External Morphology of Antennae and Mouthpart Sensillae of the Granary Weevil (Coleoptera: Curculionidae)[1]

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Abstract The ultrastructure of the antennal and mouthpart sensillae for the granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae), adult was examined with scanning electron microscopy. The cephalic capsule bears two types of sensillae—a multibranched sensillum and a multiporous peg sensillum. The multibranched sensillum was a structure not previously reported for the granary weevil or any other insect species, and is distinguished by its torpedo-shaped appearance. The multiporous peg sensillae, formed from multiple straight tubules, are distributed on the distal one-third of the rostrum. The mouthparts bear two types of sensillae—the multiporous peg sensillae on the labrum and mandibles, and short basiconic sensillae on the labial palps. The antenna consists of eight segments arranged on the scape, pedicel, and flagellum. Each antennal segment bears sqaumiform sensillae, and five types of sensillae were identified on the club segment. These sensillae are a trichodea Type I, trichodea Type II, chaetica Type I, chaetica Type II, and double-walled basiconic. Chaetica Types I and II represented >60% of the total sensillae on the club segment. Trichodea Type II sensillae were also observed and were distinguished by the characteristic bifurcate apex. Finally, the double-walled basiconic sensillae were distinguished by the fluted cuticular surface with a grooved peg-like appearance distally.

Key Words granary weevil, antenna, mouthparts, sensillae, scanning electron microscopy

The granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae), is a cosmopolitan pest of wheat, *Triticum aestivum* L. and barley, *Hordeum vulgare* L. (Mebarkia et al. 2010, Schwartz and Burkholder 1991), and also attacks a variety of dried stored grain and bean products. The adult weevil is chestnut-brown or reddish-brown to shiny black in color (Mason 2003). The head is elongated into a characteristic slender rostrum with elbowed antennae distally. The head bears the sensory organs and receptors utilized in the perception of smell, taste, and vision (i.e., antennae, mouthparts, compound eyes), and the antennae consist of eight segments that are often carried in an extended position when the insect is walking.

The characterization of sensory organs in insects, particularly the curculionids, has been studied by Bland (1986), Chaika and Tomkovich (1997), Tomkovich and Chaika (2001), Salama and Abd El-Aziz (2001, 2002), Yan et al. (2011), and Mahmoud et al. (2012). The present study aims to identify and determine the

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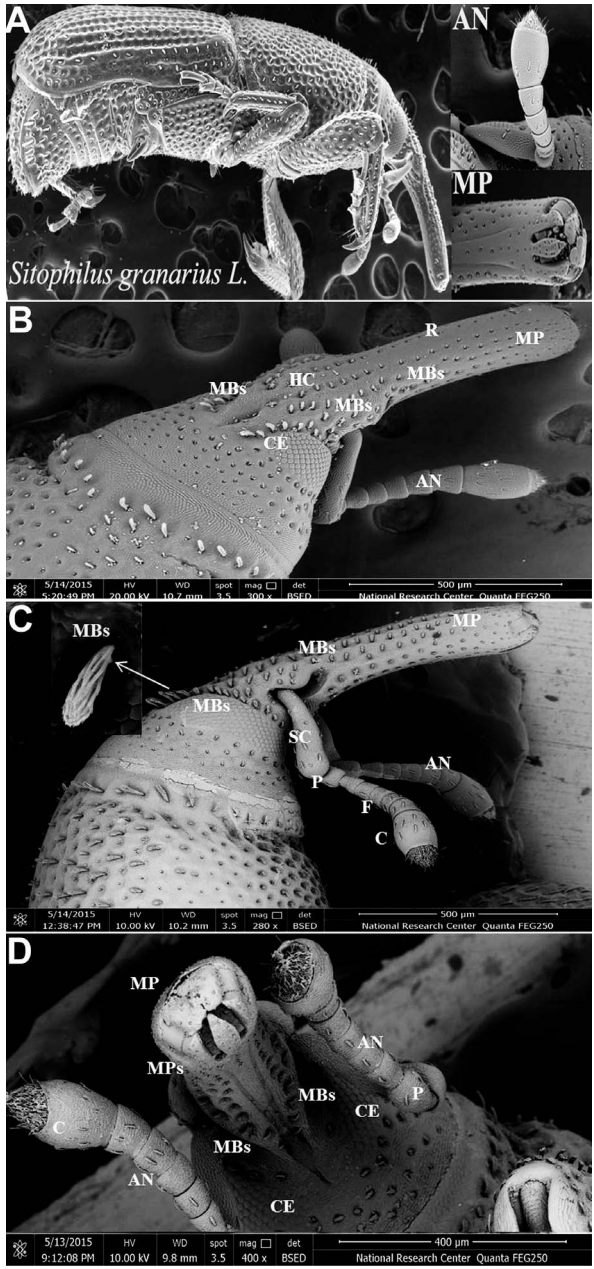

Fig. 1. SEM photomicrographs of the granary weevil, *S. granarius.* (A) Whole view of the granary weevil adult showing body structure, antenna (AN), and mouthparts (MP). (B) Dorsal view of the head capsule (HC) showing antenna (An), compound eyes (CE), the rostrum (R), multibranched sensillae (MBs), and multiporous peg sensillae (MPs).

distribution of the olfactory and gustatory sensory receptors on the antennae and mouthparts of the granary weevil, using scanning electron microscopy (SEM).

Materials and Methods

Thirty-five adults from a laboratory colony of *S. granarius* were used in these SEM studies. Insects from the laboratory colony were placed in 2.5% glutaraldehyde and 0.2 M sodium cocodylate buffer (pH 7.3) for 4 to 6 h and then removed and washed in three changes of sodium cocodylate buffer (10 min per wash). They were then immersed in the buffer for 24 h at 4°C. The fixed insects were then treated with an osmium tetraoxide buffer for 2 h or until the insect darkened in color. They were then washed again three times with glutaraldehyde buffer and fixed again for 24 h at 4°C and then dehydrated with ascending concentrations of ethyl alcohol (30, 50, 70, 80, 90, 100%) (two times for 5 min in each concentration). Insects were then dried using critical-point drying to preserve the morphological characters and mounted on aluminum stumps with adhesive (double sticky tape), then sputter-coated with gold film using Gold Sputter Coater (S150A Edward, Bolton, United Kingdom). Samples were photographed using a Quanta SEM field emission gun 250 (Koninklijke Philips N.V., Amsterdam, Netherlands).

Results and Discussion

The head appendages of *S. granarius* are distinguished by a long slender rostrum, elbowed antennae, and a compound eye on each side of the head capsule (Fig. 1). The head bears the sensory organs and receptors for chemoreception and vision. The cephalic capsule has two types of sensillae, a multibranched sensillum and a multiporous peg sensillum (Fig. 1). The observed multibranched sensillae were identified for the first time as a new type of sensillum that has not been previously reported from any insect species. This type of sensillum is characterized by its torpedo-shaped appearance. It is distributed on the dorsal surface of the cephalic capsule (Fig. 1A), arranged longitudinally along two-thirds of the length of the rostrum as five symmetrically parallel rows on the dorsolateral surface of the rostrum (Fig. 1B) and arranged as two symmetrically parallel rows on the ventral surface of the rostrum (Fig. 1C). These sensillae also surround the frontal edge of the compound eyes. The multiporous peg sensillae were found on the distal one-third of the frontal part of the rostrum along the dorsoventral surface of the rostrum (Fig. 2A). Multiporous peg sensillae are distinguished by their elongate fingerlike appearance, formed from multiple straight tubules (Fig. 2B). The mean ($\pm$ SD) length of these sensillae was 5.56 $\pm$ 0.04 μm.

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(C) Lateral view of the head showing different segments of the antenna: the scape (SC), the pedicel (P), the flagellum (F), and the club (C). Also, magnified view of multibranched sensillum (MBs) is illustrated. (D) Ventral view showing antenna (An), the mouthparts (MP), compound eyes (CE), the pedicel (P), multibranched sensillae (MBs), and multiporous peg sensillae (MPs).

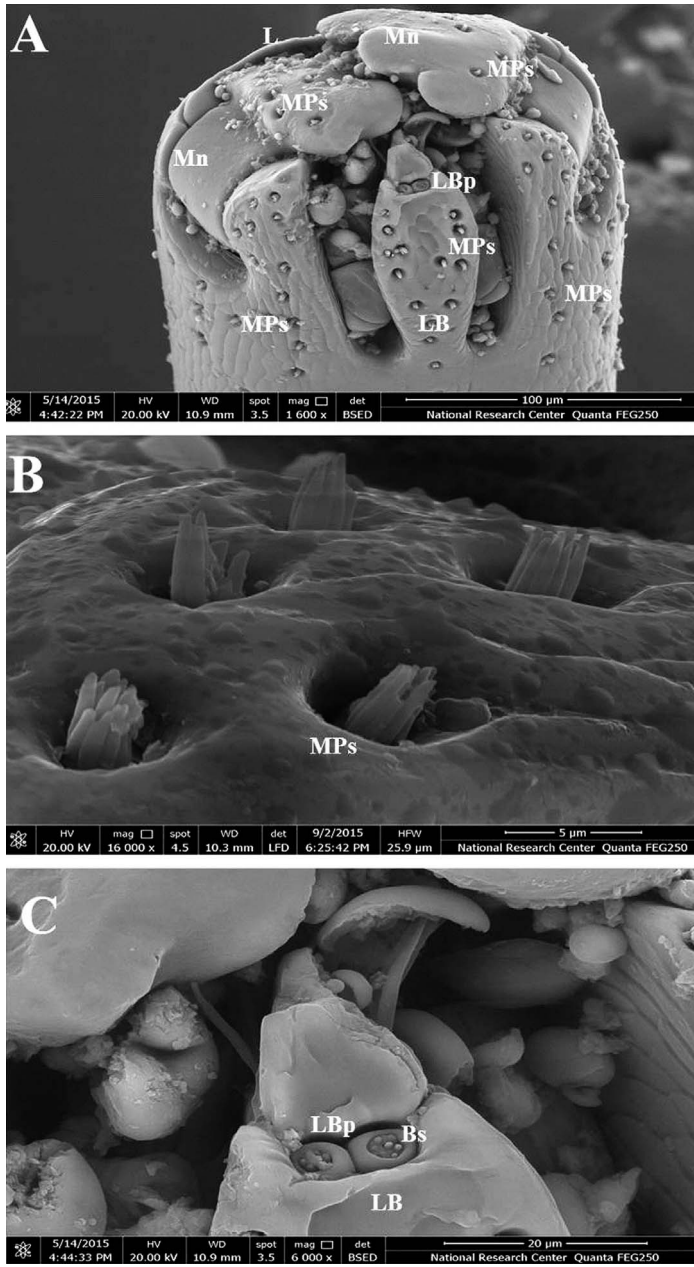

Fig. 2. SEM photomicrographs of the mouthparts of the adult granary weevil, *S. granarius*. (A) The mouthparts composed of labrum (L), a pair of mandibles (Mn), labium (LB), and a pair of labial palps (LBp). Numerous numbers of multiporous peg sensillae (MPs) were distributed on mouthparts. (B) Magnified view of multiporous peg sensillae

In contrast, Fouda et al. (2016) showed only two subtypes of basiconic sensillae distributed on the snout of *S. oryzae* (*Linnaeus*) and *S. granarius*. Our observations, therefore, do not agree with their report. Images presented herein show differences from the Fouda et al. (2016) report that included no basiconic sensillae on the snout of *S. granarius*. Our study clearly shows other types of sensillae—a multibranched sensillum and a multiporous peg sensillum (Figs. 1, 2), the latter of which has been suggested as a chemoreceptor in the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Salama and Abd El-Aziz 2001). Craig and Batz (1982) also found these sensillae in Simuliidae (Diptera) larvae which tended to be elongated.

The mouthparts are composed of a labrum, a pair of mandibles, a pair of maxillae, and labium (Fig. 2A). The labrum is a simple broad lobe forming the roof of the preoral cavity. The outer surface of the labrum carries multiparous peg sensillae. The mandibles are strongly developed and sclerotized, and each mandible bears three distal teeth-like structures. Multiporous peg sensillae were identified on the outer surface of the mandibles and on the labium (Fig. 2A). The labium has a pair of labial palps which bear sensory organs represented as seven short basiconic sensillae on the apical part of each palp (Fig. 2C). This is the first report of these sensillae on *S. granarius*; Fouda et al. (2016) did not report these types of sensillae on the labrum and mandibles of *S. granarius* or *S. oryzae*.

The granary weevil antenna is geniculate, arising from the elongated antacava (Fig. 1B) in front of the anterior margin of the compound eye and at the base of the rostrum. Each antenna consists of eight segments representing the scape, the pedicel, and the flagellum. Sqaumiform sensillae, distinguished by their long and fluted surfaces, were detected on all antennal segments (scape, pedicel, and flagellum). The scape is the longest segment and measured more than one-third of the length of the antenna. Its proximal part is slender, with a hook to fit in the antennal socket (Fig. 1). The scape carries various numbers of sqaumiform sensillae and measures a mean ($\pm$ SD) of 23.30 $\pm$ 2.59 μm in length (Fig. 4A). The pedicel has a small triangular base that fits in a comparatively large cavity at the distal end of the scape (Fig. 3B). The pedicel bears various numbers of sqaumiform sensillae (17.28 $\pm$ 0.49 μm). A magnified view of sqaumiform sensillae is illustrated in Fig. 3C. Few sqaumiform sensillae were observed in comparison with other types of sensillae present. These have been previously described on the antennae of some beetles and weevils (Dai and Honda 1990, Hix et al. 2003, Kang et al. 2012) and speculated to be mechanoreceptive in function. Our observations differ from those of Fouda et al. (2016), who characterized these type of receptors as chaetica sensillae for *S. oryzae* and *S. granarius*.

The flagellum consists of six segments of which the first five, known as the funicle, resemble the pedicel in form (Fig. 3B). Each funicle segment bears sqaumiform sensillae arranged as one row encircling the middle of the segment.

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(MPs) distinguished by their fingerlike appearance, which is formed from multiple straight tubules. (C) Magnified view of labium (LB) showing a pair of labial palps (LBp) bearing short basiconic sensillae (Bs), seven on each labial palp.

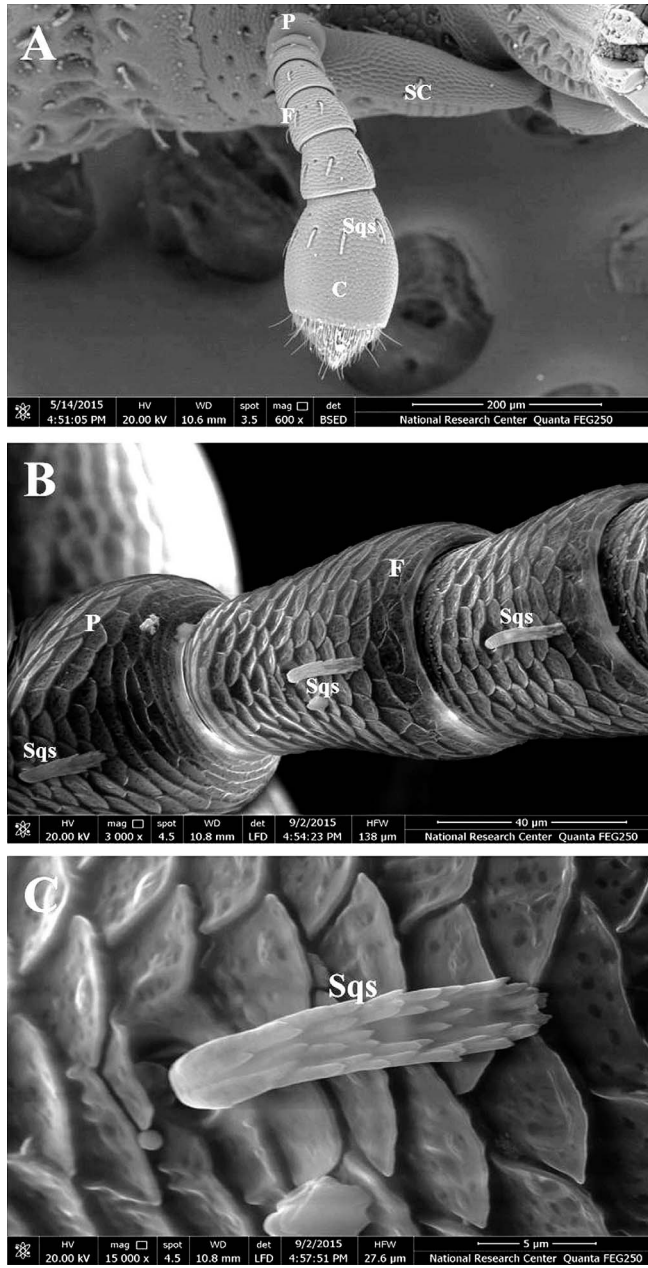

Fig. 3. SEM photomicrographs of the antennae of the adult granary weevil, *S. granarius*. (A) Showing general morphology of the antenna consisting of four parts: the scape (SC), the pedicel (P), and the flagellum (F) bears largest segment; the club (C). (B) Showing various sqaumiform sensillae (Sqs) distributed on the antennal segments. (C) Magnified view of sqaumiform sensillae (Sqs).

The club or the sixth flagellar segment (Figs. 1, 3A) is longer and broader than the other funicle segments and bears sqaumiform sensillae arranged as three rows on its distal surface. The club is elongate-oval and carries five types of sensillae apically. These olfactory sensillae have been identified as trichodea Type I, trichodea Type II, chaetica Type I, chaetica Type II, and double-walled basiconic sensillum (Fig. 4). These observations also differed from those of Fouda et al. (2016), who reported only three types of sensillae (two trichodea types [TS1, TS2] and a basiconic sensillum) on the club segment of *S. granarius*. Trichodea Type I sensillae are distinguished by their smooth surfaces, socketless bases, and tapered ends, and had a mean (± SD) length of 11.06 ± 5.07 μm. These sensillae represent about 15% of the total sensillae found on the antennae. Trichodea-type sensillae are found on the antennae of many curculionids and are thought to be olfactory receptors (Hix et al. 2003, Kang et al. 2012, Yan et al. 2011). Trichodea Type II sensillae have bifurcate apices (e.g., two-thirds of its length is a stalk and the third peripheral is branched to form a V-shape). These sensillae also represent about 15% of the total antennal sensillae. Their mean (± SD) length is 15.45 ± 1.2 μm (Fig. 4B) and are similar to the sensillae described by Bland (1981) for the alfalfa weevil, *Hypera postica* (Gyllenhal), and the red palm weevil by Salama and Abd El-Aziz (2001). In contrast, Fouda et al. (2016) reported one type of trichodea sensillae on the club segment and did not report the trichodea Type II (bifurcate apices) on *S. oryzae* and *S. granarius*. The trichodea sensillae are relatively slender and gradually taper to a sharp point, or they may be stout and taper to a blunted or tetrafurcate point. Chaetica sensillae are similar to trichodea sensillae except that these bristles and hairs are set in sockets and can be identified by their thick walls and blunt apexes. Trichodea and chaetica sensillae represented >60% of the antennal sensillae observed. Chaetica sensillae are reported for many curculionids as antennal olfactory receptors (Wang et al. 2012, Yan et al. 2011). Chaetica Type I sensillae are longer (18.38 ± 5.63μm) than Type II sensillae (12.88 ± 1.29 μm) (Fig. 4A, B). Double-walled basiconic sensillae, characterized by a short, fluted cuticular surface with a grooved peg-like appearance distally, also were observed (Fig. 4C). The mean (± SD) length of these sensillae was 5.79 ± 0.69 μm and is similar to the basiconic sensillae pegs in *Callosobruchus maculatus* (F.) and *C. subinnotatus* (Pic) adults (Mbata et al. 1997) and the fluted sensillae described from *Ips paraconfusus* LeConte by Borden and Wood (1966). These types of sensillae have not been recorded previously for any *Sitophilus* species. Only the basiconic sensillae which, characterized by smooth cuticle and blunt tip, were reported by Fouda et al. (2016).

These results reported herein add to our understanding of the granary weevil biology. Knowledge of the morphology of the types of sensillae on the antennae and mouthparts of the granary weevil will provide a foundation for understanding olfaction and feeding preferences of the granary weevil in order to develop improved control methods for this pest.

Acknowledgments

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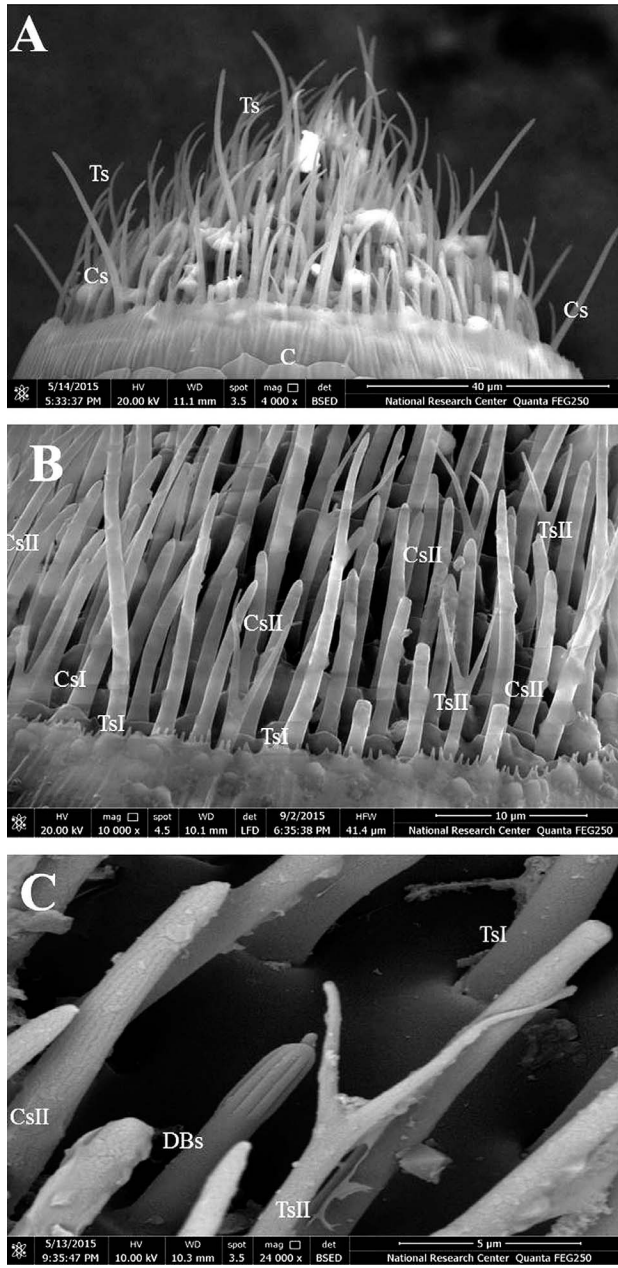

Fig. 4. SEM photomicrographs of the antennal club segment of the adult granary weevil, *S. granarius*. (A) Showing distal end of the club (C) containing different types of trichodea (Ts) and chaetica sensillae (Cs). (B) Showing various types of olfactory sensillae in the club segment, identified as: trichodea Type I (TsI), trichodea Type II (have

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bifurcate apices) (TsII), chaetica Type I (CsI), and chaetica Type II (CsII). (C) Double-walled basiconic sensillum (DBs) characterized by a fluted cuticular surface with grooved peg-like appearance distally.

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