

Observation on the ultrastructure of the oral appendages of *Glenea cantor* Fabricius (Coleoptera: Lamiinae)

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Abstract

We investigated the gross and ultrastructure morphology of the mandibles, labial palpus and mandibular palpus of adult male and female *Glenea cantor* beetles using scanning electron microscopy. There were significant differences between males and females in the gross and ultrastructure morphology of these parts. Observation of the gross morphology of the mandible, labial palpus and mandibular palpus revealed that their lengths were significantly greater in females, which is mainly related to the oviposition mechanism. In terms of the ultrastructural morphology of the mandibles, labial palpus and mandibular palpus, seven types of sensilla were found on these parts: sensilla twig basiconica (STB I, II, III, and IV), sensilla chaetica (SCh IV and V), Böhm's bristles (Bb), sensilla placodea (SP), sensilla trichodea (ST II), sensilla plate (SP), sensilla coeloconica (SC) and sensilla campaniformia (SCa). Females have significantly more ST I and SCh III on their mandibles than males. The mechanical sensors SCh and Bb, olfactory sensor ST II, taste sensor STB IV, and carbon dioxide sensor and temperature and humidity sensors SC, SP and SCa were significantly more developed on female than male labial and maxillary palps, which also further explained the important role of the oral appendages in oviposition behavior. These results will help us further understand the host selection and oviposition behavior of adult *G. cantor* and promote the information-based design of pest control methods.

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ABSTRACT

We investigated the gross and ultrastructure morphology of the mandibles, labial palpus and mandibular palpus of adult male and female *Glenea cantor* beetles using scanning electron microscopy. There were significant differences between males and females in the gross and ultrastructure morphology of these parts. Observation of the gross morphology of the mandible, labial palpus and mandibular palpus revealed that their lengths were significantly greater in females, which is mainly related to the oviposition mechanism. In terms of the ultrastructural morphology of the mandibles, labial palpus and mandibular palpus, seven types of sensilla were found on these parts: sensilla twig basiconica (STB I, II, III, and IV), sensilla chaetica (SCh IV and V), Böhm's bristles (Bb), sensilla placodea (SP), sensilla trichodea (ST II), sensilla plate (SP), sensilla coeloconica (SC) and sensilla campaniformia (SCa). Females have significantly more ST I and SCh III on their mandibles than males. The mechanical sensors SCh and Bb, olfactory sensor ST II, taste sensor STB IV, and carbon dioxide sensor and temperature and humidity sensors SC, SP and SCa were significantly more developed on female than male labial and maxillary palps, which also further explained the important role of the oral appendages in oviposition behavior. These results will help us further understand the host selection and oviposition behavior of adult *G. cantor* and promote the information-based design of pest control methods.

KEYWORD

Glenea cantor , oral appendage, scanning electron microscopy, plant protections, stem boring insects

RESEARCH HIGHLIGHTS

1. The external morphology and distribution of the oral appendages of *G. cantor* between sexes has been researched through scanning electron microscopy (SEM) for the first time.
2. Several significant differences between males and females has been found by analyzing the oral appendages of *G. cantor* with SEM.
3. The differences in the structure of oral appendages of *G. cantor* between sexes reflect functional differences in reproductive behaviors.

1 INTRODUCTION

Cerambycidae is one of the main members of the order Coleoptera, with more than 36,000 recorded species worldwide, and it is one of the most important pests of forests, street trees, and fruits (Lu et al., 2007; Wang et al., 2017). *Glenea cantor* Fabricius is a member of the subfamily Lamiinae, which is mainly distributed in southern China and Southeast Asia countries (Lu et al., 2013a). It is a stem-boring insect pest that survives as a larva by burrowing into tree trunks or branches. However, traditional control methods, such as pesticide application, have a poor effect on preventing and controlling stem-boring insects (Brockerhoff et al., 2006). Research has shown that pheromones can be used to disturb *G. cantor* (Lu et al., 2013b), including two main types of pheromone reception, short-distance and contact pheromones (Wong et al., 2017), which has been reported on a number of longhorns, including the subfamily Lamiinae (Zhang et al., 2003; Li et al., 2008). So many ideas are to prevent the stem-boring insect pest by blocking the reception of pheromones. This step belongs to the field of insect chemical ecology, which has attracted an increasing number of scholars aiming to provide theoretical guidance for the prevention of multiple pests.

The structural basis of pheromone chemosensory recognition is the sensilla ultrastructure. Studies have shown that the common sensilla types of beetles include sensilla basiconica, sensilla chaetica, sensilla twig basiconica, sensilla coeloconica, sensilla trichodea, sensilla campaniformia, sensilla placodea (Wu et al., 2018; Ma et al., 2019). Furthermore, a large amount of these sensilla are mainly distributed in the antennae, labial papilla, mandibular papilla and ovipositor (MacKay et al., 2014; Wang et al., 2014; Liu et al., 2018; Dong et al., 2020). The morphology, distribution, number and function of the different sensors differ, and the different functions of the sensilla work in synergy to receive and recognize signals (Dong et al., 2020), which are converted into electrical signals and transmitted to the brain for unified regulation, thus ensuring that the various behaviors are carried out efficiently and smoothly. For example, the main function of the spiny sensor is mechanosensory (Zhang et al., 2003; MacKay et al., 2014) sensilla trichodea and sensilla twig basiconica have olfactory functions (Dong et al., 2020); and the plate sensor, bell sensor and cavity sensor

are reported to be mainly distributed in the plate. Moreover, the bell and cavity sensors were reported to be mainly located in the labial palpus and mandibular palpus and mainly functioned to sense carbon dioxide and temperature and humidity (Ochieng et al., 2000; Dong et al., 2020). To date, however, research on these sensilla has focused on the beetle’s antennae, and sensillum in specific parts of the antennae have been shown to be involved in the recognition of oviposition sites (Peng et al., 2012). In addition, studies have also reported that labial palpus and mandibular palpus have similar roles. For instance, in *Oedaleus decorus asiaticus* Bey-Bienko (Wang et al., 2022) and *Locusta migratoria manilensis* Meyen (Zhang et al., 2020), the labial palpus and mandibular palpus of female beetles may also play a certain role in finding spawning sites.

Studies further have shown that the labial palpus and mandibular palpus of *G. cantor* plays an important role in reproductive behavior (Dong et al., 2020), but the ultrastructure of labial palpus and mandibular palpus of *G. cantor* has not been reported. Therefore, the purpose of our study was to analyze the ultrastructure of the oral appendages of male and female longhorn beetles through scanning electron microscopy and to determine the structural differences between male and female longhorn beetles and their specific role in reproductive behavior to provide new insights for pest control and forest protection.

2 MATERIAL AND METHOD

2.1 Insects

In July 2020, we collected three kapok branches that had withered in the upper stalk due to *G. cantor* infestation from Qingxiu Mountain (22°12′-23deg32′N, 107deg45′-108deg51′E), Guangxi Zhuang Autonomous Region Nanning, China. The stems were incubated in four bubble containers in the yarn in a cage (50 cm x 70 cm x 80 cm) and sprayed with water every 5 days in a controlled environment (25 ± 1degC, 75 ± 5% RH, 14 L: 10 D; Lu et al., 2011). After 2 months, five males and five females were randomly selected from our caged adults and transferred to separate glass containers (5 cm diameter x 16 cm length) in a refrigerator at -20 degC. After 25 minutes, the adult insect returned to normal and its mandibles, labial papules and mandibular papules were cut under a stereomicroscope PX-1 (Camsonar Technology Co., Ltd., Beijing, China). The mandibles, labial papules and mandibular papules are prepared.

2.2 Preparation of Specimens

The mandibles, labial papules and mandibular papules prepared as above were cleaned three times in an ultrasonic bath JP-010T (Skymen Cleaning Equipment CO., Ltd., Shenzhen, China) at 250 W for 350 s each time. The mandibles, labial papules and mandibular papules were fixed with 3% glutaraldehyde at 5 for 15 h, and dehydrated with 60%, 70%, 80%, 90%, and 100% liters of ethanol, with an interval of 8 min. The prepared mandibles, labial papules and mandibular papules were stored in glass containers which was already cleared and dried for 36 h. After drying, the specimens were mounted on a holder using double-sided sticky tape (dorsal, ventral), sputter coated with gold-palladium. Subsequently, the prepared were scanned by using an electron microscope (model S-3400N, Hitachi, Ibaraki, Japan) at an accelerating voltage of 3-10 kV. The images were named separately and stored on a computer.

2.3 Data Analysis

In this paper, according to the morphological criteria described by Moeck (1968) mainly introduce the terminology and classification of sensilla length and the intermediate width of mandibles, labial papules and mandibular papules, and various sensillum was quantified using Image J Launcher Version software at 1.44p (Broken Symmetry Software, National Institutes of Health, Bethesda, MD). On the basis of scanning electron microscopy images, the general morphological characteristics of mandibles, labial papules and mandibular papules of *G. cantor* were studied. The differences in the distribution patterns of these sensillum were accurately described by Adobe Photoshop CC 2019 software. T-test ($t = 0.05$) was used to compare the length, width, and number of each individual sensilla subtype in adults between sexes using SPSS 25.0 (IBM, Armonk, NY) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). Values are reported as mean ± SE (standard error).

3 RESULT

3.1 Gross Morphology of oral appendages

3.1.1 Mandible

The length and basal width of the mandibles of females are significantly more developed than those of males, but there is no significant difference in basal thickness (Table 1).

Table 1 Lengths, base widths and base thickness of mandible of both sexes in *G. cantor*

| Index (mm) | Sex | Sex | Significance <i>t</i> | Significance <i>P</i> |
|----------------|--------------|-------------|--------------------------|--------------------------|
| Lengths | 1.71 ± 0.07* | 1.26 ± 0.03 | 5.92 | 0.00 |
| base widths | 1.09 ± 0.05* | 0.88 ± 0.03 | 3.45 | 0.00 |
| base thickness | 0.67 ± 0.04 | 0.59 ± 0.03 | 1.46 | 0.16 |

Note: Data are presented as means ± SE, n = 10. *Indicating significant difference between males and females at 0.05 level.

3.1.2 Labial palpus

The length and basal width of the mandibles of females were significantly more developed than those of males, but there was no significant difference in basal thickness. Figure 1 shows that the length and width of the female labial palpus are different from those of males. The basal segment 1 of the labial palpus is shorter, the terminal segment is expanded and conical at the top, and the base of each segment forms an articulated structure that is embedded within the terminal part of the preceding segment, and it is flexible, soft and bendable (Table 2).

Table 2 Lengths and base widths of labial palpus of both sexes in *G. cantor*

| Labial palpus | Length (mm) | Length (mm) | Significance <i>t</i> | Significance <i>P</i> | Width (mm) | Width (mm) | Significance <i>t</i> | Significance <i>P</i> |
|-----------------|--------------|--------------|--------------------------|--------------------------|--------------|--------------|--------------------------|--------------------------|
| 1 st | 0.17 ± 0.01c | 0.14 ± 0.01b | 3.22 | 0.01 | 0.09 ± 0.01b | 0.06 ± 0.00b | 6.45 | 0.00 |
| 2 nd | 0.52 ± 0.02b | 0.42 ± 0.02a | 3.13 | 0.01 | 0.10 ± 0.01a | 0.08 ± 0.01a | 4.69 | 0.00 |
| 3 rd | 0.60 ± 0.01a | 0.46 ± 0.01a | 7.11 | 0.00 | 0.10 ± 0.01a | 0.08 ± 0.01a | 5.87 | 0.00 |
| Whole | 1.28 ± 0.03 | 1.02 ± 0.02 | 7.91 | 0.00 | 0.09 ± 0.00a | 0.07 ± 0.00 | 8.87 | 0.00 |

Note: Data are presented as means ± SE, n = 10; Different letters in each column indicate significant difference at 0.05 level using Tukey's.

3.1.3 Mandibular palpus

There is also clear dimorphism in the mandibular palpus of male and female adults, especially in length, with each subsegment and overall length showing significant differences, except for the basal width of segment 4 of the mandibular palpus, which is not significantly different (Table 3).

Table 3 Lengths and base widths of mandibular palpus of both sexes in

G. cantor

| Mandibular palpus | Length (mm) | Length (mm) | Significance <i>t</i> | Significance <i>P</i> | Width (mm) | Width (mm) | Significance <i>t</i> | Significance <i>P</i> |
|-------------------|--------------|--------------|--------------------------|--------------------------|---------------|--------------|--------------------------|--------------------------|
| 1 st | 0.22 ± 0.01d | 0.19 ± 0.01d | 2.84 | 0.01 | 0.08 ± 0.00b | 0.07 ± 0.00a | 2.39 | 0.02 |
| 2 nd | 0.46 ± 0.01b | 0.34 ± 0.01b | 7.17 | 0.00 | 0.10 ± 0.01a | 0.08 ± 0.01a | 5.31 | 0.00 |
| 3 rd | 0.41 ± 0.01c | 0.30 ± 0.01c | 8.46 | 0.00 | 0.10 ± 0.00a | 0.08 ± 0.00a | 3.75 | 0.00 |
| 4 th | 0.60 ± 0.01a | 0.48 ± 0.00a | 12.53 | 0.00 | 0.09 ± 0.00ab | 0.08 ± 0.01a | 1.11 | 0.27 |

| | | | | | | | |
|-------|-------------|-------------|-------|------|-------------|-------------|------|
| Whole | 1.47 ± 0.02 | 1.13 ± 0.02 | 12.64 | 0.00 | 0.10 ± 0.00 | 0.08 ± 0.00 | 4.27 |
|-------|-------------|-------------|-------|------|-------------|-------------|------|

Note: Data are presented as means ± SE, n = 10; Different letters in each column indicate significant difference at 0.05 level using Tukey's.

3.2 Ultrastructural morphology of oral appendages

3.2.1 Mandible

The mandible sensors of adult *G. cantor* are relatively simple and highly ossified and are mainly used for feeding and making oviposition grooves. The lateral and medial sides of the palate have numerous receptive hair areas, with a small number of hair-shaped sensors type I (ST I) distributed on the outer side (Fig. 1), while the ventral and dorsal edges of the palate have sensilla chaetica (SCh III) in addition to ST I, and the dorsal surface has significantly more sensilla than the ventral surface (Fig. 2). Females have significantly more ST I and SCh III on their mandibles than males.

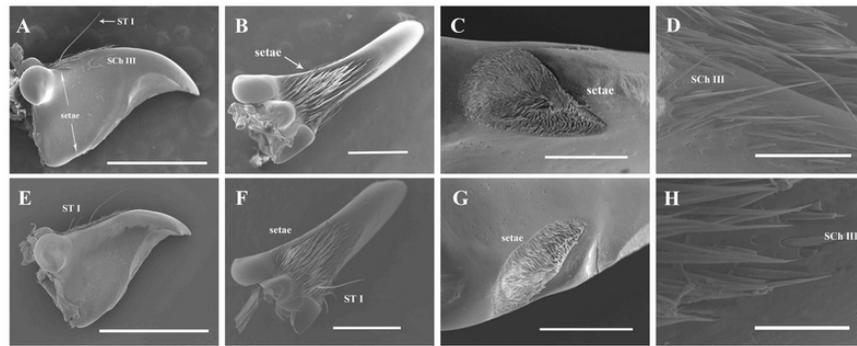


Fig. 1 The morphological characteristics of mandible of both sexes of *G. cantor*

A, Venter view of mandible of female; B, Lateral view of mandible of female; C, Interior view of mandible of female; D, Dorsal view of mandible of female; E, Venter view of mandible of male; F, Lateral view of mandible of male; G, Interior view of mandible of male; H, Dorsal view of mandible of male. (Scale bars): A, E = 1000 μm; B, F = 500 μm; C, D, G = 200 μm; H = 50 μm.

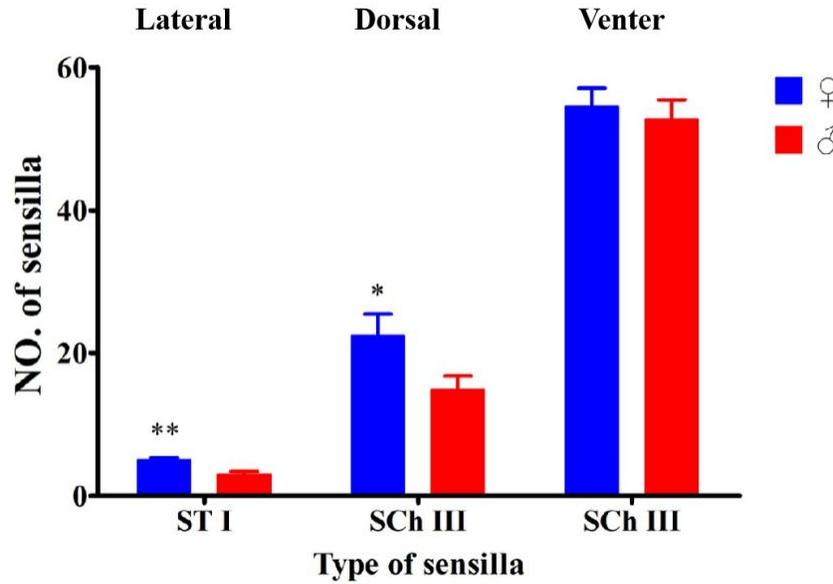


Fig. 2 Numbers and distributions of sensilla on the mandible of both sexes of *G. cantor*

Lateral; Dorsal; Venter, n = 10. ** Indicating significant difference between males and females at 0.01 level, * indicates significant difference between males and females at 0.05 level, n = 10.

3.2.2 Labial palpus

The labial palps of *G. cantor* have a rich variety of sensor species, with no differences between male and female sensor types but some variability in the number of sensors (Fig. 3). The sensor species on the labial palps can be divided into seven main groups (11 subtypes): sensilla chaetica (SCh IV, SCh V), sensilla twig basiconica (STB I, STB II, STB III, STB IV), Böhm's bristle sensor (Bb), sensilla trichodea (ST II), sensilla plate (SP), sensilla coeloconica (SC) and sensilla campaniformia (SCa) (Table 4).

Table 4 The morphological characteristics of sensilla on the labial palpus of both sexes in *G. cantor*

| Type | Subtype | Sex | Length (µm) | Width (µm) | Morphological character | Morphological character |
|---------------------------|---------|-----|----------------|--------------|---------------------------|-------------------------|
| Sensillia Twig Basiconica | STB I | | 2.21 ± 0.08 | 2.09 ± 0.07 | Tip | Basal |
| | | | 2.70 ± 0.10* | 2.04 ± 0.05 | Flat blunt cavity | None |
| | STB II | | 2.37 ± 0.22 | 1.81 ± 0.05 | Rounded blunt projection | None |
| | | | 3.52 ± 0.12* | 1.78 ± 0.04 | | |
| | STB III | | 2.52 ± 0.12 | 1.50 ± 0.04 | Finger-shaped protrusions | None |
| | | | 2.85 ± 0.16 | 2.03 ± 0.08* | | |
| | STB IV | | 2.88 ± 0.20 | 1.78 ± 0.08* | Rounded blunt | None |
| | | | 2.70 ± 0.11 | 1.54 ± 0.08 | | |
| Sensilla Chaetica | SCh IV | | 52.58 ± 1.96 | 3.37 ± 0.23 | Sharp pointed | dense |
| | | | 50.79 ± 2.04 | 3.15 ± 0.18 | | |
| | SCh V | | 249.79 ± 32.10 | 9.08 ± 0.67* | Sharp pointed | dense |
| Böhm bristles | Bb | | 185.09 ± 22.23 | 5.65 ± 0.23 | | |
| | | | 10.90 ± 1.28 | 2.46 ± 0.16 | Sharp pointed | Loose |
| Sensilla Trichodea | ST II | | 11.30 ± 0.99 | 2.45 ± 0.12 | | |
| | | | 58.57 ± 3.33 | 4.38 ± 0.16* | Blunt | Loose |

| | | | | | |
|------------------------|-----|---------------|--------------|--------------------------|-------|
| Sensilla Plate | SP | 59.14 ± 2.92 | 3.61 ± 0.14 | Board shape | Loose |
| | | 20.63 ± 1.44 | 1.77 ± 0.10 | | |
| | | 31.31 ± 0.88* | 1.88 ± 0.11 | | |
| Sensilla Coeloconica | SC | — | 0.43 ± 0.03 | Hole cavity | None |
| | | — | 0.37 ± 0.02 | | |
| Sensilla Campaniformia | SCa | — | 2.87 ± 0.17* | Rounded blunt protrusion | loose |
| | | — | 1.80 ± 0.11 | | |

Note: Data are presented as means ± SE, n = 10. * Indicating significant difference between males and females at 0.05 level (The same to the following table). “—” indicate the sensilla were absent.

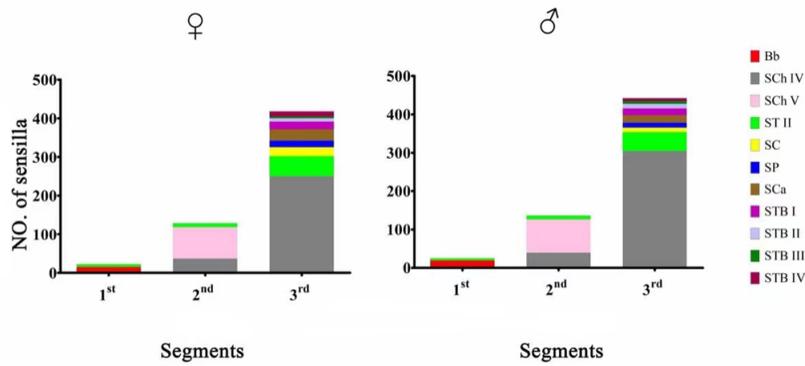


Fig. 3 Numbers and distributions of sensilla on the labial palpus of both sexes of *G. cantor*

Sensillia twig basiconica

Sensilla twig basiconica (STB) is found only at the apical end of adult labial palps and consists of four subtypes (STB I, STB II, STB III, and STB IV). The shape of these sensors varies markedly between subtypes (Fig. 4): STB I has a smooth surface and a flat and deeply concave apical part with a distinct chamber and no basal fossa; STB II has a generally smooth surface and a rounded apical part with chrysanthemum shaped granules and no basal fossa; STB III has a smooth surface and a flower-like wrapping around a finger-like projection at the tip; STB IV has an overall smooth surface and a rounded apical part with a dome.

The terminal tapered inductor of the labial palpus exhibits external morphological features that are significantly longer in males than in females, including STB I ($t = -4.00$, $P < 0.01$) and STB II ($t = -4.59$, $P < 0.01$), while the basal width of STB III ($t = -5.76$, $P < 0.01$) in males is similarly significantly wider than in females; however, the STB IV basal width ($t = 2.15$, $P < 0.05$) in females was significantly wider than males. Quantitatively, females had significantly more STB IV than males (Fig. 3).

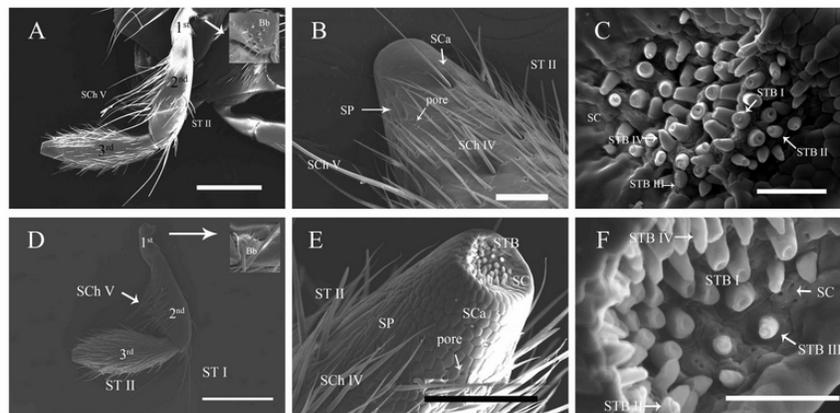


Fig. 4 The ultrastructural of labial palpus of both sexes of *G. cantor*

A, Labial palpus of female beetle; B, Sensilla on 3rd of labial palpus of female beetle; C, Sensilla styloconica on 3rd of labial palpus of female beetle; D, Labial palpus of male beetle; E, Sensilla on 3rd of labial palpus of male beetle; F, Sensilla styloconica on 3rd of labial palpus of male beetle. Scale bars: A, D = 300 μ m, B, E = 50 μ m, C, F = 10 μ m.

Sensilla chaetica

The sensilla chaetica of the adult labial palpus differ from those on the antennae, with two subtypes (SCh IV, SCh V). SCh IV is found on segments 2 and 3 of the labial palpus, while SCh V is found on segment 2 of the labial palpus. These two subtypes of inductors differ considerably in length and basal width (Table 4).

The basal width of the SCh V on the labial palpus of females was significantly wider than that of males. In contrast, the number of SCh IV ($= 250.00 \pm 10.32$, $= 304.20 \pm 7.69$, $t = -4.21$, $P = 0.00$) on the third segment of the labial palpus was significantly greater in males than in females (Fig. 3).

Bohm bristles

Bohm's bristle sensor (Bb) is present at the base of the labial palpus and mandibular palpus only at the junction of the base of the first segment with the mouthparts, and the sensor has a smooth epidermal wall with an acute tip and a loose basal fossa (Fig. 5). This sensor is a relatively common sensor at the junction of insect organs, and no subtypes exist, which it shows no significant differences between males and females as a whole (Fig. 3).

Sensilla trichodea

The adult labial palps have a relatively small number of sensilla trichodea, with only one subtype, ST II, which is more abundant and is found in all segments of the labial palpus in both sexes. It has a blunt apex, a V-shaped pattern in the epidermal wall and a loose basal fossa (Fig. 4).

There was no significant difference in the number of labial palpus ST II between males and females (Fig. 3), but the basal width of ST II was significantly wider in females than in males.

Sensilla placodea

The sensilla placodea (SP) of brown-spotted *G. cantor* was long and lamella-plate-shaped with a loose basal fossa and smooth epidermal walls (Figs. 4, 6), and in adults, the labial palpus and mandibular palpus sensilla placodea were distributed on the outer side of the terminal segment.

Females had significantly more SP than males in both labial palps ($= 17.00 \pm 0.32$, $= 12.80 \pm 0.58$, $t = 6.33$, $P < 0.01$) (Fig. 2), but the length of the SP of the labial palpus was significantly longer in males than in females (Table 4).

Sensilla coeloconica

Sensilla coeloconica (SC) are found mainly at the top of the terminal segments of the labial palpus of adults, scattered and interspersed along the outer edges of the terminal conical sensor group. The shape of the sensor is elevated to form a chamber, with a raised and ruffled surface (Fig. 4).

Sensilla campaniformia

Sensilla campaniformia (SCa) are more common on the labial palpus and mandibular palpus of insects of the family Araneae and are named after their bell-shaped appearance. The sensor consists of a broad basal fossa and a rounded, blunt projection with a smooth epidermal wall and is found mainly on the terminal segments of the labial palpus and mandibular palpus of adults.

There is clear male and female dimorphism in the morphological characteristics of the insect. The basal width of the SCa was significantly greater in females than in males (Table 4), and the number of SCa ($= 29.40 \pm 2.52$, $= 19.20 \pm 1.07$, $t = 3.73$, $P < 0.05$) was significantly greater in females than in males (Fig. 3).

3.2.3 Mandibular palpus

The mandibular palps of *G. cantor* also have a rich variety of sensor types, with no difference between male and female sensor types but some variation in the number of sensors (Fig. 5). The sensor species on mandibular palps can also be divided into seven main groups (11 subtypes): sensilla chaetica, sensilla twig basiconica, Böhm's bristle sensor (Bb), sensilla trichodea (ST II), sensilla plate (SP), sensilla coeloconica (SC) and sensilla campaniformia (SCa) (Table 5).

Table 5 The morphological characteristics of sensilla on mandibular palpus of both sexes in *G. cantor*

| Type | Subtype | Sex | Length (μm) | Width (μm) | Morphological character | Morphological c |
|--------------------------|---------|-----|--------------------------|-------------------------|---------------------------|-----------------|
| Sensilla Twig Basiconica | STB I | | 2.26 ± 0.10 | 2.04 ± 0.04 | Flat blunt cavity | None |
| | | | 2.33 ± 0.10 | 1.96 ± 0.05 | | |
| | STB II | | 2.35 ± 0.13 | 1.74 ± 0.08 | Rounded blunt projection | None |
| | | | 2.69 ± 0.14 | 1.60 ± 0.06 | | |
| Sensilla Chaetica | STB III | | 2.42 ± 0.16 | 1.89 ± 0.10 | Finger-shaped protrusions | None |
| | | | 2.63 ± 0.13 | 2.01 ± 0.10 | | |
| | STB IV | | $3.21 \pm 0.10^*$ | 2.28 ± 0.12 | Rounded blunt | None |
| | | | 2.62 ± 0.08 | 1.71 ± 0.10 | | |
| Sensilla Chaetica | SCh IV | | $50.24 \pm 1.57^*$ | 2.99 ± 0.13 | Sharp pointed | dense |
| | | | 42.56 ± 1.07 | 3.46 ± 0.14 | | |
| | SCh V | | $226.98 \pm 12.67^*$ | 7.40 ± 0.26 | Sharp pointed | dense |
| Böhm bristles | Bb | | 81.31 ± 4.93 | 3.29 ± 0.09 | Sharp pointed | Loose |
| | | | 8.62 ± 0.96 | $2.49 \pm 0.07^*$ | | |
| Sensilla Trichodea | ST II | | 8.46 ± 0.58 | 1.92 ± 0.07 | Blunt | Loose |
| | | | 37.19 ± 4.09 | 2.84 ± 0.18 | | |
| Sensilla Plate | SP | | $50.68 \pm 3.85^*$ | 3.04 ± 0.09 | Board shape | Loose |
| | | | 29.39 ± 0.84 | $2.65 \pm 0.14^*$ | | |
| Sensilla Coeloconica | SC | | 30.75 ± 0.76 | 1.27 ± 0.05 | Hole cavity | None |
| | | | — | 0.35 ± 0.02 | | |
| Sensilla Campaniformia | SCa | | — | $0.45 \pm 0.02^*$ | Rounded blunt protrusion | loose |
| | | | — | $2.60 \pm 0.13^*$ | | |

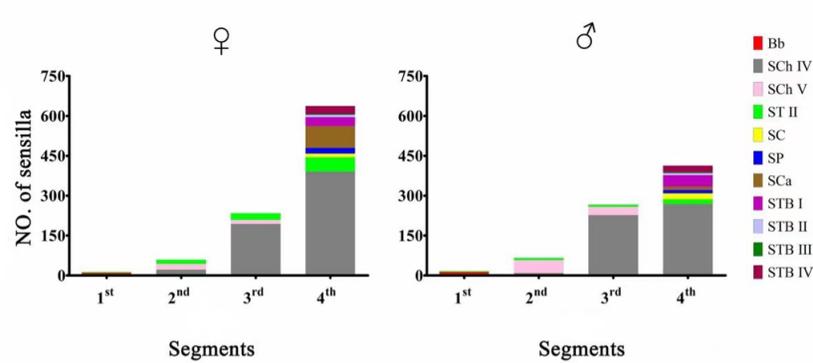


Fig. 5 Numbers and distributions of sensilla on mandibular palpus of both sexes of *G. cantor*

Sensilla twig basiconica

Similar to the labial palps, sensilla twig basiconica (STB) also occur only at the apical end of adult mandibular palps and can also be divided into four subtypes (STB I, STB II, STB III, and STB IV). The shape of these sensors varies markedly between subtypes (Fig. 6).

The terminal tapered inductor of the mandibular palpus showed little overall difference in external morphological features, but females likewise differed significantly from males in the length of STB IV ($t = 4.54$, $P < 0.01$). In terms of number, STB I ($t = -5.62$, $P < 0.01$) were more numerous in males than females, while females had significantly more STB IV ($t = 2.63$, $P < 0.05$) than males.

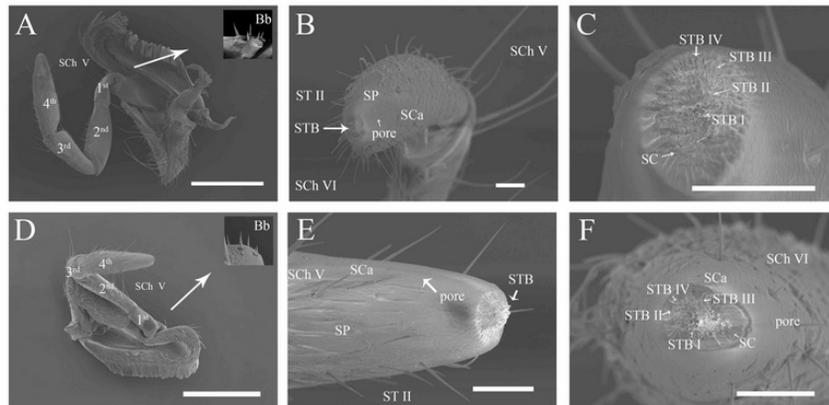


Fig. 6 The ultrastructure of mandibular palpus of both sexes of *G. cantor*

A, mandibular palpus of female beetle; B, Sensilla on 4th of mandibular palpus of female beetle; C, Sensilla styloconica on 4th of labial palpus of female beetle; D, mandibular palpus of male beetle; E, Sensilla on 4th of mandibular palpus of male beetle; F, Sensilla styloconica on mandibular palpus of male beetle Scale bars: A, D = 500 μm , B, C, E, F = 50 μm .

Sensilla chaetica

The sensilla chaetica of the adult mandibular palpus has two subtypes (SCh IV, SCh V). SCh IV is found on segments 2, 3 and 4 of the mandibular palpus, while SCh V is found on segments 2 and 3 of the mandibular palpus. These two inductor subtypes differ considerably in length and basal width (Table 5).

The lengths of SCh V and SCh IV of the female mandibular palpus were both longer than those of the males, but there were no significant differences in width. Quantitatively, females outnumbered males only in SCh IV ($= 226.98 \pm 12.67 \mu\text{m}$, $= 81.31 \pm 4.93 \mu\text{m}$, $t = 10.71$, $P < 0.01$) in segment 4 of the mandibular palpus, whereas males outnumbered females in SCh IV ($= 321.60 \pm 1.21$, $= 24.60 \pm 1.47$, $t = 7.58$, $P < 0.01$), and SCh V ($= 22.00 \pm 2.79$, $= 48.60 \pm 4.12$, $t = -5.35$, $P < 0.01$) were significantly more numerous in males than in females, and SCh IV ($= 193.60 \pm 6.52$, $= 226.60 \pm 5.84$, $t = -3.77$, $P < 0.05$) and SCh V ($= 14.80 \pm 3.38$, $= 31.60 \pm 1.44$, $t = -4.57$, $P < 0.01$) in segment 3 of the mandibular palpus were significantly more numerous in males than in females. Bohm's bristle sensor (Bb) also occurred at the junction of the base of the first segment with the mouthparts. There were no significant numerical differences in Bb between males and females in the labial palpus and mandibular palpus as a whole (Fig. 5).

Bohm bristles

The Bohm's bristle sensor (Bb) also founded that it just exists at the junction of the base of the 1st segment with the mouthparts. There were no significant numerical differences between males and females in the labial papuls and mandibular papuls as a whole (Figs. 5). The overall variability in morphological characters was also not significant, except for the presence of a significantly wider Bb base width ($= 2.49 \pm 0.07 \mu\text{m}$, $= 1.92 \pm 0.07 \mu\text{m}$, $t = 5.81$, $P < 0.01$) in the mandibular palpus of females than males (Tables 5).

Sensilla trichodea

Sensilla trichodea also occur in a small number in adult mandibular palps but only in one subtype, ST II. The length of female mandibular palpus ST II was significantly greater than that of males; female mandibular palpus segment 2 ($= 15.40 \pm 0.81$, $= 7.80 \pm 0.66$, $t = 7.25$, $P < 0.01$), segment 3 ($= 25.40 \pm 1.50$, $= 7.80 \pm 0.37$, $t = 11.36$, $P < 0.01$) and segment 4 ($= 53.80 \pm 7.45$, $= 17.60 \pm 0.68$, $t = 4.84$, $P < 0.01$) were significantly more numerous in ST II (Fig. 5).

Sensilla placodea

In the mandibular palpus, the SP ($= 2.65 \pm 0.14 \mu\text{m}$, $= 1.27 \pm 0.05 \mu\text{m}$, $t = 9.20$, $P < 0.01$) was significantly wider basally in females than in males. The basal width of the SP of the mandibular palpus was significantly wider in females than in males, but the length of the SP of the labial palpus was significantly longer in males than in females (Table 5). The number of SP in females was much greater than that in males in the mandibular palpus ($= 21.20 \pm 1.02$, $= 12.40 \pm 0.24$, $t = 8.39$, $P < 0.01$) (Figs. 3).

Sensilla coeloconica

Sensilla coeloconica (SC) also mainly occurred at the top of the terminal segments of the mandibular palpus of adults (Fig. 6).

Sensilla campaniformia

Both the basal width and the number of SCa in the mandibular palpus were significantly greater in females than in males (Table 5). SCa in the mandible palpus in females were all significantly more numerous than in males ($= 82.60 \pm 1.17$, $= 13.20 \pm 1.07$, $t = 43.89$, $P < 0.01$) (Figs. 5).

4 DISCUSSION

As largely consistent with reports for other pests, the olfactory system of *G. cantor* is also an ideal target for pest control (Wu et al., 2022). In this study, by analyzing the oral appendages of *G. cantor* with scanning electronic microscopy, we found several significant differences between males and females. On the one hand, in the gross morphology of the mandible, we found that the length and basal width of the mandibles of females were significantly more developed than those of males, but there was no significant difference in basal thickness. This suggests that the functional differences between the ossified parts of the mandible of

males and females are mainly the depth of groove production and have little effect on their shared feeding behavior. This is because the palate of females is involved in the grooving process and has a greater demand for mechanical sensing, especially for sensing the force required by the palate during the grooving process, so it is related to the sex difference in basal width and length. This has also been reported in insects such as the desert locust *Schistocerca gregaria* Forskal (Katel et al., 2021), in which the mandible participates in the grooving process, and there are some behavioral differences between males and females. On the other hand, in the ultrastructural morphology of the mandible, we found that the number of ST I in the female mandible was significantly higher than that in the male mandible. As an olfactory sensor, ST I can help females identify and confirm oviposition grooving more quickly through its olfactory in addition to feeding function. This result indicated that this olfactory sensor could help females recognize more chemical odors, which is consistent with the results of previous studies on other insects (Dong et al., 2020).

In addition, the oral appendages of adult *G. cantor* (labial palpus and mandibular palpus) are mainly involved in host selection, oviposition, mating and other behaviors (Zhang et al., 2018; Hall et al., 2019). In the gross morphology of the labial palpus and mandibular palpus, we also found that there was obvious dimorphism, which is consistent with *Aphis gossypii* Glover and *Diaphorina citri* Kuwayama (Ettay et al., 2001; Bento et al., 2017), especially in terms of length. Each subsegment and overall length showed obvious differences, and only the basal width of the fourth segment of mandibular palps did not show significant sex differences. This is related to the dominant role of labial and mandibular papillae in female oviposition.

It is worth mentioning that our results on the ultrastructural morphology of the labial palpus and mandibular palpus also confirmed this conjecture, and we found that the SCh V, SCh IV and Bb of female beetles are more developed than those of male beetles. A large number of studies have shown that the main function of the SCh is to focus on force and vibration induction, and the sex difference in the Bb in *G. cantor* is mainly related to its function of sensing the position and movement frequency of labial and mandibular cumulus hair during oviposition (Sun et al., 2011). Therefore, we hypothesized that this was because *G. cantor* needs more developed mechanical sensors to detect the vibration frequency and force of the oral appendages in the process of oviposition localization and recognition. Other sensilla showing female superiority mainly distributed in the labial and mandibular palpus of *G. cantor* also include ST II, STB IV, SC, SP and SCa. The functions of ST II have been confirmed in at least two different types of olfactory sensilla cells in the antennae of male cotton bollworms (Lopes et al., 2002; Dong et al., 2020). Similar functions of this sensor have also been confirmed in our study of the labial palpus and mandibular palpus of *G. cantor*. Moreover, some studies have shown that STB IV in *Liriomyza sativae* Blanchard is a kind of contact chemosensory apparatus and has taste function (Zhang et al., 2017). Based on this, we conducted a further investigation and found that STB IV shows female superiority in external morphology and quantity both in the labial palpus and mandibular palpus of *G. cantor*. In addition, SC, SP and SCa have been reported in a variety of insects, such as *Dyseriocrania subpurpurella* and *Pissodes yunnanensis* (Faucheux et al., 2008), showing that the main functions are carbon dioxide sensing and temperature and humidity sensing. We found that these functions also apply to the reproductive behavior of *G. cantor* because the oviposition process is highly complex, which requires the female to be sensitive to the appropriate temperature, humidity and carbon dioxide concentration in the environment, which is also in line with their relatively developed results in females.

In summary, the present study investigated the external morphology of the sensillum types of the oral appendages of *G. cantor* using scanning electron microscopy to contribute to a better understanding of host selection and courtship behavior in *G. cantor* and to future studies linking these behaviors to electrophysiological mechanisms. The observed differences in sensillum distribution and function will greatly facilitate the design of more effective standardized control methods against this pest.

5 CONCLUSION

We investigated, by means SEM, the external morphology and distribution of the oral appendages of *G. cantor*. We find that the gross morphology and ultrastructural morphology of mandible, labial and mandibular palpus are significantly different between sexes of *G. cantor* using scanning electron microscopy. Observation

of the gross morphology of the mandible, labial palpus and mandibular palpus revealed that their lengths were significantly greater in females. In terms of the ultrastructural morphology of the mandibles, females have significantly more ST I and SCh III on their mandibles than males. The mechanical sensors SCh and Bb, olfactory sensor ST II, taste sensor STB IV, and carbon dioxide sensor and temperature and humidity sensors SC, SP and SCa were significantly more developed on female than male labial and maxillary palps. These play an important role in reproductive behavior to provide new insights for the pest control and forest protection.

AUTHOR CONTRIBUTION

Yao Wu: Conceptualization, Methodology, Data curation , Investigation, Validation, Formal analysis, Writing - original draft; Zhixin Li: Software; Liangshan Ming: Validation; Zhongyan Huang: Software, Resources; Gaochuan Zhang: Visualization; Wen Lu: Supervision, Writing - review & editing; Hongning Liu: Project administration, Writing - review & editing; Zishu Dong: Conceptualization, Methodology, Data curation , Funding acquisition, Project administration, Resources, Writing - original draft, Conceptualization, Methodology, Data curation , Funding acquisition, Project administration, Resources, Writing - original draft

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DISCLOSURE STATEMENT

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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