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Authors: Zhou, Chang-Xiang, Sun, Xiao, Mi, Feng, Chen, Jingyuan, and Wang, Man-Qun

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#### Research

# Antennal Sensilla in the Parasitoid Sclerodermus sp. (Hymenoptera: Bethylidae)

Chang-Xiang Zhou, <sup>1</sup> Xiao Sun, <sup>1</sup> Feng Mi, <sup>1</sup> Jingyuan Chen, <sup>2</sup> and Man-Qun Wang <sup>1,3</sup>

<sup>1</sup>Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

<sup>2</sup>Hubei Academy of Forestry, Wuhan 430075, People's Republic of China

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ABSTRACT. Parasitoid wasps of the genus *Sclerodermus* (Hymenoptera: Bethylidae) are an important natural enemy of the Japanese pine sawyer beetle *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). In this study, we used scanning electron microscopy to examine the external morphology of the antennal sensilla of *Sclerodermus* sp. Antennae of females and males comprised the scape, pedicel, and 11 flagellomere segments. Based on the morphology of the sensilla in each sex, seven types of sensillum were identified: sensilla trichodea (Tr.1, Tr.2 and Tr.3), sensilla basiconica (Ba.1, Ba.2, and Ba.3), sensilla styloconica (St.1 and St.2), sensilla placodea, sensilla coeloconica, sensilla squamiforma, and Bohm's bristles. Tr.2, Ba.1, and St.1 were only found in females, whereas Ba.2, Ba.3, and St.2 were only observed in males. Sensilla placodea were the most common, given that they occur on the antennae of many parasitoid Hymenoptera, whereas sensilla Tr were the most abundant, being distributed over the entire antennal surface. These sensilla are likely to have roles in the host locating and habitat searching behavior of adult *Sclerodermus* wasps. Therefore, our findings provide a basis for further studies of the host location behavior of this and other species of parasitic wasp.

Key Words: Sclerodermus sp., Monochamus alternatus, antennal sensilla, parasitoid, scanning electron microscopy.

The Japanese pine sawyer beetle, Monochamus alternatus Hope (Coleoptera: Cerambycidae), is an important insect vector of the pine wood nematode Bursaphelenchus xylophilus Steiner et Buhrer in Asia, which is the causal agent of pine wilt disease (Morimoto and Iwasaki 1972, Linit 1988, Arakawa and Togashi 2002). Many studies have suggested that an effective way to control pine wilt disease is to control the pine wood nematode by reducing the number of its beetle host (Yang 2004, Togashi 2008). In addition to intensified quarantine efforts, tree removal, and phytosanitary measures using methyl bromide fumigation, biological control primarily with the parasitic wasp Scleroderma guani, has shown great promise in combating pine wilt disease through the suppression of the Japanese pine sawyer beetle (Xu et al. 2008). The parasitoid wasp *Sclerodermus* sp. (Hymenoptera: Bethylidae), which has been newly discovered in Yunnan Province, China, is an important natural enemy of M. alternatus. This Sclerodermus sp. can not only parasitize pine sawyer larvae, but also kill them as for supplementary food (Zhang et al. 2012). However, little is known about the host location mechanisms of Sclerodermus sp.

On insect antennae, there are various kinds of sensillum with different functions that have important roles throughout the life history of the insect (Schneider 1964). Parasitic hymenopterans have specialized sensory organs, such as antennal sensilla, to facilitate their biological behaviors, such as habitat searching, host localization, recognition, selection, and acceptance, courtship, mating and oviposition (Das et al. 2011). To better understand the chemical communication of these potential biological control agents, the ultrastructure of antennal sensilla has been studied extensively using electron microscopy techniques in various species of parasitoid Hymenoptera (Norton and Vinson 1974, Le Rü et al. 1995, Amornsak et al. 1998, Cônsoli et al. 1999, van Baaren et al. 1999, Bleeker et al. 2004, Roux et al. 2005, Gao et al. 2007, Rocha et al. 2007, Onagbola and Fadamiro 2008, Dweck 2009, Onagbola et al. 2009, Wang et al. 2010, Das et al. 2011, Li et al. 2011).

To understand the host location behavior and mechanisms of *Sclerodermus* sp., the antennal morphology, antennal sensillum morphology and their distribution in both male and female adult wasps

were observed using a scanning electron microscope (SEM). This study provides a basis for future electrophysiological and behavioral studies.

#### **Materials and Methods**

**Insects.** Adult male and female *Sclerodermus* sp. were supplied by the Chinese Academy of Forestry Science and were reared under laboratory conditions [25°C, 75% relative humidity, and a photoperiod of 16:8 (L:D) h].

**Scanning Electron Microscope.** In total, 10 females and 10 males were used for the SEM analysis. Before the SEM analysis, each head with antennae was removed and fixed in 2.5% glutaraldehyde (pH 7.2) at 4°C for 24 h. The heads were then cleaned three times in an ultrasonic wave cleaner for 15 s. The antennae were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%) for 20 min each and then fully dehydrated twice in 100% ethanol solution (for 30 min each). Following drying to the critical point, the antennae were mounted on a holder using double-sided adhesive tape and were sputter-coated with gold (40:60). A Hitachi jsm-63901 (Tokyo, Japan) SEM was used to take micrographs from different locations of the antennal surface at 20 kV

**Data Analysis.** Sensilla on each segment of the *Sclerodermus* antennae were identified, measured, and counted. Classification of the sensilla was based on the terminology of Schneider (1964). The length and width of each segment, and the length and width at the base of each sensillum type were measured. Measurements obtained from micrographs of at least 10 individuals were used to calculate the means. Significant differences in the number, length and width between males and females were determined using the Student *t*-test.

#### Results

**General Description of the Antennae.** The antennae of *Sclerodermus* sp. were geniculate in shape and comprised three regions: an elongated proximal scape, a medial pedicel, and a

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<sup>&</sup>lt;sup>3</sup>Corresponding author, e-mail: mqwang@mail.hzau.edu.cn

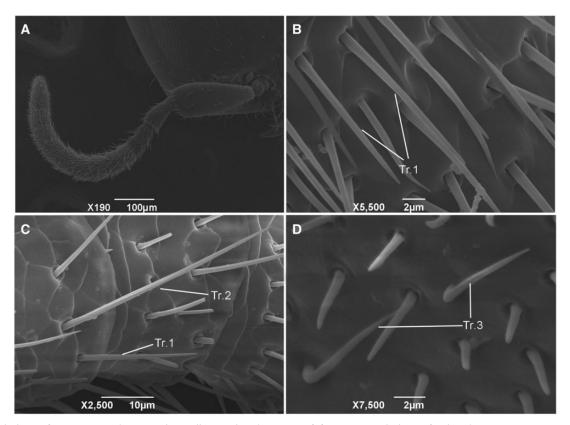


Fig. 1. Morphology of antennae and antennal sensilla in *Sclerodermus* sp. (A) Gross morphology of *Sclerodermus* sp. antenna, showing the scape, pedicel, and 11 flagellomeres. (B) Type 1 sensilla trichodea (Tr.1). (C) Type 1 and type 2 sensilla trichodea (Tr.1 and Tr.2). (D) Type 3 sensilla trichodea (Tr.3).

multi-segmented distal flagellum (Fig. 1A). The length of antennae from the females and males was  $810.1 \pm 3.7 \, \mu m$  and  $996.7 \pm 48.6 \, \mu m$ , respectively, although there was no significant difference in the length between the sexes (P > 0.05). The scape gradually broadened toward the antennal tip and was the longest antennal segment, measuring  $200.1 \pm 9.1 \, \mu m$  and  $147.0 \pm 10.4 \, \mu m$  in females and males, respectively, with the lengths being significantly different (P < 0.05). The shorter, triangular-shaped pedicel  $[76.7 \pm 5.2 \,\mu m]$  in females and  $78.7 \pm 1.4 \,\mu\text{m}$  in males; no significant difference (P > 0.05)] linked the scape to the flagellum. The elongated flagellum comprised 11 flagellomeres and abundant sensilla. The first 10 flagellomeres were similar in size. The last flagellomere was clavate in shape, tapering to a rounded shape, and was longer than the other flagellum segments, being significantly shorter in females  $(102.3 \pm 6.8 \,\mu\text{m})$  than in males  $(128.4 \pm 5.1 \,\mu\text{m}) (P < 0.05)$ . The length and width of each segment of male and female antennae are shown in Table 1.

Morphology of Antennal Sensilla. According to the morphology of each type of sensilla, seven different sensillum types were identified in both sexes, including three morphological types of sensilla trichodea (Tr.1, Tr.2, and Tr.3), three morphological types of sensilla basiconica (Ba.1, Ba.2, and Ba.3), two morphological types of sensilla styloconica (St.1 and St.2), one sensillum placodeum (Pl), one sensillum coeloconicum (Co), one sensillum squamiformum (Sq), and Bohm's bristles (B.b). However, Tr.2, Ba.1, and St.1 were only found in the females, whereas Ba.2, Ba.3, and St.2 were only observed in the males. The other sensillum types were identified in both male and female wasps.

The average number and distribution of the different types of are shown in Table 2, whereas their characteristic morphological features are shown in Table 3.

Sensilla Trichodea. The entire antennal segments of both male and female wasps were abundantly covered with sensilla Tr. In general,

these blunt-end hair-like structures were inclined and straight or slightly curved toward the apex of the segment.

Sensilla trichodea type 1 (Tr.1) inserted into sockets that were slightly elevated above the cuticle; each sensillum had a straight, blunt tip that narrowed at the distal region, and were distributed from the scape to the 11th flagellomere in both sexes (Fig. 1B and C). The average length and basal diameter of Tr.1 were  $16.24 \pm 0.48 \, \mu m \, (n=40)$  and  $0.93 \pm 0.02 \, \mu m \, (n=40)$ , respectively. The number of Tr.1 from the second to the eighth flagellomere in females was significantly higher than in males (P < 0.05), but there were no significant differences between two sexes on the other flagellomeres (P > 0.05).

Sensilla trichodea type 2 (Tr.2) were similar to Tr.1 in terms of their structure, but were longer and thicker than Tr.1 (Fig. 1C). Tr.2 were only found in antennae from females from the scape to the 11th flagellomere, and their average length and basal diameter were  $22.19 \pm 0.84 \, \mu m \, (n=20)$  and  $1.19 \pm 0.05 \, \mu m \, (n=20)$ , respectively.

Sensilla trichodea type 3 (Tr.3) were extremely curved at the basal region, and inserted flatly against the surface of the antenna (Fig. 1D). Tr.3 were distributed on the female antennae from the ninth to the 11th flagellomere, and in the male antennae from the second to the 11th flagellomere. The average length and basal diameter of Tr.3 were  $12.36 \pm 0.36 \,\mu\text{m}$  (n = 39) and  $0.92 \pm 0.02 \,\mu\text{m}$  (n = 39), respectively. There was no significant difference in the number of Tr.3 between males and females on the last three segments (P > 0.05).

Sensilla Basiconica. Compared with the other types of sensilla Tr, the base of each sensilla Ba was on a basal ring. Based on the differences in their morphological features, three types of these sensilla were identified.

Sensilla basiconica type 1 (Ba.1) had a thumb-like shape and were characterized by a grooved surface, which projected perpendicularly to the axis of the antennae (Fig. 2A). Their spatial arrangement followed a specific pattern on the last flagellomere (Fig. 2B), and there were 10

Table 1. Length and basal diameter of each segment of male and female Sclerodermus sp. Antennae

|  |   | Scape           | Pedicel        | f1             | f2             | f3             | f4             | f5             | f6             | f7             | f8             | f9             | f10            | f11             |
|--|---|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| Length   | М | 147.0 ± 10.4    | 78.7 ± 1.4     | 49.3 ± 1.3     | 48.7 ± 3.5     | 55.5 ± 5.4     | 65.0 ± 4.6     | 63.8 ± 4.1     | 65.4 ± 3.5     | 64.1 ± 3.2     | 58.7 ± 5.0     | 62.2 ± 6.0     | 58.0 ± 6.9     | 128.4 ± 5.1     |
| (μm)   | F | $200.1 \pm 9.1$ | $76.7 \pm 5.2$ | $39.8 \pm 2.6$ | $33.0 \pm 1.0$ | $37.2 \pm 0.4$ | $37.1 \pm 1.6$ | $39.3 \pm 2.5$ | $42.9 \pm 1.9$ | $43.4 \pm 6.3$ | $38.1 \pm 4.2$ | $41.6 \pm 4.7$ | $43.1\pm6.6$   | $102.3 \pm 6.8$ |
| Diameter   | M | $70.1 \pm 4.9$  | $53.4 \pm 1.1$ | $42.6 \pm 1.6$ | $48.2\pm2.0$   | $51.2 \pm 1.4$ | $53.2 \pm 1.2$ | $51.8\pm0.7$   | $50.5 \pm 1.6$ | $49.6 \pm 1.5$ | $47.4 \pm 1.2$ | $47.7 \pm 1.4$ | $44.9 \pm 1.2$ | $42.3 \pm 0.7$  |
| (µm)   | F | $69.1 \pm 9.5$  | $47.4\pm0.6$   | $39.2\pm1.4$   | $45.0\pm2.3$   | $47.9 \pm 1.9$ | $51.7\pm1.8$   | $59.1 \pm 1.1$ | $57.8\pm0.5$   | $58.6\pm1.7$   | $55.1 \pm 1.1$ | $51.3\pm0.3$   | $49.3\pm1.1$   | $47.3 \pm 0.4$  |
| Note: values of length and diameter are mean ( $\pm$ standard deviation) length and diameter of 10 antennae. f1–f2 = flagellomere 1–9. |   |                 |                |                |                |                |                |                |                |                |                |                |                |                 |

Table 2. Numbers and distribution of the antennal sensilla of Sclerodermus sp.

| Туре | Gender         | Scape                    | Pedicel        | Flagellomeres           |                |                |                |                           |                          |                                 |                 |                                |                |                          |
|------|----------------|--------------------------|----------------|-------------------------|----------------|----------------|----------------|---------------------------|--------------------------|---------------------------------|-----------------|--------------------------------|----------------|--------------------------|
|      |                |                          |                | 1                       | 2              | 3              | 4              | 5                         | 6                        | 7                               | 8               | 9                              | 10             | 11                       |
| Tr.1 | Male<br>Female | 47.0 ± 4.5<br>24.0 ± 3.4 |                | 8.8 ± 0.5<br>11.5 ± 2.2 |                |                |                | 22.4 ± 3.5<br>95.0 ± 15.0 | 19.6 ± 6.6<br>84 0 + 4 2 |                                 |                 | 23.6 ± 4.7<br>42.0 ± 9.9       |                | 42.0 ± 8.0<br>74.7 ± 8.7 |
| Tr.2 | Male           | -                        | -              | -                       | -              | -              | -              | -                         | -                        | _                               | _               | _                              | -              | _                        |
|      | Female         | $43.3 \pm 5.2$           | $22.0 \pm 6.5$ | $22.0 \pm 2.6$          | 24.0 ± 2.9     | $21.0 \pm 1.0$ |                |                           |                          | $46.0 \pm 6.0$                  | $33.0 \pm 7.0$  |                                | $32.0 \pm 2.0$ | $101.3 \pm 8.7$          |
| Tr.3 | Male           | _                        | -              | _                       | $6.0 \pm 5.2$  | $8.7 \pm 0.7$  | $16.0 \pm 6.1$ | $14.0 \pm 4.0$            | $12.0 \pm 0.0$           | $11.3 \pm 1.8$                  | $14.0 \pm 2.3$  | $11.3 \pm 1.3$                 |                | $24.0 \pm 4.2$           |
| D 4  | female         | _                        | -              | _                       | _              | -              | _              | _                         | _                        | _                               | _               | $15.0 \pm 5.0$                 | $19.3 \pm 3.7$ | $36.0 \pm 4.0$           |
| Ba.1 | Male           | -                        | -              | -                       | -              | -              | _              | _                         | _                        | _                               | _               | _                              | _              | _                        |
| D 2  | Female         | -                        | 452 + 27       | -                       | -              | -              | $1.0 \pm 0.0$  | $1.3 \pm 0.3$             | $2.0 \pm 0.0$            | $2.0 \pm 0.0$                   | $2.0 \pm 0.0$   | $2.0 \pm 0.0$                  | $2.0 \pm 0.0$  | $10.0 \pm 0.0$           |
| Ba.2 | Male           | -                        | $15.3 \pm 3.7$ | $11.3 \pm 2.9$          | $10.0 \pm 1.2$ | $14.0 \pm 3.1$ | $11.0 \pm 1.0$ | $16.0 \pm 4.0$            | $16.0 \pm 2.0$           | $18.0 \pm 2.0$                  | $16.7 \pm 5.2$  | $11.3 \pm 1.8$                 | $6.7 \pm 3.5$  | $21.0 \pm 7.0$           |
| D 2  | Female         | _                        | -              | -                       | -              | -              | -              | -                         | -                        | -                               | -               |                                | -              |                          |
| Ba.3 | Male           | -                        | $36.4 \pm 3.7$ | $26.4 \pm 2.3$          | $31.2 \pm 2.9$ | $38.8 \pm 4.7$ | $43.6 \pm 6.9$ | $48.8 \pm 3.0$            | $50.0 \pm 3.3$           | $39.6 \pm 6.5$                  | $39.6 \pm 5.9$  | $36.0 \pm 7.3$                 | $33.2 \pm 6.0$ | $89.3 \pm 20.5$          |
| 6. 4 | Female         | _                        | -              | -                       | -              | -              | _              | _                         | _                        | _                               | _               | _                              | -              | -                        |
| St.1 | Male           | -                        | -              | -                       | -              | -              | _              | -                         | -                        | -                               | -               | -                              | _              | -                        |
|      | Female         | _                        | -              | -                       | -              | _              | _              | $1.0 \pm 0.0$             | $1.0 \pm 0.0$            | $1.0 \pm 0.0$                   | $1.0 \pm 0.0$   | $1.0 \pm 0.0$                  | $2.0 \pm 0.0$  | $3.0 \pm 0.0$            |
| St.2 | Male           | -                        | -              | -                       | _              | $1.0 \pm 0.0$  | $1.0 \pm 0.0$  | $1.0 \pm 0.0$             | $1.0 \pm 0.0$            | $1.0 \pm 0.0$                   | $1.0 \pm 0.0$   | $1.0 \pm 0.0$                  | $1.0 \pm 0.0$  | -                        |
| D.I  | Female         | _                        | -              | -                       | -              | -              | _              |                           | -                        |                                 | -               |                                | -              |                          |
| Pl   | Male           | -                        | _              | _                       | _              | $4.0 \pm 0.0$  |                | $3.3 \pm 0.5$             | $3.3 \pm 0.5$            | $3.3 \pm 0.5$                   | $3.0 \pm 0.4$   | $3.2 \pm 0.4$<br>$4.0 \pm 0.0$ | $3.4 \pm 0.2$  | $3.7 \pm 0.3$            |
| C-   | Female         | _                        | _              | _                       | _              | _              | $2.0 \pm 0.0$  | $2.0 \pm 0.0$             | $4.0 \pm 0.0$            | $4.0 \pm 0.0$                   | $4.0 \pm 0.0$   |                                | $4.0 \pm 0.0$  | $4.0 \pm 0.0$            |
| Со   | Male           | _                        | _              | _                       | _              | _              | -              | _                         | _                        | $1.0 \pm 0.0$                   | -               | $1.0 \pm 0.0$                  | $1.0 \pm 0.0$  | _                        |
| C    | Female<br>Male | _                        | _              | _                       | 40+00          | 100+20         | -<br>05 ± 10   | 110+20                    | 10.0 ± 2.3               | $1.0 \pm 0.0$<br>$10.7 \pm 1.8$ | -<br>10 7 + 0 7 | $1.0 \pm 0.0$ $11.0 \pm 1.3$   | $1.0 \pm 0.0$  | $\frac{-}{10.7 \pm 0.7}$ |
| Sq   |                | _                        | _              | _                       | 0.0            | 20.0 = 2.0     | $8.5 \pm 1.0$  | $11.0 \pm 3.0$            |                          |                                 | 2017 = 017      | 22.0 = 2.0                     |                |                          |
| n l- | Female         | -                        | -<br>          | -                       | $5.0 \pm 1.0$  | $7.0 \pm 1.0$  | $12.0 \pm 2.0$ | $10.0 \pm 0.0$            | $9.0 \pm 1.0$            | $11.0 \pm 1.0$                  | $9.0 \pm 1.0$   | $11.0 \pm 1.0$                 | $11.0 \pm 1.0$ | $13.0 \pm 1.0$           |
| B.b  | Male           | -                        | $5.7 \pm 0.7$  | -                       | -              | -              | _              | _                         | _                        | -                               | _               | _                              | -              | _                        |
|      | Female         | _                        | $5.7 \pm 0.9$  | _                       | _              | _              | -              | _                         | -                        | -                               | -               | -                              | _              | -                        |

Note: values are mean (  $\pm$  standard deviation) number of different types of sensilla on each antennal segment (n=10 antennae). Tr.1 = sensilla trichodea 1, Tr.2 = sensilla trichodea 2, Tr.3 = sensilla trichodea 3, Ba.1 = sensilla basiconica 1, Ba.2 = sensilla basiconica 2, Ba.3 = sensilla basiconica 3, St.1 = sensilla styloconica 1, St.2 = sensilla styloconica 2, Pl = sensilla placodea, Co = sensilla coeloconica, Sq = sensilla squamiforma, B.b = bohm's bristles.

Table 3. Morphological characteristics of antennal sensilla of Sclerodermus sp.

| Types | Morphological characteristics of sensilla |                     |         |                |                 |  |  |  |  |  |  |
|-------|---|---------------------|---------|----------------|-----------------|--|--|--|--|--|--|
|       | Length (μm)                               | Basal diameter (μm) | Wall    | Tip            | Shape           |  |  |  |  |  |  |
| Tr.1  | 16.24 ± 0.48                              | 0.93 ± 0.02         | Grooved | Sharp          | Straight        |  |  |  |  |  |  |
| Tr.2  | $22.19 \pm 0.84$                          | $1.19 \pm 0.05$     | Grooved | Sharp          | Straight        |  |  |  |  |  |  |
| Tr.3  | $12.36 \pm 0.36$                          | $0.92 \pm 0.02$     | Smooth  | Sharp          | Curved          |  |  |  |  |  |  |
| Ba.1  | $8.90 \pm 0.41$                           | $2.89 \pm 0.04$     | Grooved | Blunt          | Straight        |  |  |  |  |  |  |
| Ba.2  | $24.57 \pm 0.29$                          | $1.83 \pm 0.03$     | Smooth  | Sharp          | Straight        |  |  |  |  |  |  |
| Ba.3  | $14.23 \pm 0.19$                          | $1.85 \pm 0.04$     | Smooth  | Sharp          | Curved          |  |  |  |  |  |  |
| St.1  | $10.67 \pm 3.83$                          | $1.25 \pm 0.25$     | Grooved | Sharp or blunt | Slightly curved |  |  |  |  |  |  |
| St.2  | $8.86 \pm 0.55$                           | $1.22 \pm 0.06$     | Grooved | Sharp          | Slightly curved |  |  |  |  |  |  |
| Pl    | $9.92 \pm 0.27$                           | $5.98 \pm 0.19$     | Smooth  | Sharp          | Straight        |  |  |  |  |  |  |
| Co    | _   | $0.68 \pm 0.04$     | _       | Blunt          | Rounded         |  |  |  |  |  |  |
| Sq    | _   | _                   | Smooth  | Sharp          | Slightly curved |  |  |  |  |  |  |
| B.b   | $4.08\pm0.23$                             | $0.96\pm0.05$       | Smooth  | Sharp          | Straight        |  |  |  |  |  |  |

Note: values are mean ( $\pm$  standard deviation) length and diameter of at least 20 sensilla. Tr.1 = sensilla trichodea 1, Tr.2 = sensilla trichodea 2, Tr.3 = sensilla trichodea 3, Ba.1 = sensilla basiconica 1, Ba.2 = sensilla basiconica 2, Ba.3 = sensilla basiconica 3, St.1 = sensilla styloconica 1, St.2 = sensilla styloconica 2, Pl = sensilla placodea, Co = sensilla coeloconica, Sq = sensilla squamiforma, B.b = bohm's bristles.

Ba.1 on the last section of flagellum. Ba.1 were relatively thick, measuring  $8.90 \pm 0.41 \, \mu m \ (n=20)$  in length and with a basal diameter of  $2.89 \pm 0.04 \, \mu m \ (n=20)$ , and were only located from the fourth flagellomere to the 11th flagellomere in the female wasps.

Sensilla basiconica type 2 (Ba.2) were similar in shape to the sensilla trichodea, but were longer and thicker. They had a smooth wall

and sharp tips (Fig. 2C). Ba.2 were found on all segments except the scape in the males, and their average length and basal diameter were  $24.57 \pm 0.29 \, \mu m \, (n=20)$  and  $1.83 \pm 0.03 \, \mu m \, (n=20)$ , respectively.

Sensilla basiconica type 3 (Ba.3) were similar to Ba.2, but the distal region was short and curved (Fig. 2C). As seen for Ba.2, Ba.3 were only distributed from the pedicel to the 11th flagellomere in the males.

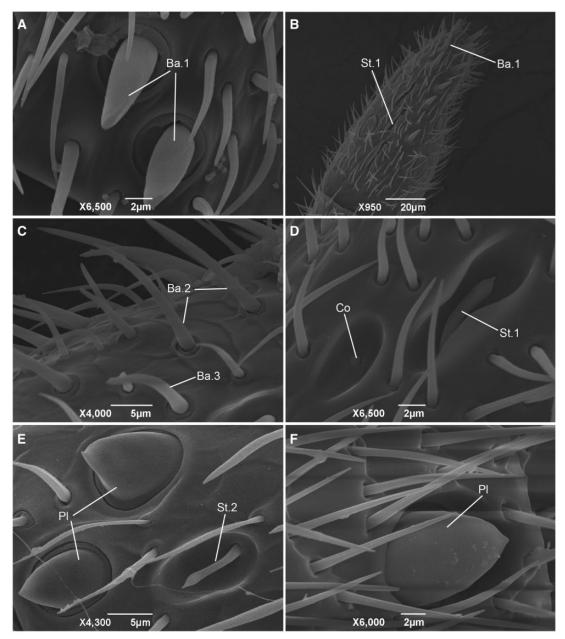


Fig. 2. Antennal sensilla in *Sclerodermus* sp. (A) Sensilla basiconica 1 (Ba.1). (B) Continuously arranged sensilla basiconica 1 (Ba.1) and sensilla styloconica 1 (St.1) on the 11th flagellomere. (C) Sensilla basiconica 2 (Ba.2) and Sensilla basiconica 3 (Ba.3). (D) Sensilla styloconica 1 (St.1) and sensilla coeloconica (Co). (E) Sensilla styloconica 2 (St.2) and sensilla placodea (Pl). (F) Sensilla placodea (Pl).

Their average length and basal diameter were  $14.23 \pm 0.19 \,\mu\text{m}$  (n=20) and  $1.85 \pm 0.04 \,\mu\text{m}$  (n=20), respectively.

Sensilla Styloconica. Sensilla styloconica type 1 (St.1) had a fingerlike shape with longitudinal grooves, fluted walls, a nipple at the distal region and a stem plug in a deep hole of the antenna. The whole sensillum was surrounded by a barbell chamber (Fig. 2D). St.1 were only found from the fifth to the 11th flagellomere in the females and were continuously arranged on the last segment of the antennae (Fig. 2B). Their average length and basal diameter were  $10.67 \pm 3.83 \,\mu m$  (n=20) and  $1.25 \pm 0.25 \,\mu m$  (n=20), respectively.

Sensilla styloconica type 2 (St.2) had an expanded head with grooves, a nipple in the distal region and a long smooth stem plug in a deep hole. They were surrounded by an elliptical chamber whose long axis was  $11.56\,\mu m$ , and whose minor axis was  $5.17\,\mu m$  (Fig. 2E). In contrast to St.1, St.2 were only found in the males from the third to the tenth flagellomere and with only one St.2 for each segment. Their

average length and basal diameter were  $8.86 \pm 0.55 \, \mu m \, (n=20)$  and  $1.22 \pm 0.06 \, \mu m \, (n=20)$ , respectively.

Sensilla Placodea. Pl had an oval structure and a cone-shaped tip, with the long axis being parallel to the long axis of the flagellum (Fig. 2E and F). At higher magnification, many wall pores were observed on the surface of Pl. These sensilla were distributed from the fourth to the 11th flagellomere in the females and from the third to the 11th flagellomere in the males. Their average length and basal diameter were  $9.92 \pm 0.27 \,\mu\text{m}$  (n = 20) and  $5.98 \pm 0.19 \,\mu\text{m}$  (n = 20), respectively. Other than there being significantly more Pl on the fourth flagellomere of males than on that of the females (P < 0.05), there was no significant difference in Pl between the males and females (P > 0.05) (Table 2).

Sensilla Coeloconica. Co were characterized by short pegs, with a juglans-shape and a deep grooved head, and a short smooth-surfaced stem. They were located in deep depressions surrounded by a clock-shaped ring whose long axis was 7.24 µm, and minor axis was 4.36 µm

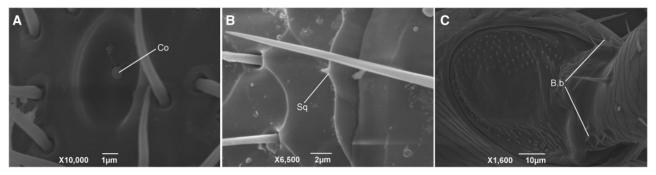


Fig. 3. Antennal sensilla in Sclerodermus sp. (A) Sensilla coeloconica (Co). (B) Sensilla squamiforma (Sq). (C) Bohm's bristles (B.b).

(Figs. 2D and 3A). Co were found on the seventh, ninth, and tenth flagellomeres, with only one Co on each segment in both sexes. Their mean basal width was  $0.68 \pm 0.04 \, \mu m \, (n=20)$ .

Sensilla Squamiforma. Sq were inserted into the cuticle without a basal pedestal and narrowed from their base to their tip, which had a spine-like shape. They were squamaceous and parallel to the long axis of the flagellum (Fig. 3B). They were located on the joint region between each segment from the second flagellomere to the 11th flagellomere in both sexes, but there was no significant difference in number of Sq between males and females (P > 0.05).

Bohm's Bristles. B.b were spine-like structures with smooth cuticles, lying straight and almost perpendicular to the antennal surface (Fig. 3C). They were relatively short compared with the other sensilla, measuring  $4.08 \pm 0.23 \, \mu \text{m} \, (n=20)$  in length and with a basal diameter of  $0.96 \pm 0.05 \, \mu \text{m} \, (n=20)$ . They were mainly distributed in clusters on the joint region between the scape and the pedicel in both sexes, with six being present on average. No significant difference in the number of B.b was found between males and females (P > 0.05).

#### **Discussion**

This study is the first to describe the antennal sensilla of *Sclerodermus* sp. and was based on previous reports on antennal ultrastructures and functions of other parasitic wasp species. The results of the SEM analyses show that seven different types of sensillum were identified in both sexes, including three sensilla Tr, three sensilla Ba, two sensilla St, one sensilla Pl, one sensilla Co, one sensilla Sq, and B.b. Most of these antennal sensillum types have been described in other parasitic wasps, although many of the studies used different nomenclature to describe them.

As the most abundant sensilla, sensilla Tr have a hair- or peg-like structure, with multiple, one, or no pores on the wall. These sensilla have also been reported in other species of parasitic wasp (Bleeker et al. 2004, Roux et al. 2005, Gao et al. 2007, Rocha et al. 2007, Onagbola and Fadamiro 2008, Onagbola et al. 2009, Wang et al. 2010, Das et al. 2011, Li et al. 2011). In *Sclerodermus* sp., three types of Tr were found on males and females. Tr.2 were much longer and thicker than Tr.1 but both were straight, whereas Tr.3 were extremely curved at basal region. In general, nonporous sensilla Tr have been described as having putative mechanoreceptive functions (Alm and Kurczewski 1982, Das et al. 2011). In this study, the absence of pores on sensilla Tr in *Sclerodermus* sp. suggests that they function as mechanoreceptors in both sexes.

Sensilla Ba mainly have a thicker body than that of Tr, with multiple pores on the sensilla tips or around the walls (Amornsak et al. 1998, Cônsoli et al. 1999, van Baaren et al. 1999, Gao et al. 2007, Das et al. 2011, Li et al. 2011). However, Ba are nonporous in some cases (Gao et al. 2007). In our study of *Sclerodermus* sp., there were three types of Ba on the antennae. Ba.1 had a thumb-like shape, which was similar to the long sensilla Ba with tip and wall pores of the ant-like bethylid wasp *S. guani* (Li et al. 2011). Ba.2 were similar to Ba.3, but Ba.3 were shorter and curved at the distal region. Ba.2 are similar to the sensilla Ba type II of *Microplitis pallidipes* (Gao et al. 2007) and the sensilla

trichodea of two closely related parasitoid wasp species, *Cotesia glomerata* and *Cotesia rubecula* (Bleeker et al. 2004). Ba.2 have been considered to have an olfactory function (Gao et al. 2007). Ba.3 have also been described as sensilla Ba type 2 with wall pores in the endoparasitic wasps *Microplitis croceipes* and *Cotesia marginiventris* (Das et al. 2011), multiparous sensilla Tr with pores (Pettersson et al. 2001, Ryan 2002, Bleeker et al. 2004), and multiporous pitted sensilla Tr C in *Trichogramma nubilale* (Olson and Andow 1993). In general, sensilla Ba type 2 are presumed to function as olfactory receptors in many insects (Steinbrecht 1987, Hansson et al. 1991, Steinbrecht 1997, Bleeker et al. 2004, Das et al. 2011). Thus, we suggest that Ba.3 in *Sclerodermus* sp. are putative olfactory sensilla.

Sensilla St have a long fluted or smooth stem plug in a deep hole. These sensilla are rare in other parasitic wasp species. In *Sclerodermus* sp., two types of St were found on the antennae. St.1 were surrounded by a barbell chamber and only in females. St.2 were surrounded by an elliptical chamber and only distributed in the males. A few St have been found on the female antennae of *Spathius agrili*, an important larval ectoparasitoid of the emerald ash borer (Wang et al. 2010), and only one St is located on the antennae of *Gryon gallardoi* (Rocha et al. 2007). Our observations of St.1 and St.2 are in agreement with previous findings from the ant-like bethylid wasp *S. guani*, from which they were described as double-walled multiporous sensilla type 1 and type 2 (Li et al. 2011). Sensilla St are chemoreceptors (Pitts and Zwiebel 2006; Wang et al. 2010), and might detect sex pheromones. Therefore, we suggest that St in *Sclerodermus* sp. have a role in detecting sex pheromones.

Sensilla Pl are the most common sensilla on the antennae of Hymenoptera parasitoid species, although they occur in various sizes and shapes (Cônsoli et al. 1999, van Baaren et al. 1999, Bleeker et al. 2004, Roux et al. 2005, Gao et al. 2007, Onagbola and Fadamiro 2008, Wang et al. 2010, Das et al. 2011, Li et al. 2011). In most parasitic wasps, Pl are elongated in shape and arranged in alternate rings around the antennae. In this study, both male and female Sclerodermus sp. had Pl. These sensilla had an oval structure with their long axis being parallel to the long axis of the flagellum. However, the external structure of Pl was different from that observed in other parasitic wasps, except for S. guani, described as Pl (Li et al. 2011) and G. gallardoi, described as papillary sensilla (Rocha et al. 2007). The closest resemblance of this type of Pl has been found in honeybees, Apis mellifera adansonii (Dietz and Humphreys 1971) and Apis mellifera ligustica (Gramacho et al. 2003). The multiple wall pores on Pl suggest an olfactory function (Ochieng et al. 2000, Bleeker et al. 2004, Roux et al. 2005, Marques-Silva et al. 2006, Gao et al. 2007).

Sensilla Co are recessed in deep pits (Ryan 2002), and are the least abundant sensilla in parasitic wasps (Li et al. 2011). This sensillum type is found in many Hymenoptera parasitoid species (van Baaren et al. 1999, Bleeker et al. 2004, Roux et al. 2005, Gao et al. 2007, Onagbola and Fadamiro 2008, Das et al. 2011, Li et al. 2011). In *Sclerodermus* sp., Co were also one of the least abundant sensilla types, and only located on the seventh, ninth, and tenth flagellomeres with only one Co

for each segment in both sexes. Co have been previously described as 'pit organs' in bees because they are recessed into deep pits (Wcislo 1995), and as sensilla Co type I in *C. glomerata* and *C. rubecula* (Bleeker et al. 2004). These nonporous sensilla are generally presumed to be associated with thermo- or hygroperception (Altner et al. 1983, Bleeker et al. 2004; Onagbola and Fadamiro 2008). The Co in *Sclerodermus* sp. might have a similar role owing to the few sexual differences in their abundance and the absence of pores.

Sensilla Sq is an uncommon sensillum type in parasitic wasps. In the antennae of *Sclerodermus* sp., Sq were distributed on the joint region between two segments in both sexes. These sensilla have only been found on the pulvilli of *S. agrili* and could be mechano- and chemoreceptors that might detect vibrational signals derived from the hosts (Wang et al. 2010).

B.b found on the antennae of *Sclerodermus* sp. have not been observed in other Hymenoptera parasitoid species. However, B.b have been described on the antennae of the parasitoid beetle *Dastarcus helophoroides* (Fairmaire), which is an important parasite of longicorn beetles (Ren et al. 2012). B.bs are usually found in areas opposite the membrane between the head capsule and the scape, as well as between the scape and the pedicel, or on the scape and pedicel (Schneider 1964). Merivee et al. (2002) thought that B.b were probably mechanoreceptors, sensing the position and movement of the antennae (Merivee et al. 2002).

In conclusion, the present study identified and characterized for the first time both the morphology and distribution of seven types of sensillum located on the antennae of *Sclerodermus* sp. The results provide direct morphological evidence that sensilla on the antennae might have important roles in the biological behavior and life cycle of this new parasitic wasp. Future studies on the functional morphology of antennal sensilla using transmission electron microscopy coupled with electrophysiological recordings are likely to confirm the functions of the different antennal sensilla identified in this study.

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