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Ultrastructure of Sensory Equipments on the Heads of *Kallitaxila granulata* (Stål) (Hemiptera: Fulgoromorpha: Tropiduchidae)

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ABSTRACT The polyphagous planthopper, *Kallitaxila granulata* (Stål) (Hemiptera: Fulgoromorpha: Tropiduchidae), has been recently introduced into southeastern China, the Philippine islands, and Hawaii, where it has done significant damage to agricultural and forest ecosystems. The external morphology of the heads of adult male and female *K. granulata* was examined using scanning electron microscopy. Seven types of sensilla were reported: trichoid sensilla and campaniform sensilla on the antennal pedicel, antennal scape and maxilla; plate organs on the antennal pedicel; coeloconic sensilla in Bourgoin's organ on the expanded flagellar base; ampullaceal sensilla on the antennal pedicel; wavy-pit sensilla on the antennal pedicel and antennal scape; and coin-shaped sensilla on each lateral side of the labium. Evans' organ was described as placoid sensilla sunk into shallow cuticular cavities below the antennae. The external morphology, distribution, and abundance of sensilla located on antennae, maxillae, and labium in *K. granulata* were illustrated, with a brief discussion of their taxonomic, phylogenetic, and putative functional significance. *Microsc. Res. Tech. 75:1659–1665, 2012.* © 2012 Wiley Periodicals, Inc.

INTRODUCTION

The infraorder Fulgoromorpha, also known as planthoppers, include some of the world's most devastating agricultural pests, which feed on major agricultural crops across the globe. They reduce crop yield through feeding and oviposition and they can transfer phytoplasmas and viruses to a wide range of plants (Denno and Perfect, 1994). The planthoppers of economic importance in the United States have been reviewed by Wilson and O'Brien (1987), who recorded 150 species from 99 economic plants.

Kallitaxila granulata (Stål) (Hemiptera: Fulgoromorpha: Tropiduchidae), a polyphagous planthopper previously found causing damage to crop plants, e.g., guava (*Psidium guajava* L.), grapefruit (*Citrus paradisi* Macfarlane), hapuu (*Cibotium chamissoi* Kaulfuss), uluhe (*Dicranopteris linearis* Underwood), and kukui (*Aleurites moluccana* (L.) Willdenow) in southeastern China, the Philippines, and Hawaii (Yang et al., 2001). Because of its wide host-plant and range, its feeding and oviposition effects on host plants, and its relationships with other phytophagous insects via shared natural enemies, *K. granulata* probably plays a complex role in ecosystems where the species is present (Yang et al., 2001). For other planthoppers, this role is probably mediated mainly by semiochemicals produced by conspecifics and other associated taxa, particularly the host plants (Bourgoin and Deiss, 1994; Obata et al., 1981; Roberto et al., 2009; Srinivas et al., 1997; Young, 2002). A better knowledge of the sensory equipments of this species may therefore be of use in the development of control plans.

Although some sensory equipments on the antennae, maxillae, and labium in several other species of planthoppers have been reported, little information is available regarding Tropiduchidae (Stroiński et al., 2011). *K. granulata* has yet to be studied in this way.

The present article provides detailed information regarding the external morphology and the number and distribution of these sensilla found in adult male and female *K. granulata*. Scanning electron microscopy (SEM) observations provided new character sets for future comparative morphological studies in Tropiduchidae and Fulgoroporpha.

MATERIALS AND METHODS

Adult of *K. granulata* (six males and eight females) were obtained from China (Fujian and Taiwan) and the Philippines. For scanning electron microscopy (SEM), the head together with antennae and labium was

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removed from the body. Because *K. granulata* was covered with a waxy powder, several cleaning methods were tested to obtain the best results. The specimens were first immersed in chloroform bath or lukewarm 10% KOH bath in an ultrasonic cleaner for 1 min to remove the cuticular wax powder, and cleaned twice in 75% alcohol (2 min for each case). The specimens were then dehydrated in a graded ethanol series followed by critical point drying and coated by gold. Observations were made with a HITACHI S34Q SEM (Hitachi, Tokyo, Japan) at the Microscopy Core Facility, Biological Technology Center, Beijing Forestry University. Terminology for the sensilla description follows Bourgoin and Deiss (1994), completed in Stroiński et al. (2011).

RESULTS

Gross Morphology of the Antennae

In *K. granulata*, the antennae were found laterally on the head capsule underneath the eyes (Fig. 1A). In both male and female specimens, each antenna was about 700 μm long, with a long, wire-like flagellum pointing toward the external side (Fig. 1A). The antennae were comprised of three segments: a short ring-like antennal scape connecting the antenna to the head capsule, a stout cylindrical antennal pedicel, and a long bristle-like flagellum (Fig. 1B). The antennal scape was about 35 μm long and bore few sensilla (Fig. 1E), and the antennal pedicel was about 130 μm long and was covered with sensory equipments (Figs. 1B and 1C). The flagellum was found to be composed of two distinct portions, a basal bulb and an apical arista (Fig. 1B). The expanded flagellar base was inserted in the apex of the antennal pedicel at the level of a disk-like, concave socket (Figs. 1C and 1D). This area was encircled by concentrically arranged cuticular spines (Fig. 1D). The distal part of the bulb gave rise to a thread-like arista, which was about 450 μm long and ended in a sharp apex (Fig. 1B). At the base of the arista, an evident Bourgoin's organ, with an aperture surrounded by petal-like walls, was observed (Fig. 3D).

Types and Distribution of Antennal and Maxillary Sensilla

Serial SEM images showed six types of sensilla: trichoid sensilla, plate organs, campaniform sensilla, coeloconic sensilla, ampullaceal sensilla, and wavy-pit sensilla. The trichoid sensilla, having three subtypes, were found to be widely distributed on the antenna, maxilla and labium, while plate organs were situated around the apex of the antennal pedicel (Figs. 1C and 2A). The coeloconic sensilla were presented only inside the Bourgoin's organ at the flagellar basal bulb (Fig. 3D), while ampullaceal sensilla were discovered at the apex of the antennal pedicel (Figs. 3E–3G). Two campaniform sensilla were situated near the apical sockets of the antennal pedicel (Figs. 1C and 1D) and on the lateral surface of the antennal scape (Figs. 1E and 1F) respectively. Wavy-pit sensilla, which were found to be distributed on both antennal pedicel (Fig. 2C) and antennal scape (Figs. 1E and 1G), were here reported for the first time. On the head capsule, three campaniform sensilla with similar structures were found at the base of the antennal scape (Figs. 1I–1K and 2D).

Trichoid Sensilla Subtype I (TrI). TrI were hair-shaped and scattered on the surface of the antennal pedicel (Figs. 1C, 2A, 2B, and 3G). They were found to be 28.6–66.7 μm long (1.9–3.1 μm in basal diameter), with straight grooves on the surface (Fig. 2B). Each of them was inserted into an evident raised socket, bearing a basal pore of 0.8–1.5 μm in diameter and protruding between 30° and 45° from the antenna (Figs. 2A, 2B, and 3G). TrI in the proximal part of the antennal pedicel tended to be shorter than those in more distal part (Fig. 2A). TrI with bi-forked apexes were occasionally observed (Fig. 2B). Cross sections of TrI show a fine channel surrounded by longitudinal fibers in each TrI (Fig. 2A). Trichoid sensilla on frons, seemingly similar with TrI on the antennal pedicel, were also observed (75.0–84.5 μm long, Fig. 2E).

Trichoid Sensilla Subtype II (TrII). TrII were usually limited on dorsal-lateral apex of the antennal pedicel around the plate organs (Figs. 1C, 3A, and 3F). They were found to be 16.9–27.1 μm long (2.5–2.7 μm in diameter at base), blunt-tipped (Fig. 2F), bent shafts (Figs. 1C, 3A, and 3F), and hollow (Fig. 2I) or with fibers inside (Fig. 2H). This subtype of trichoid sensilla was smaller than TrI. They were found inserted into depressions (4.2–4.8 μm in diameter) lacking specialized raised sockets (Fig. 2F). Straight patterns and several tiny pits were found on the surfaces of TrII (Figs. 2F and 2G).

Trichoid Sensilla Subtype III (TrIII). TrIII were widely distributed on the antennae (14.6–20.3 μm long, Figs. 1C and 2C), labium (47.7–81.5 μm long, Fig. 4F), and maxillae (5.4–12.1 μm long, Figs. 1I and 2D). TrIII had the structure similar to that of TrI but could be distinguished from TrI by the raised socket lacking a pore.

Plate Organs (PO). In *K. granulata*, only 10 PO were circularly situated on the distal surface of the antennal pedicel (Fig. 1C), each including a plate externally surrounded by 5–8 cuticular denticles (Figs. 1C, 2A, 3A, 3B, and 3E–3G). The edge of the plate (6.0–7.5 μm in height, 13.1–19.0 μm in diameter, and 0.8–1.2 μm thick) turned up in four to seven places giving rise to petal-like structures (Figs. 3A and 3B). Some of them were double-petalled in places (Figs. 3A and 3B). Ten or more shallow pits have been detected on the flat central area of PO (Fig. 3C). PO in *K. granulata* could be considered folded-flattened plates or clover leaf-like structures (Stroiński et al., 2011).

Campaniform Sensilla Subtype I and II (CaI, CaII). A single CaI (about 8.5 μm in diameter) was found at the apical surface of the antennal pedicel near the expanded flagellar base (Figs. 1C, 1D, and 3F). Another CaII (about 9.5 μm in diameter) was situated on the ventral surface of the antennal scape (Figs. 1E and 1F). Both of them were dome-shaped and surrounded by thick walls (Figs. 1D and 1F). CaI was found in a deeper cavity with two big denticles (Fig. 1D), whereas CaII rose up from the surface by about 3.4 μm and had no cuticular denticles surrounding it (Fig. 1F).

Campaniform Sensilla Subtype III (CaIII). Three CaIII (11.0–13.7 μm in diameter) with structures similar to CaII were found at the base of the antennal scape (Figs. 1I–1K and 2D). They could be distinguished from CaI easily due to the lack of cuticular denticles. CaIII also had a tiny pit (about 0.5 μm in diameter, Fig. 1K) on its surface in the center.

Coeloconic Sensilla (Co) and Bourgoin's Organ (BO). At the base of the flagellar arista, an evident BO was detected (Fig. 3D) with an aperture surrounded by

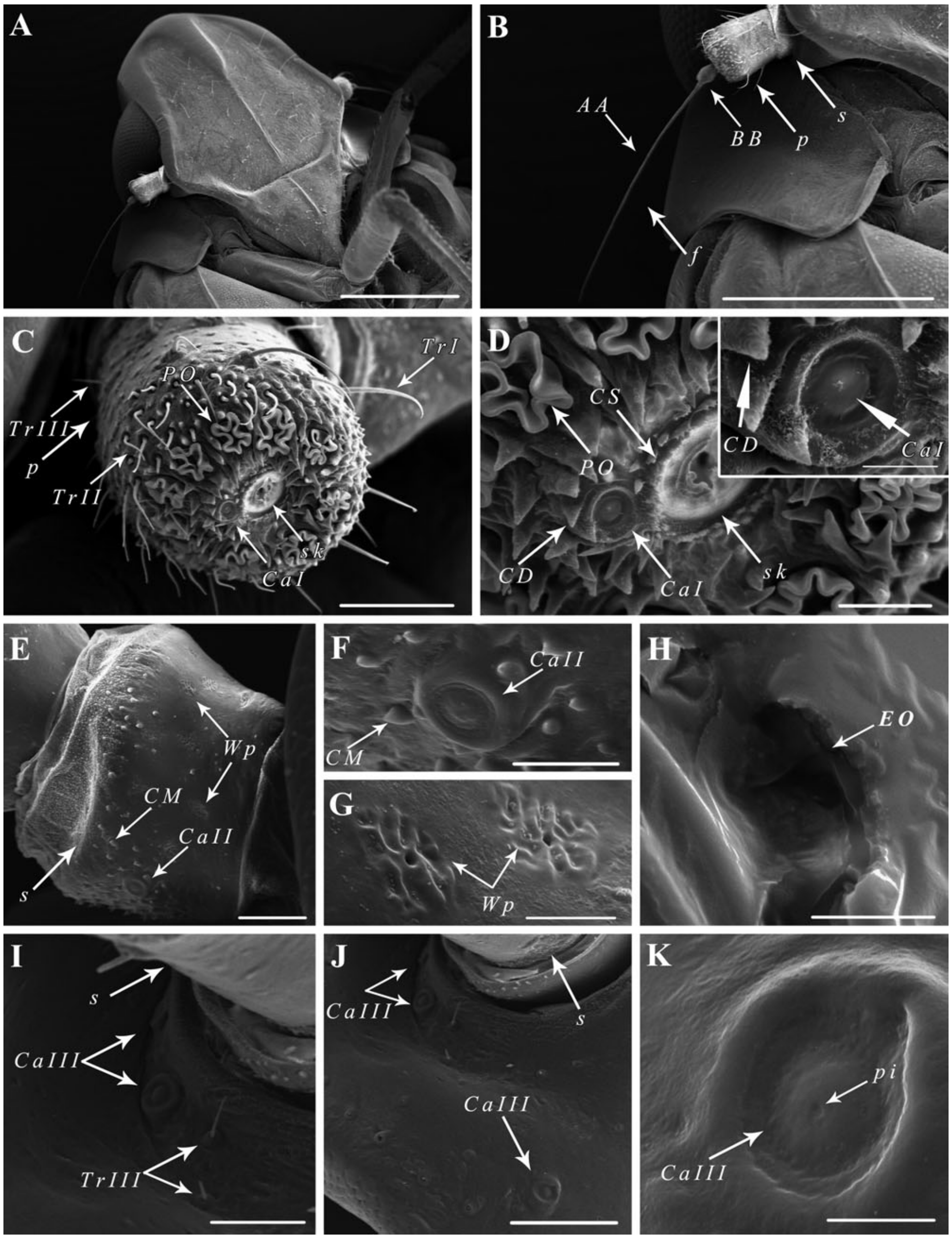

Fig. 1. SEM images of *Kallitaxila granulata.* **A**: Head capsule of *K. granulata* showing the position of the antennae. **B**: General view of the antenna. **C**: General view of the antennal pedicel. **D**: Distal part of the antennal pedicel. **E**: General view of the antennal scape. **F**: Campaniform sensillum subtype II and cuticular microtubercles on the antennal scape. **G**: Wavy-pit sensilla on the antennal scape. **H**: Evans' organ on the maxilla. **I**: Maxillary part under the antennal scape. **J**: Campaniform sensilla subtype III on the maxilla under the antennal scape. **K**: Campaniform sensillum subtype III on the maxilla. AA, apical arista of the flagellum; BB, basal bulb of the flagellum; CaI, campaniform sensilla subtype I; CaII, campaniform sensilla subtype II; CaIII, campaniform sensilla subtype III; CD, cuticular denticles; CM, cuticular microtubercles; CS, cuticular spines; EO, Evans' organ; f, flagellum; p, antennal pedicel; pi, pit; PO, plate organs; s, antennal scape; sk, socket; TrI, trichoid sensilla subtype I; TrII, trichoid sensilla subtype II; TrIII, trichoid sensilla subtype III; Wp, wavy-pit sensilla. [(A) scale bar 500 µm, (B) scale bar 400 µm, (C, J) scale bar 50 µm, (D) scale bar 15 µm, 5 µm in box, (E) scale bar 25.5 µm, (F, H) scale bar 10 µm, (G, K) scale bar 5 µm, (I) scale bar 25 µm.]

four to five petal-like walls, each about 2.5 µm long (Fig. 3D). The antennal second projections (Shih and Yang, 1996), also called basal flagellar processes (Liang, 2001) were absent from this species. Additionally, about 10 cone-shaped cuticular processes were observed below the walls, consecutively circled around the flagellar basal bulb (Fig. 3D). Co were usually sheltered by BO (Bourgoin, 1986; Roberto et al., 2009).

Ampullaceal Sensilla (Am). The Am were some tiny basiconic structures inserted in depressions (1.7–2.3 µm in diameter) beside PO (Figs. 3E–3G). With blunt tips and rough surfaces, the basiconic projections were 1.3–2.2 µm long and 0.8–1.1 µm in basal diameter.

Wavy-Pit Sensilla (Wp). Only after the waxy powder was completely removed could we find Wp, which

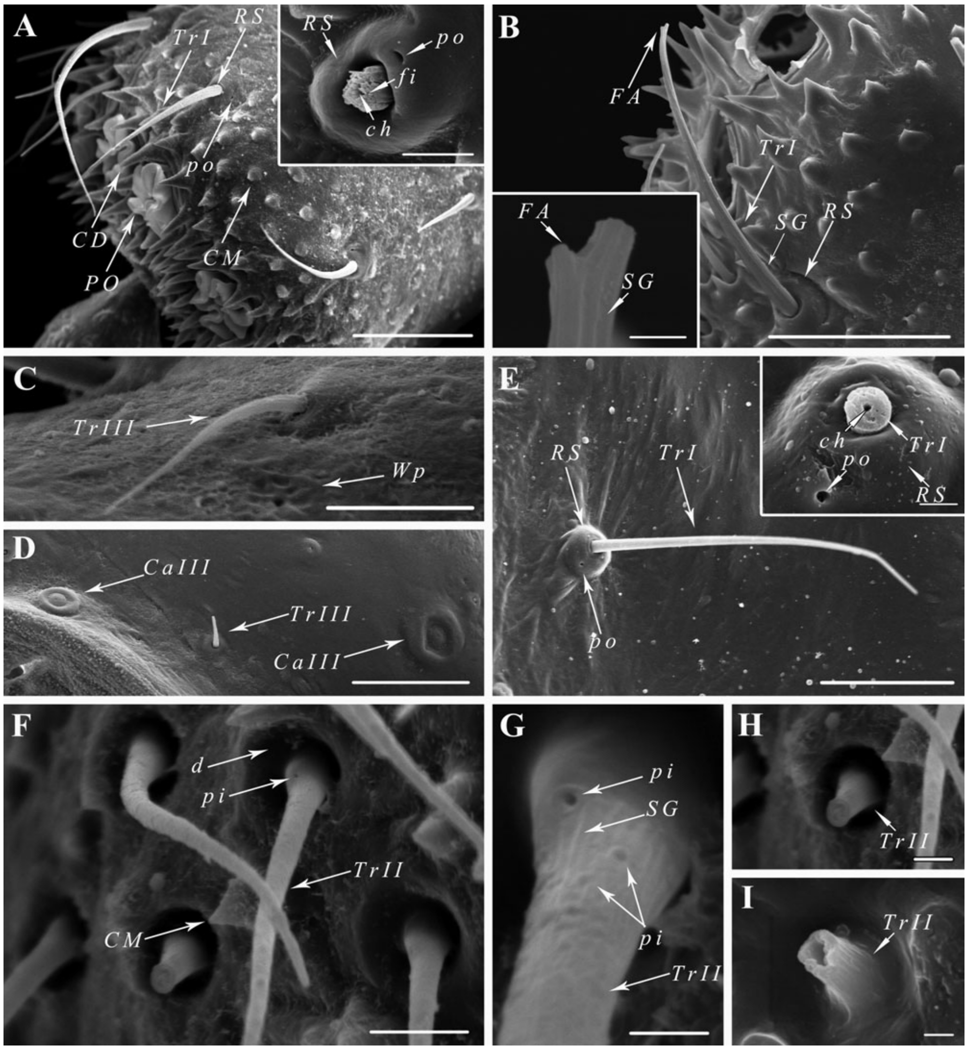

Fig. 2. SEM images of trichoid sensilla on the antennal pedicel, frons and maxilla of *Kallitaxila granulata.* **A**, **B**: Trichoid sensilla subtype I on the antennal pedicel. **C**, **D**: Trichoid sensilla subtype III on the antennal pedicel and the maxilla, with wavy-pit sensilla and campaniform sensilla subtype III nearby. **E**: Trichoid sensilla subtype I on the frons. **F**–**I**: Trichoid sensilla subtype II on the antennal pedicel. CaIII, campaniform sensilla subtype III; CD, cuticular denticles; ch, channel; CM, cuticular microtubercles; d, depression; FA, forked apex; fi, fibers; pi, pit; po, pore; PO, plate organs; RS, raised socket; SG, straight grooves; TrI, trichoid sensilla subtype I; TrII, trichoid sensilla subtype II; TrIII, trichoid sensilla subtype III; Wp, wavy-pit sensilla. [(A) scale bar 30 μm, 5 μm in box, (B) scale bar 30 μm, 1 μm in box, (C) scale bar 10 μm, (D) scale bar 30 μm, (E) scale bar 30 μm, 2 μm in box, (F) scale bar 5 μm, (G, I) scale bar 1 μm, (H) scale bar 2 μm.]

were widely distributed on the surfaces of the antennal pedicel (Fig. 2C) and antennal scape (Figs. 1E and 1G). This type of sensilla consisted of a wrinkled cuticular surface (5.0–8.0 μm in diameter), like a wave, with a tiny pore (about 0.7 μm in diameter) in the center (Fig. 1G).

Evans' Organs (EO). EO were present at the dorsal margin of the maxillary plates in both male and female specimens (Fig. 1H), with a distance of roughly 70.6 μm above the frontoclypeal structure and below the antennae and eyes. Each EO was formed by a shallow cavity (10.8–13.5 μm in diameter) with some cuticular infoldings in the center (Fig. 1H).

Other Antennal Structures

Cuticular Microtubercles (CM). CM were nonsensory cuticular structures present in great numbers on the dorsal surfaces of the antennal pedicel and antennal scape (Figs. 1E, 1F, 2A, 2F, 3E, and 3G). In *K. granulata*, they were oblate cone-shaped structures with blunt tips (1.0–3.0 μm in length).

Cuticular Denticles (CD). These structures were regularly present with five to eight in number surrounding the petal-like projections of each PO (Figs. 1D, 2A, 3A, 3B, 3E, and 3G). The CD were cone-shaped (8.2–11.9 μm long, 3.5–4.3 μm in basal diameter) with a smooth surface and an acute apex. They were more sclerotized and straighter than the petal-like projections of PO.

Gross Morphology of the Labium

K. granulata had a highly modified labium adapted to piercing and sucking. The labium was three-segmented: the shortest proximal segment, which was partially obscured by the overlapping clypeus, the longest

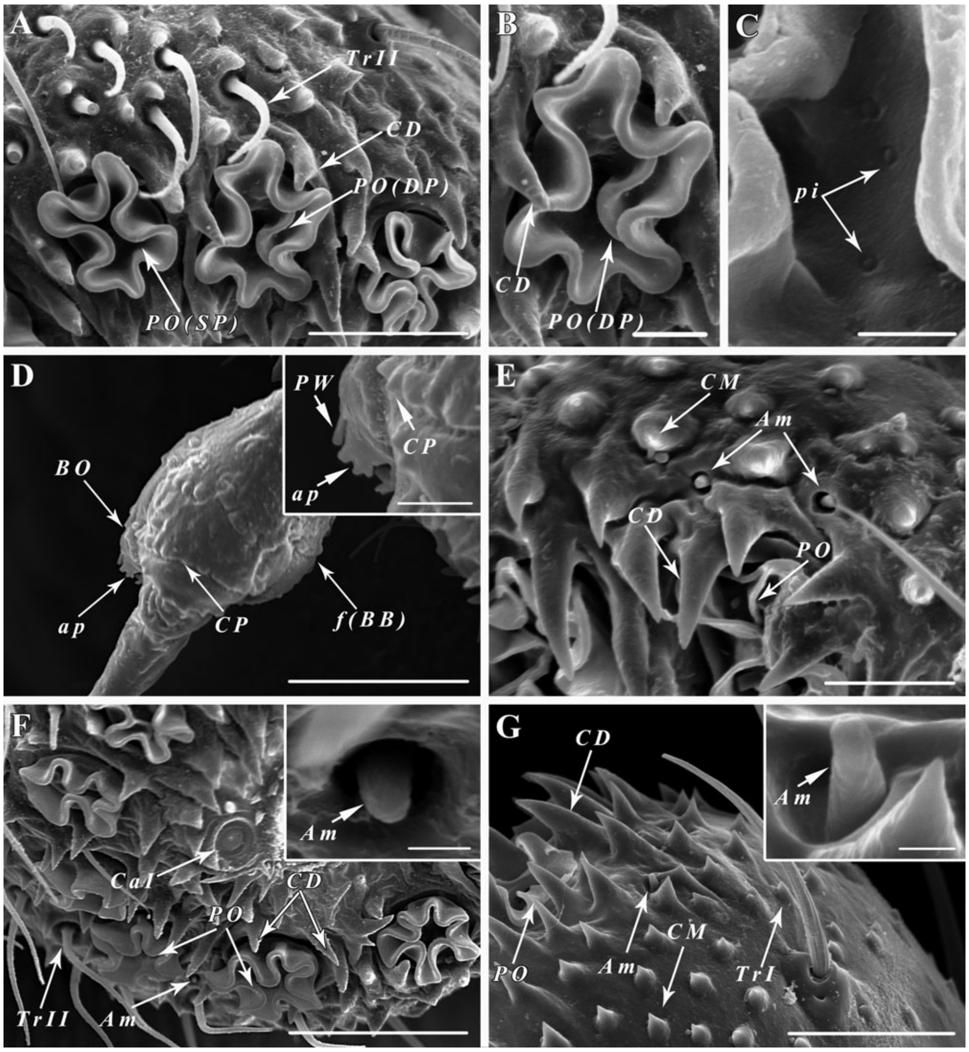

Fig. 3. SEM images of the plate organs, Bourgoin's organ and ampullaceal sensilla in *Kallitaxila granulata.* **A**–**C**: Plate organs at apical part of the antennal pedicel, with cuticular denticles around it and several shallow pits on its central flat. **D**: Bourgoin's organ on flagellar basal bulb, with cone-shaped processes around it. **E**–**G**: General view of part of the antennal pedicel showing the position of ampullaceal sensilla. Am, ampullaceal sensilla; ap, aperture; BB, basal bulb of the flagellum; BO, Bourgoin's organ; CaI, campaniform sensilla subtype I; CD, cuticular denticles; CM, cuticular microtubercles; CP, cone-shaped processes; DP, double petal; pi, pit; PO, plate organs; PW, petal-like walls; SP, single petal; TrI, trichoid sensilla subtype I; TrII, trichoid sensilla subtype II. [(A) scale bar 20 μm, (B) scale bar 6 μm, (C) scale bar 2 μm, (D) scale bar 30 μm, 5 μm in box, (E) scale bar 10 μm, (F) scale bar 30 μm, 1 μm in box, (G) scale bar 20 μm, 1 μm in box.]

middle segment, and the shorter distal segment narrowing to a circular tip (Fig. 4D). It had a stylet bundle formed from the mandibles and maxillae lying encased within a groove in the labium. The dorsal surface of the labium was bisected by a stylet groove within which lie the outer mandibular and inner maxillary mouthparts, forming the stylet bundle (Figs. 4A and 4D).

Numerous cone-shaped cuticular processes (0.6–2.8 μm long) with acute tips were found on the dorsal surface of the labrum (Figs. 4A and 4B). Trichoid sensilla (TrIII) with high-rise bases could be seen on dorsal surfaces of the anteclypeus (16.9–72.3 μm long) and labium (12.3–81.5 μm long) (Figs. 4A, 4C, and 4F).

Despite the insertion of the trichoid sensilla (TrIII) into the raised socket (Figs. 4A, 4C, and 4F), a distinct coin-shaped sensilla, close to the labium apex, was found on each lateral side of the distal segment of the labium.

Coin-Shaped Sensilla (Cs). Each Cs was identified with 46.2–57.5 μm to the labial apex at each lateral side of the labium (Figs. 4D–4F), and it was 14.0–14.6 μm in diameter, rose 2.2–3.8 μm above the cuticle. Only after the waxy powder was completely removed could we find a bacilliform process (about 2.4 μm high, 3.4 μm long), which was set in the center with a pore (about 3.2 μm in diameter) underneath (Fig. 4E). These sensilla could be classified as subapical sensory organs under the system described by Backus (1985) and as latero-subapical labial sensilla under the system described by Liang (2005).

DISCUSSION

The antennal morphology of planthoppers is autapomorphic for the Fulgoromorpha (Asche, 1988; Bourgoin, 1985). The morphology of the antennae in *K. granulata*

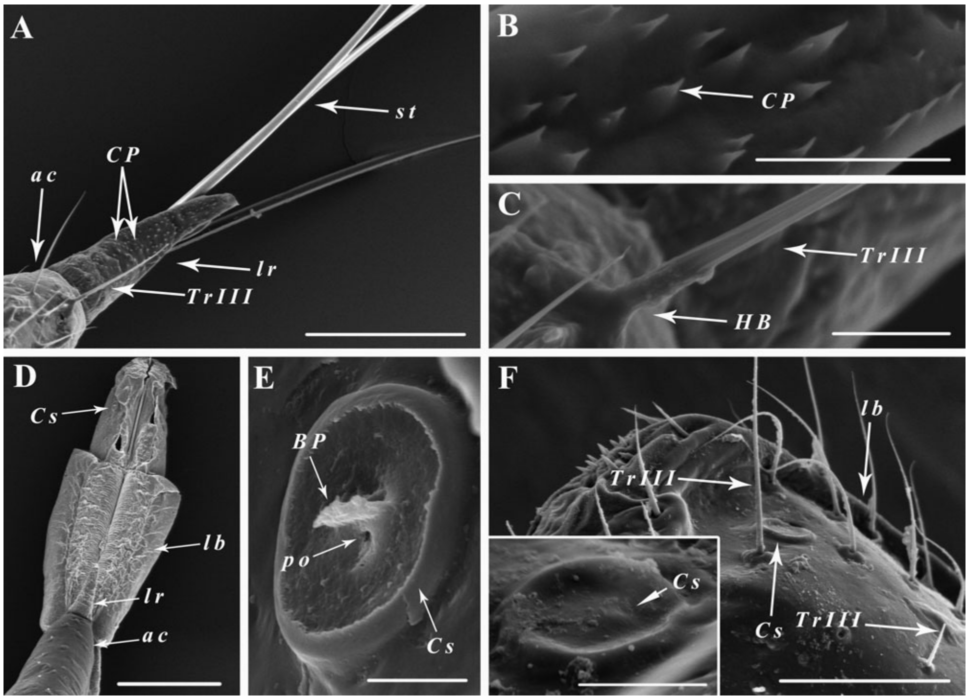

Fig. 4. SEM images of the sensilla on the mouthparts of *Kallitaxila granulata*. **A**: General view of stylets, a labrum and an anteclypeus. **B**: Cone-shaped processes on the labrum. **C**: Trichoid sensilla subtype III on the anteclypeus. **D**: Ventral view of apical mouthparts. **E**: A coin-shaped sensillum on the labium. **F**: Lateral view of labium. ac, anteclypeus; BP, bacilliform process; CP, cone-shaped process; Cs, coin-shaped sensillum; HB, high-rise base; lb, labium; lr, labrum; po = pore; st, stylets; TrIII, trichoid sensilla subtype III. [(A) scale bar 100 μm, (B, C) scale bar 10 μm, (D) scale bar 150 μm, (E) scale bar 5 μm, (F) scale bar 50 μm, 10 μm in box.]

is similar with that in other fulgoromorphan species, which has three segments: a basal antennal scape, a bulbous antennal pedicel, and an unsegmented bristle-like flagellum composed of an expanded base and a long arista (Fig. 1B).

TrI has straight grooves on the surface (Fig. 2B) and a distinct pore at the raised socket (Figs. 2A and 2E). TrII are inserted into a depression without any specialized raised socket (Fig. 2F), with straight patterns and many tiny pits at their surfaces (Figs. 2F and 2G). Both TrI and TrII have been identified as chemoreceptors (Aljunid and Anderson, 1983).

Wp scattered on the antennal pedicel and antennal scape (Figs. 1E, 1G, and 2C) in *K. granulata* have been found in Cercopidae.

Ca have been found in a variety of places on insects, e.g., halters, palps, legs, bases of wings, and even the eyes (Bromley et al., 1980; Schneider, 1964). Ca have been reported in planthoppers by Bourgoin (1985) and Roberto et al. (2009). The number and distribution of these sensilla can be differed significantly across species. In Tettigometridae, three Ca can be found near the basal part of the antennal pedicel (Bourgoin, 1985). However, in Tropiduchidae and Cixiidae, there is only one Ca in the antennal pedicel near the expanded flagellar base (Roberto et al., 2009). The Ca is thought to monitor general stresses in the cuticle, possibly through some directional sensitivity but it is certainly not as specific as other types of mechanoreceptive sensilla (Bromley et al., 1980). Ca may respond to stresses arising from movement of the flagella, as already reported in aphids (Dunn, 1978). However, in *K. granulata*, the inner ultrastructure must be studied further to provide evidence of this.

PO exhibit important structural variations in planthoppers. PO concentrated at the apex of the antennal pedicel of *K. granulata* are the folded flattened plates, which are similar to the clover leaf-like structures observed in *Ossoides lineatus* (Tropiduchidae), *Microflata stictica* (Flatoidae), and *Lophops carinatus* (Lophopidae) (Bourgoin and Deiss, 1994; Marshall and Lewis, 1971; Stroiński et al., 2011). However, they are not similar to those found in other Tropiduchidae, such as *Z. gressitti* and *Kusuma* sp., in which the projected digitate type replaces the clover-leaf type (Marshall and Lewis, 1971; Stroiński et al., 2011). PO in *K. granulata* may function as olfactory sensilla (Aljunid and Anderson, 1983; Marshall and Lewis, 1971; Stroiński et al., 2011).

BO in *K. granulata* has petal-like walls around the aperture (Fig. 3D). The Co inside BO can function either as olfactory and chemosensory receptors or as thermo/hygroreceptors (Altner et al., 1977; Kellogg, 1970; Loftus, 1976; Zacharuk, 1980). These sensilla may provide thermosensory information that, in synergy with olfactory information, plays an important role on the orientation behavior of insect species towards oviposition sites (Angioy et al., 2004; Setzu et al., 2011; Stensmyr et al., 2002). In Cercopidae, Co with numerous pores appear to be the main olfactory receptors for host selection (Liang and Fletcher, 2002). The exact function of these sensilla in *K. granulata* is still unclear, and related studies of inner ultrastructure are necessary.

Am (Figs. 3E–3G), situated near PO on the antennal pedicel, are sunken below the cuticular surface with basiconic-shape peg in the center. This structure was shown in Tettigometridae by Bourgoin (1985) for the first time. The morphology of Am in *K. granulata* are consistent with those in previous records and first found in Tropiduchidae.

Cs (Figs. 4D–4F), which correspond to a special sensory organ known as subapical sensory organ (Backus, 1985) and latero-subapical labial sensilla (Liang, 2005) are situated on each side of labium and close to the labial apex, are present in other planthoppers taxa but appear in different conformations. It is peg-like in many cixiid species (e.g., *Borysthenes maculata*, *Andes marmorata*; Liang, 2005: Figs. 1 and 2), and some other fulgoromorphan families (Liang, 2005: Table 1). It is multilobed in *Nilaparvata lugens* and other Delphacidae (Foster et al., 1983: Fig. 1a; Sōgawa, 1977, 1981). This kind of sensilla can also be found in *Cimex hemipterus* as a baton-shaped structure (Hemiptera: Cimicidae) (Liang, 2005). According to Zacharuk (1980), and Keil and Steinbrecht (1984), peg-like basiconic sensilla (called latero-subapical labial sensilla by Liang, 2005) are mainly chemosensory and mechanosensory, while the functions of multilobed sensilla are considered not only olfactory but also hygroreceptive by Sōgawa (1977, 1981). As such, Cs in *K. granulata* may have a similar function, but the inner ultrastructure must be studied further to provide evidence of this.

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