± 33B	1,299 ± 39a
	♀	198 ± 35B	215 ± 13B	362 ± 14A	339 ± 25A	1,113 ± 38b
COS	♂	138 ± 16B	71 ± 23C	128 ± 39B	212 ± 11A	549 ± 42a
	♀	177 ± 15B	87 ± 10D	149 ± 28C	202 ± 19A	614 ± 53b
AUS	♂	68 ± 6B	62 ± 7B	105 ± 9A	40 ± 19C	275 ± 25a
	♀	71 ± 10C	76 ± 7BC	110 ± 14A	85 ± 11B	343 ± 16b
BAS	♂	21 ± 4A	5 ± 2B	—	6 ± 2B	31 ± 4a
	♀	11 ± 3A	5 ± 2B	—	5 ± 2B	21 ± 3b

* DL, distal lamella; MLD, distal surface of middle lamella; MLP, proximal surface of middle lamella; PL, proximal lamella.

** Mean numbers of each sensilla type on a lamellar surface were compared between sexes (*t*-test, $P = 0.05$, $n = 10$); paired means followed by the same lowercase letter are not significantly different. Mean numbers of the same sensilla on the same sex were compared among the lamellar locations by one-way ANOVA ($P = 0.05$; $n = 10$); means followed by the same uppercase letter are not significantly different (Tukey's HSD multiple comparisons).

† PLAS, sensilla placodea; COS, sensilla coeloconica; AUS, sensilla auricilica; BAS, sensilla basiconica.

by pheromone compounds. The abundance of PLAS was significantly greater in males than in females, similar to previous reports in other beetles, reflecting mate-searching behavior of males (Kim and Leal 2000). COS were significantly more abundant in females than in males, suggesting that COS could be involved in the reception of plant volatiles (Kim and Leal 2000, Romero-López et al. 2004).

PLAS were randomly scattered on lamellae along the lamellar periphery, while COS, AUS, and BAS were mainly distributed in the centers of lamellae (Fig. 5). This demonstrates that the lamellae of *H. parallela* open slightly to recognize sex pheromones and open widely to recognize plant volatiles, exposing more sensilla, especially COS. PLAS were mainly distributed in the proximal surfaces of middle lamellae in both sexes (♂: $F = 29.92$; df = 3, 36; $P < 0.0001$; ♀: $F = 125.1$; df = 3, 36; $P < 0.0001$), as were AUS (♂: $F = 55.68$; df = 3, 36; $P < 0.0001$; ♀: $F = 26.56$; df = 3, 36; $P < 0.0001$). COS were mainly distributed in the proximal lamellae in both sexes (♂: $F = 56.27$; df = 3, 36; $P < 0$ .0001; ♀: $F = 65.68$; df = 3, 36; $P < 0.0001$). BAS were mainly distributed in the distal lamellae in both sexes (♂: $F = 108.5$; df = 2, 27; $P < 0.0001$; ♀: $F = 29.39$; df = 2, 27; $P < 0.0001$).

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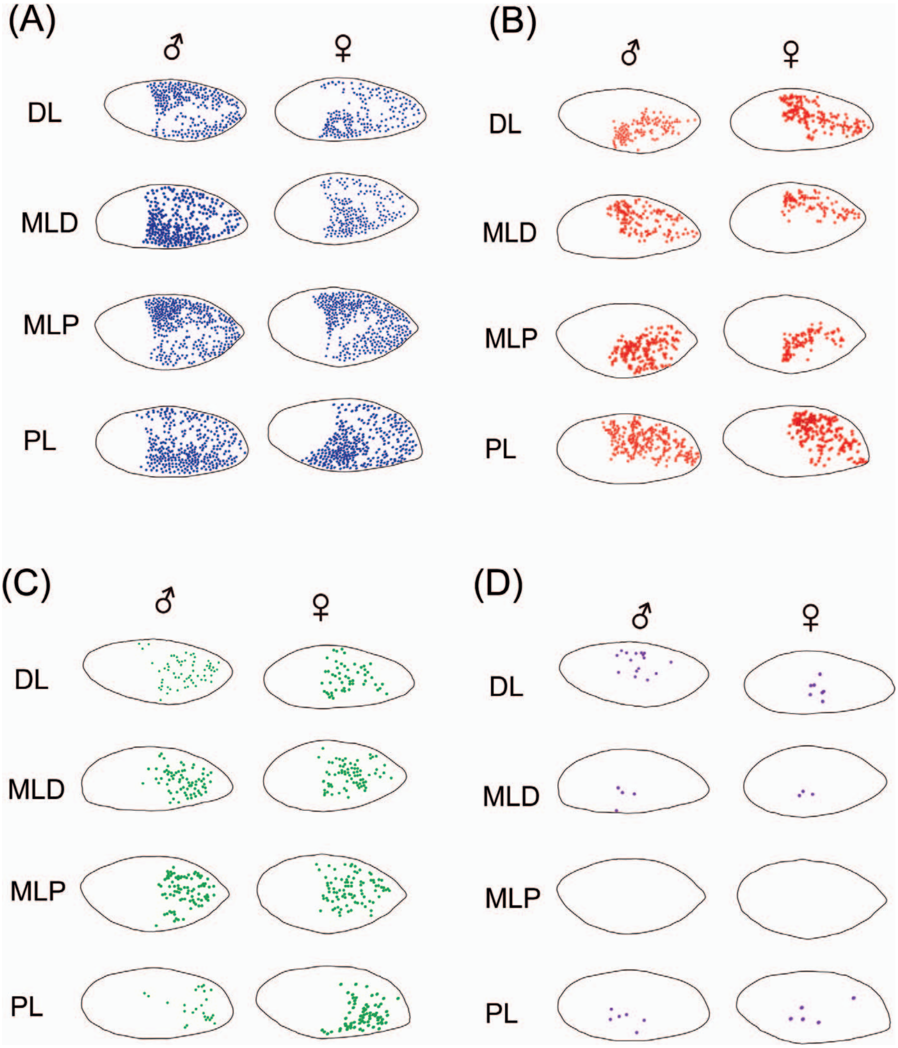

Fig. 5. **Distribution of sensilla on the inner surfaces of the lamellae in both sexes of *H. parallela*. (A) Sensilla placodea (PLAS); (B) sensilla coeloconica (COS); (C) sensilla auricilica (AUS); (D) sensilla basiconica (BAS). Abbreviations: DL, distal lamella; MLD, distal surface of middle lamella; MLP, proximal surface of middle lamella; PL, proximal lamella.**

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