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Morphology of the sensilla of larval antennae and mouthparts of the oriental fruit moth, *Grapholita molesta*

Yue Qin SONG[1,2], Hui Zhong SUN[1], Jun Xiang WU[2]
[1]*College of Forestry, Henan University of Science and Technology, Luoyang, China*
[2]*State Key Laboratory of Crop Stress Biology for Arid Areas, Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education / College of Plant Protection, Northwest A&F University, Yangling, China*

Abstract

The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera Tortricidae) is an important pest of stone and pome fruits worldwide, with its larvae boring in the fruit and shoot, frequently causing serious economic damage. The morphology of the larval antennae and mouthparts is described and illustrated with the aid of scanning electron microscopy, with particular focus on the sensilla. The antennae possess three types of sensilla: two sensilla chaetica, six sensilla basiconica, and a sensillun styloconicum. The labrum bears six pairs of sensilla chaetica. The epipharynx carries three pairs of flatten sensilla chaetica, three pairs of small epipharyngeal sensilla, and one pair of broad sensillum digitiformium. Each dentate mandible carries two sensilla chaetica on outer face. On the maxilla, both cardo and stipes carry one sensillum chaeticum. Each galea has three short-sharp sensilla basiconica, two large sensilla styloconica and three sensilla chaetica. The distal segment of the maxillary palp possesses seven sensilla basiconica, one sensillun styloconicum, laterally one sensillum digitiformium and two sensillum placodeum. Each labial palp bears distally a cone-shaped sensillum chaeticum and an elongate sensillum styloconicum. In addition, the functions of these sensilla are discussed by comparing them with those of other Lepidopteran larvae.

Key words: Lepidoptera, *Grapholita molesta*, larva, scanning electron microscope, ultrastructure.

Introduction

The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera Tortricidae), is one of the most destructive pests of stone and pome fruit (Rothschild and Vickers, 1991). The antennal morphology and sensilla of *G. molesta* adults have also been studied (George and Nagy, 1984), but no studies have investigated the micromorphology of its larvae. Here we provide the first description of the larval antennae and mouthparts and illustrate them by means of scanning electron microscopy.

Materials and methods

The *G. molesta* larvae were obtained from infested peach fruits in a peach orchard in Yangling, Shaanxi, China, in July 2010. Larvae were reared in a chamber with a photoperiod of 15:9 h (light: dark) at 24 ± 0.5 °C and 70 ± 10% relative humidity (RH) with an artificial diet (Yokoyama *et al.*, 1987).

We selected 20 fifth instars larvae for analysis using scanning electron microscopy (SEM) investigation. All larvae were fixed in Carnoy's fixative solution (95% ethanol: glacial acetic acid = 1:1 v/v) for 12 h. Samples were then preserved in 2.5% glutaraldehyde prepared in 0.2 M phosphate buffer (PBS) (pH 7.2) overnight at 4 °C. Subsequently, we rinsed samples three times with 0.2 M PBS for 30 min each time. Mouthparts were dissected from heads and heads with antennae were sonicated for 2 min in 0.2 M PBS. Then, the heads with antennae and each mouthpart were dehydrated in a graded series of 70, 80, 90, 95 and 100% ethanol for 15 min each. Next, the samples were transferred into acetone and isoamyl acetate three times for 30 min each and were kept in isoamyl acetate overnight. The next day, the samples were dried in a CO_2-criticalpoint dryer (Hitachi koki Co. Ltd., Tokyo, Japan). The dried samples were attached on the sample block with double-sided adhesive tape and sputtered with gold in Hitachi E-1030 (Tokyo, Japan). The specimens were examined in a Hitachi S-4800 SEM (Tokyo, Japan) operated at 10 kV accelerating voltage.

The images were labeled using Adobe Photoshop CS4. The length and basal width of each sensillum type of at least five larvae were measured by ImageJ software from scanning micrographs. Data (mean ± SE) were analyzed using SPSS 17.0. The sensilla were labeled according to the criteria proposed by Albert (1980).

Results

The head of the *G. molesta* mature larva is a strongly sclerotized capsule (width: 957.1 ± 23.5 μm, length: 761.7 ± 17.8 μm), oval and smooth, and hypognathous. Its surface has many long tactile sensilla (LTS, length: 268.3-359.7 μm) and short tactile sensilla (STS, length: 83-126.4 μm). The frontal portion of the head capsule has one pair of antenna, mouthparts, and six pairs of stemmata (figure 1).

Antenna

The larval antenna of *G. molesta* is rather short (mean: 178.9 ± 16.4 μm), and located between the stemmata and mandibles (figure 1). It is composed of three segments: scapus (width: 62.5 ± 1.6 μm, length: 66.4 ± 7.5 μm), pedicel (width: 46.0 ± 1.4 μm, length: 87.2 ± 7.8 μm), and flagellum (width: 16.5 ± 1.9 μm, length: 25.3 ± 1.9 μm; figure 2A). The scapus is the broadest segment and

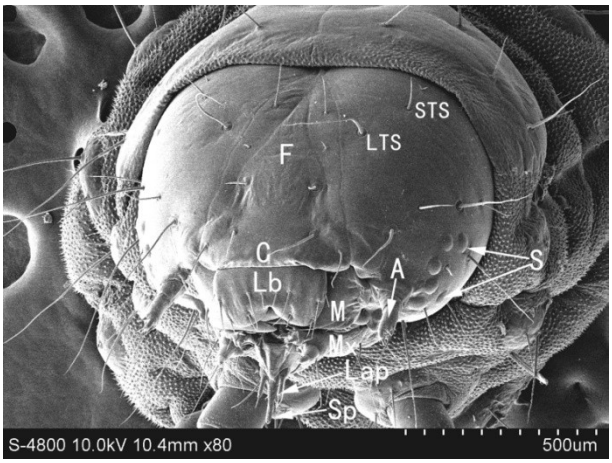

Figure 1. Frontal view of the head of *G. molesta* fifth instar larva. A, antenna; F, frontoclypeus; C, clypeus; S, stemmata; Lb, labrum; M, mandible; Mx, maxilla; LaP, labial palp; Sp, spinneret; LTS, long sensilla trichodea; STS, short sensilla trichodca.

is without sensilla. The medial pedicel bears three sensilla basiconica and two sensilla chaetica. The appearance of B1 and B2 is similar, while B3 is a slender and short peg. The aporous, smooth and socketed hairs of sensilla chaetica have very different length. C2 is much longer than C1. Both C1 and C2 are located at the distal margin of the pedicel (figure 2B-C). The distal flagellum carries three sensilla basiconica and an elongate sensillum styloconicum. The sensillum basiconicum B5, similar to B1 and B2, is much longer and larger than B4 and B6. B6 is inflated at the base and much thinner than B4 and B5. Sensillum B4 is the smallest and subcylindrical (figure 2D).

Labrum

The labrum is 142.4 ± 4.1 μm long and 281.7 ± 11.4 μm wide. Six pairs of aporous socketed sensilla chaetica are distributed over the dorsal surface (figure 3A).The membranous epipharynx carries three types of sensilla. Three broadly flattened aporous hairs (S1-3) are clustered on the margin of each lobe. There are three dome-shaped sensilla with a small sharply pointed apex located on each side of the epipharynx (Sc1-Sc3), named "epipharyngeal sensilla" by Boer *et al.* (1977). Two broad sensilla digitiformia (D) are present along the left and right lateral margin of the epipharynx extend to S3. The basal portion of the epipharynx is covered with numerous microtrichia (M) arranged in rows (figure 3B).

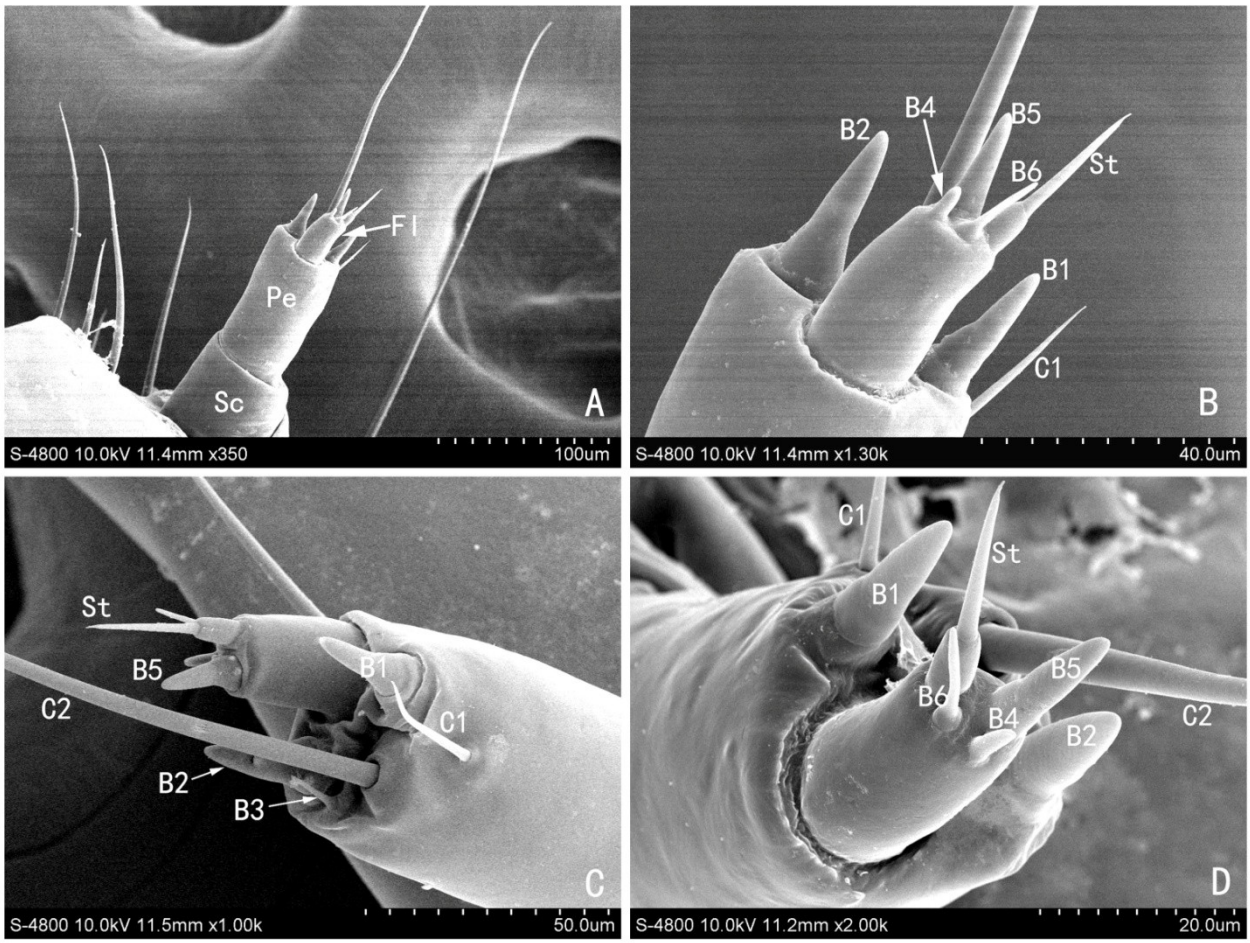

Figure 2. Morphology and structure of the antenna of *G. molesta* fifth instar larva. (**A**) ventral view of the three-segmented antenna; (**B**) ventral magnifying view of the antenna; (**C**) dorsal magnifying view of the antenna; (**D**) distal magnifying view of the antenna. Sc, scapus; Pe, pedicel; Fl, flagellum; C1-C2, sensilla chaetica; B1-B6, sensilla basiconica; St, sensillum styloconicum.

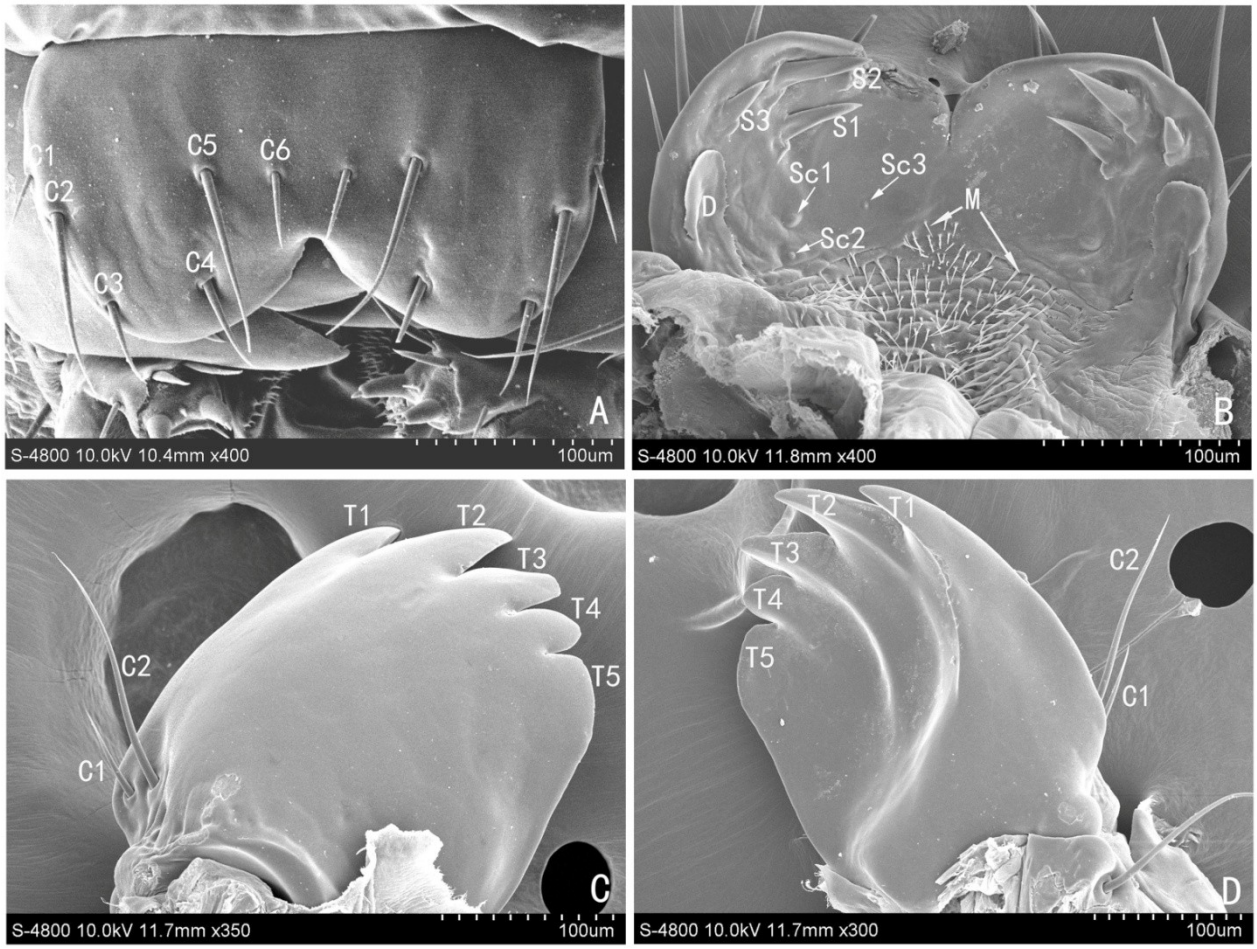

Figure 3. Labrum and mandible of *G. molesta* fifth instar larva. (**A**) Frontal view of the labrum, showing six pairs of sensilla chaetica (C1-C6). (**B**) Inner view of the epipharynx, showing three flattened sensilla chaetica (S1-S3), three epipharyngeal sensilla (Sc1-Sc3), , a sensillum digitiformium (D) and numerous microtrichia (M) in rows. (**C**) Inner view of the mandible, showing two sensilla chaetica (C1-C2). (**D**) Outer view of the mandible, showing five distal teeth (T1-T5).

Mandible

Each mandible is 256.0 ± 13.1 µm long, 222.6 ± 3.4 µm wide, strongly developed, and sclerotized. The apex of each mandible is dentate, with five teeth. Two long sensilla chaetica situated on the basal part of outer surface. Therefore C2 is almost three times longer than C1 (figure 3C-D).

Hypopharynx

The central mouthpart has a tongue. Both sides of the tongue crimp to the mouthpart centre and bear numerous microtrichia in rows on the margin of the tongue (figure 4A).

Maxillary galeae

Each maxilla consists of the proximal cardo (width: 91.5 ± 2.4 µm, length: 59.5.0 ± 7.6 µm) and distal stipes segment (width: 82.4 ± 0.2 µm, length: 41.5 ± 0.5 µm). The two-segmented lateral palp (the base segment: 52.1 ± 7.27 µm long, 32.7 ± 0.7 µm wide; the distal segment: 45.3 ± 4.1 µm long, 16.6 ± 0.6 µm wide) and the medial galea arise from the stipes distally (figure 4A-B). Both cardo and stipes segment carry each a long sensillum chaeticum C1and C2 (figure 4B-C). Other sensilla are assembled on the distal surface of the galea and maxillary palp.

The galea bears eight sensilla on its distal surface. Two large sensilla styloconica St1 and St2 are located in the medial of galea terminal. There was a medial sensillum chaeticum C3 on the lateral wall of the galea. Two sensilla chaetica C4 and C5 are flattened and long, and located along the dorsal margin of the galea. The aporous sensillum basiconicum B1 is sharp and located near hypopharynx. B2 is filiform and situated between the St1 and St2. B3 is short at the edge of the galea (figure 4C).

Maxillary palps

The two segment maxillary palps are 89 ± 13.3 µm long. The surface of the distal segment in the palp is cratered. They have not been found to traverse the inner wall (figure 5). The spatial distribution of the eight sensory pegs at the tips of the right and left palps are as follow: seven sensilla basiconica and a sensillum styloconicum. The terminal segment of the maxillary palp also bears an elongate and broad digitiform sensillum, which lies in the outer wall of the maxillary palp, which is attached at the base and free along its length. Two sensilla placodea are situated on the lateral side of the distal segment (figure 4D).

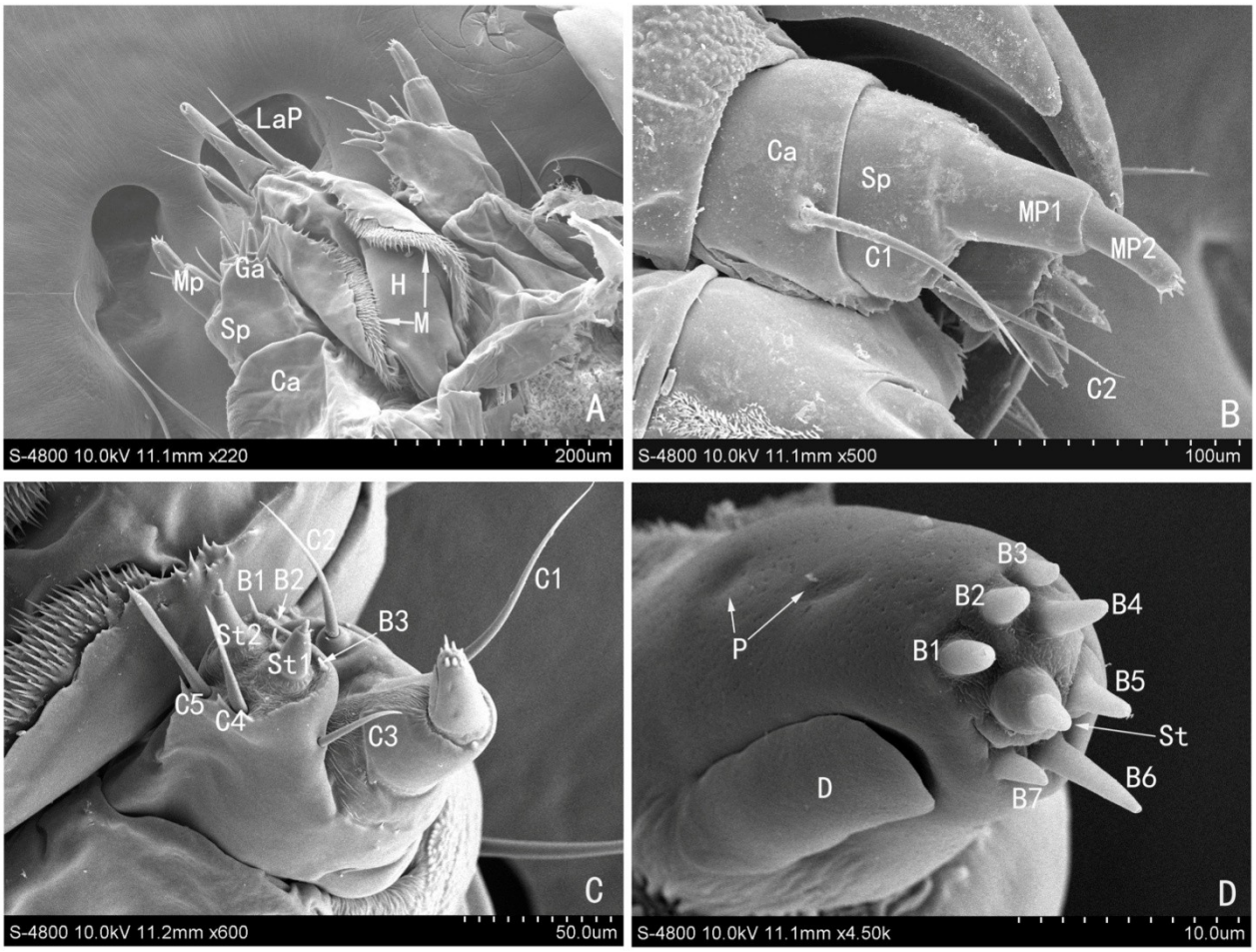

Figure 4. Maxillae of *G. molesta* fifth instar larva. (**A**) The inner view of maxilla and hypopharynx, showing the cardo (Ca), stipes (Sp), galea (Ga), maxillary palp (Mp), Hypopharynx (H) and numerous microtrichia (M). (**B**) The profile view of the maxilla, showing two sensilla chaetica (C1-C2) and two-segmented (MP1-MP2). (**C**) The distal view of the galea, showing three sensilla chaetica (C3-C5), three sensilla basiconica (B1-B3) and sensilla styloconica (St1-St2). (**D**) The distal segment of the maxillary palp, showing seven sensilla basiconica (B1-B7), a sensillum styloconicum (St), a sensillum digitiformium (D) and two sensillun placodeum (P).

Labium

The labium bears a pair of palps (length: 90.9 ± 5.0 μm) and a long tube-like spinneret (length: 109.7 ± 3.7 μm), from which silk can be secreted by the larva (figure 6A). The labial palp bears a long sensillum styloconicum and a short cone-shaped sensillum chaeticum. The sensillum styloconicum has a bulbous base and a hair-like apical portion. The sensillum chaeticum was situated on the dorsal side of the palp (figure 6B).

At the base of the labium are located two small sensilla pegs (P1 and P2, mean = 24 μm long), which possess an alveola (mean = 4.2 ± 0.7 μm) (figure 7). The surface of the palps has many multiporous.

Discussion

Insect sensilla play an important role in olfactory and gustatory sensation. In this study, sensilla on the larval antennae and mouthparts were examined in the oriental fruit moth, *G. molesta* with scanning electron microscopy. The types, numbers, and distribution of sensilla on the larval antennae of *G. molesta* were similar to *Carposina sasakii* Matsumura (Liu *et al*., 2011), *Pentateucha inouei* Owada et Brechlin (Lin, 2002), and *Homoeosoma*

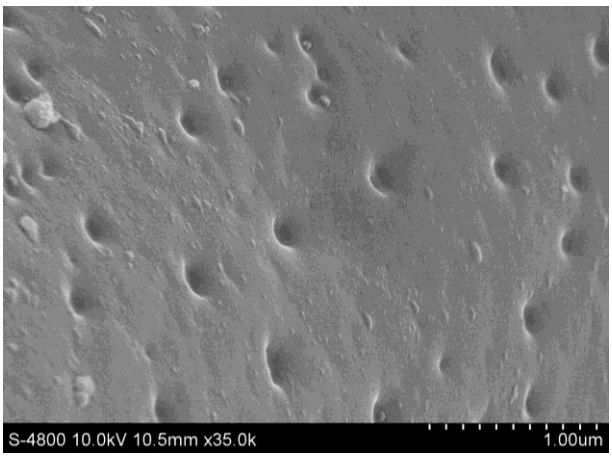

Figure 5. The cratered surface of the distal segment of the maxillae palp.

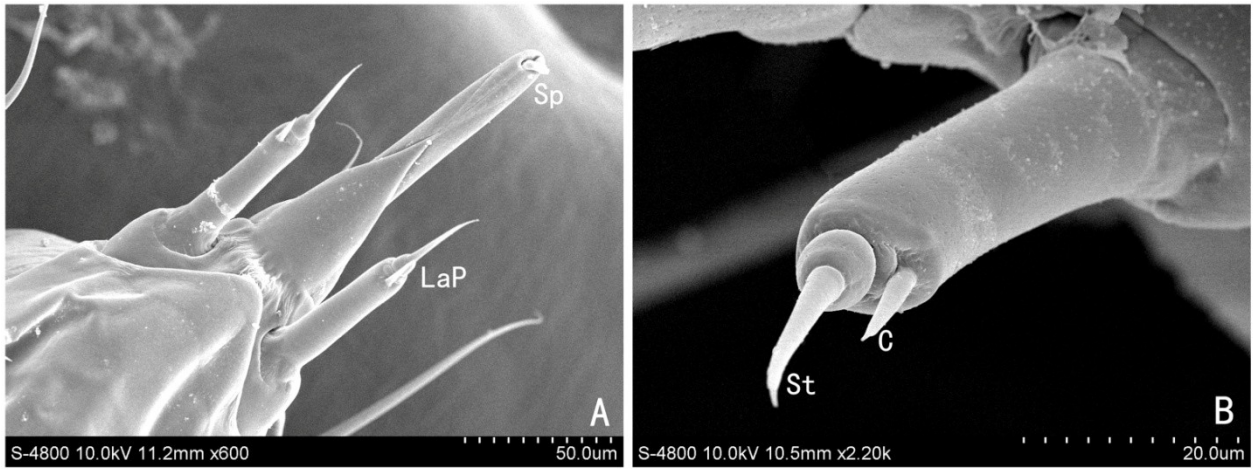

Figure 6. Labium of *G. molesta* fifth instar larva. (**A**) The dorsal view of the labium, showing two labial palps (LaP) and a spinneret (Sp). (**B**) The magnifying view of multiporous palp, showing a sensillum chaeticum (C) and a sensillum styloconicum (St).

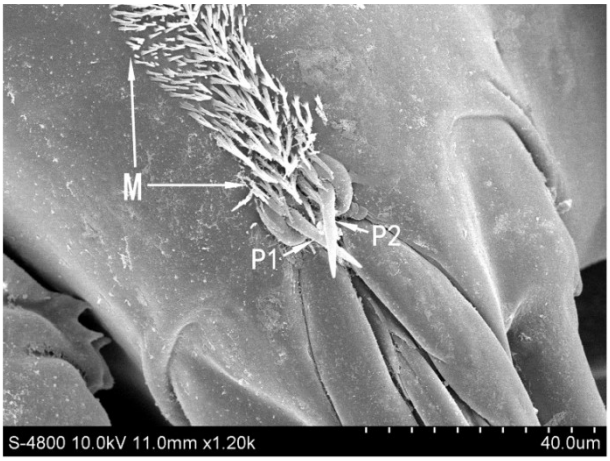

Figure 7. Ventral view of the labium of *G. molesta* fifth instar larva, showing two crossed sensilla pegs (P1-P2) with alveola at base and numerous microtrichia (M).

nebulella (Denis et Schiffermuller). (Faucheux, 1995) even though the morphological appearances are different. The structure and function of the sensilla chaetica, sensilla styloconica, and sensilla basiconica have already been established through structural, electrophysiological, and behavioral observations. The large sensilla basiconica (1, 2, 4, 5) on the antennae were reported to possess olfactory function (Zacharuk, 1985). The antennal sensilla styloconica are suggested as temperature cold-sensitive receptors, and the small sensilla basiconica 3 and 6 also were involved in this function (Schoonhoven, 1967). The antennal sensilla chaetica are detecting tactile stimuli (Schoonhoven, 1967; Albert, 1980; Zacharuk, 1985; Baker *et al*., 1986; Faucheux, 1995).

On the labrum, there are six pairs of sensilla chaetica. This number is similar to other Lepidoptera, and reported to have mechano-receptive functions in this taxonomic group (Albert, 1980; Faucheux, 1995; Davis *et al*., 2008). The epipharynx of *G. molesta* possessed six epipharyngeal sensilla, which differs from most larvae that have only two (Boer *et al*., 1977; Albert, 1980) or no epipharyngeal sensilla (Devitt and Smith, 1982). These sensilla are probably gustatory receptors (Albert, 1980). Three broadly flattened aporous hairs on the epipharynx are recorded for mechanoreceptor (Devitt and Smith, 1982; Faucheux, 1995).

The two sensilla chaetica on the mandible are mechanoreceptors (Albert, 1980; Kent and Hildebrand, 1987). Each mandibula bears five teeth for cutting off pieces of food and crushing them. There are few reports about the sensilla on the hypopharynx. Faucheux (1995) found that there are no sensilla on the hypopharynx in *H. nebulella*, while *G. molesta* has only many agnails in rows on the edge of the hypopharynx. The functions of these sensilla may be help to evaluate food quality.

The most notable variation in the larval mouthparts of Lepidoptera exists on the maxillae. On each maxillary palp, *G. molesta* possesses eight apical sensilla, seven sensilla basiconica and one sensilla styloconicum in contrast with eight sensilla basiconica in other Lepidoptera (Grimes and Neunzig, 1986; Faucheux, 1995). This situation may be attributed to its style of concealed feeder boring inside fruits (Keil, 1996; Lin, 2002; Liu *et al*., 2011). They are probably related to olfactory functions (Schoonhoven, 1972; Devitt and Smith, 1982) and may also have mechanoreceptory functions (Schoonhoven and Dethier, 1966). The sensillum digitiformium on maxillary palp seems to be a consistent structure in larvae since it has been found in 36 species of Lepidoptera (Devitt and Smith, 1982; Baker *et al*., 1986; Keil, 1996; Lin, 2002; Liu *et al*., 2011), even though the distribution and external appearance are different. Devitt and Smith (1982) reported the sensillum digitiformium is sensitive to temperature by the lamellated structure of the single dendrite. The sensilla placodeum in other larvae was reported to possess olfactory function (Devitt and Smith, 1982; Baker *et al*., 1986; Keil, 1996). The surface of the maxillary palps and labium palps is cratered, the same situation is found in the maxillary palps of *Helicoverpa armigera* (Hubner) (Keil, 1996). Their function is unclear because not been found to traverse the inner wall as should be the case in olfactory sensilla.

The sensilla chaetica C1-5 of the maxillae are tactile (Schoonhoven and Dethier, 1966; Hanson, 1970), especially sensilla C4-5 are undoubtedly stimulated by contact with food and provide information on food texture. The sensilla styloconica and sensilla basiconica on the galea have been shown to be important chemosensory function for modifying feeding behaviour (Albert, 1980). Ishikawa *et al.* (1969) used amputation experiments to confirm the important function of sensilla styloconica in food recognition.

The two sensilla on the labial palp have been examined electrophysiologically and were shown to be typical mechanoreceptors (Albert, 1980; Devitt and Smith, 1982; Faucheux, 1995). Two small sensilla pegs under the labium also are found in *H. nebulella* (Faucheux, 1995) and *C. sasakii* (Liu *et al*., 2011), however, the structures and functions are not clear. The description of sensilla types in *G. molesta* will serve as a basis for future electrophysiological studies, aiming to understand feeding preferences with a perspective to the development of future control methods.

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Authors' addresses: Jun Xiang WU (corresponding author: junxiangwu68@163.com), College of plant protection, Northwest A&F University, No.3 Taicheng Road, Yangling 712100, Shaanxi, China; Yue Qin SONG (songyueqin6@163.com); Hui Zhong SUN (huizhong66@163.com).

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