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Antennal Sensilla in the Small Banana Weevil *Polytus mellerborgi* Boheman (Coleoptera: Curculionidae)

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Authors' Contributions
DZ and JY conceived and designed the study. JY and FL prepared samples for analysis. JY performed SEM studies. WY and JG analyzed the data. YW wrote the article.

Key words
Antennal club, small banana weevil.

A B S T R A C T

The antennal morphology and sensilla of small banana weevil, *Polytus mellerborgi* Boheman (Coleoptera: Curculionidae), were examined. The antennae of *P. mellerborgi* were composed of eight antennomeres, a scape, pedicel and flagellum composed of six flagellomeres. The use of scanning electron microscopy facilitated the recognition of five different sensilla types on the antennae of *P. mellerborgi*, including sensilla trichodea, sensilla chaetica (with two subtypes: Sch1 and Sch2), sensilla basiconica and sensilla gemmiformia. These results provided necessary background information for our ongoing study on electrophysiology and chemical ecology of *P. mellerborgi*.

INTRODUCTION

The banana weevils are recognized globally as the major pest of banana and plantain (Tinzaara *et al.*, 2011), and *Polytus mellerborgi* Boheman has become widely spread (Zimmerman, 1968), which looks like a miniature *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) and therefore commonly known as the small banana weevil (Okolle *et al.*, 2009). *P. mellerborgi* is often found in numbers in decaying trunks of bananas and bores in the decaying corms and stalks of banana (Zimmerman, 1968; Yin *et al.*, 2015). Although it is more in the field followed by *C. sordidus*, damage caused by *P. mellerborgi* is yet to be assessed (Okolle *et al.*, 2009).

Many studies on other banana weevils show that traps baited with plant volatiles and/or aggregation pheromones may have great potential as an efficient monitoring and control tool against these pests (Jayaraman *et al.*, 1997). So far, we haven't been sure if the small banana weevils are attracted to plant volatiles emanating from crop residues as the same as *C. sordidus* (Budenberg *et al.*, 1993), although *P. mellerborgi* may be trapped overnight by placing freshly cut pseudostem on the ground, with the cut surface in contact with the soil (Nelson *et al.*, 2006). Apparently, the olfactory and taste sensilla located on the insect antennae are involved in the process of damaging host plants. However, there has been no published work on the antennal structure of *P. mellerborgi*. Therefore, the aim of this study is to identify the different sensilla types of *P. mellerborgi* using scanning electron microscopy (SEM). It could provide basic information for developing methods of pest control in terms of olfactory and contact chemosensory systems.

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MATERIALS AND METHODS

P. mellerborgi adults were obtained in Changjiang (N19°32', E108°95'), Hainan Province, P. R. China. For SEM observation, 10 antennae of weevils were cut randomly regardless of their sexes. After that, the antennae were rinsed in 70 % ethanol in ultrasonic cleaning instrument (JL-, Jieli Co. Ltd., Shanghai City, P. R. China) for 1 min and then dehydrated in a graded ethanol series (80, 90, 95, 100%). After air drying, the specimens were mounted on a holder using electric adhesive tape and then coated with gold and observed using HITACHI S-3000N SEM (Hitachi Corp., Tokyo, Japan) in Hainan University (Haikou City, Hainan Province, P. R. China). Five antennae on the ventral side and five on the dorsal side were studied under SEM. Thirty images of the sensilla on the surfaces of the antennae were taken at magnification of 180× to 9000×, EB acceleration voltage at 10.0 kV and image size ranging from 9.7 to 18.5 mm, then classified and measured. In order to calculate the mean length of sensilla types, at least 10 sensilla of the same type from different individuals were measured.

The terminology and classification of sensilla used here followed those of Schneider (1964) and Keil (1997). The length and width of different types of sensilla were measured using Nano Measurer 1.2 software (Department of Chemistry, Fudan University, P. R. China). The data were analyzed using SPSS19.0 for Windows (SPSS Inc., Chicago, USA). Statistical results were expressed as mean ± standard deviation.

RESULTS

The antennae of *P. mellerborgi* were composed of eight antennomeres, a scape, pedicel and flagellum composed of six flagellomeres. The last flagellomere was called as antennal club. The flagellum was the most prominent part on the antenna, on which most sensilla were located, especially on the antennal club. Five types of sensilla and cuticular grooves were found on *P. mellerborgi* antennae according to SEM, including sensilla trichodea, sensilla chaetica type 1 and 2, sensilla basiconica, and sensilla gemmiformia. These cuticular grooves occurred widely on the antennae of adult weevils (Fig.1D and 1E).

Sensilla trichodea (St.)

St were on the antennal club and towards the club apex, and identified based on their length and shape with a conical base and slightly curved tip (Fig.1B and 1C). They were distributed widely over the entire antennal club and the most extensively distributed type of sensilla on the antennae of *P. mellerborgi* in various lengths. These sensilla varied from 25.70 to 36.76 µm in length, and from 1.67 to 2.77 µm in width (Table I).

Table I.- Sizes of antennal sensilla of *P. mellerborgi*.

Sensilla type	Length (µm)	Width (µm)
sensilla trichodea	31.23±5.53	2.22±0.55
sensilla chaetica 1	48.75±4.89	2.07±0.38
sensilla chaetica 2	26.55±19.59	1.65±0.92
sensilla basiconica	16.31±0.03	1.12±0.02
sensilla gemmiformia	0.44±0.10	0.45±0.09

Mean numbers (±SD) of different types of sensilla on the flagellum.

Sensilla chaetica (Sch.)

The sensilla chaetica, the longest hairs on the antennae, were divided into two subtypes. Sch1, long, robust, bristle-like sensilla with a sharp tip, sat on the antennal club (Fig.1B). Sch2, long, robust, bristle-like sensilla, extended from the antennae at roughly a 20° angle and had shallow longitudinal veins and irregular tips (Fig.1B, 1D and 1E). Sch2 were distributed regularly along the lateral side of flagellomeres.

Sensilla basiconica (Sb)

Sensilla basiconica (Fig.1C), common and distributed randomly on antennal club, had fewer numbers with smooth wall. Sb were blunt-tipped, shorter than St, mean length was 16.31±0.03 µm and mean width was 1.12±0.02 µm (Table I).

Sensilla gemmiformia (Sg)

The sensilla gemmiformia (Fig. 1F) were only found in the joint between each two neighboring flagellomeres. Their appearance resembled a sawtooth. Sg were very small with a sharp tip and thick base.

DISCUSSION

The study demonstrated that *P. mellerborgi* contained five different types of sensilla on the antennae. Compared with other Curculionidae, *P. mellerborgi* antennae are similar to those of *Odoiporus longicollis* (Olivier) (Coleoptera: Curculionidae) (Gao *et al.*, 2011), on both of which sensilla trichodea, sensilla chaetica, sensilla basiconica and sensilla gemmiformia are all found. The antennal club of *P. mellerborgi*, only one flagellomere, was absolutely different from that of *Hypera meles* (F.) (Coleoptera: Curculionidae) which is formed by four flagellomeres (Smith *et al.*, 1976), *Eucryptorrhynchus chinensis* (Olivier) and *E. brandti* (Coleoptera: Curculionidae) both of which are composed of three flagellomeres (Yu *et al.*, 2013). St of *P. mellerborgi* widely distributed, which were more densely concentrated on the distal region of the antennal club. In general, St are usually the most common and numerous structures on insect antennae and presumed to function as olfactory receptors in many insects (Bleeker *et al.*, 2004; Mustaparta, 1975), for example, *Agriotes obscurus* L. (Coleoptera: Elateridae) (Merivee *et al.*, 1997) and *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae) (Castrejón-Gómez *et al.*, 1999).

External shape, large measurements, specific location at the antennal apex, small number and perpendicular position on the antennal surface indicate that Sch1 in *P. mellerborgi* are most probably taste sensilla which are crucial in final host plant detection and selection. Presumably the Sch1 are the first sensilla to contact with the substrate because they are the longest (protrude over all other sensilla) and project at right angles from the antennal surface. They also occur on the antennae of all beetles studied, for example, Carabidae (Merivee *et al.*, 2000, 2001, 2002), Elateridae (Merivee *et al*., 1997, 1998, 1999) and many other beetles. By electrophysiological recordings these sensilla are innervated by five neurons: four chemoreceptor neurons and one mechanoreceptor neuron. Similar set of neurons is typical for insect taste sensilla. In Carabidae, one of the neurons is sensitive to water soluble salts (Merivee *et al.*, 2004), the second neuron responds to pH (Merivee *et al.*, 2005; Milius *et al*., 2006), the third neuron responds to plant sugars and amino acids (Merivee *et al.*, 2007, 2008, 2012), the fourth neuron is sensitive to plant secondary compounds like alkaloids and glucosides (Milius *et al*.,

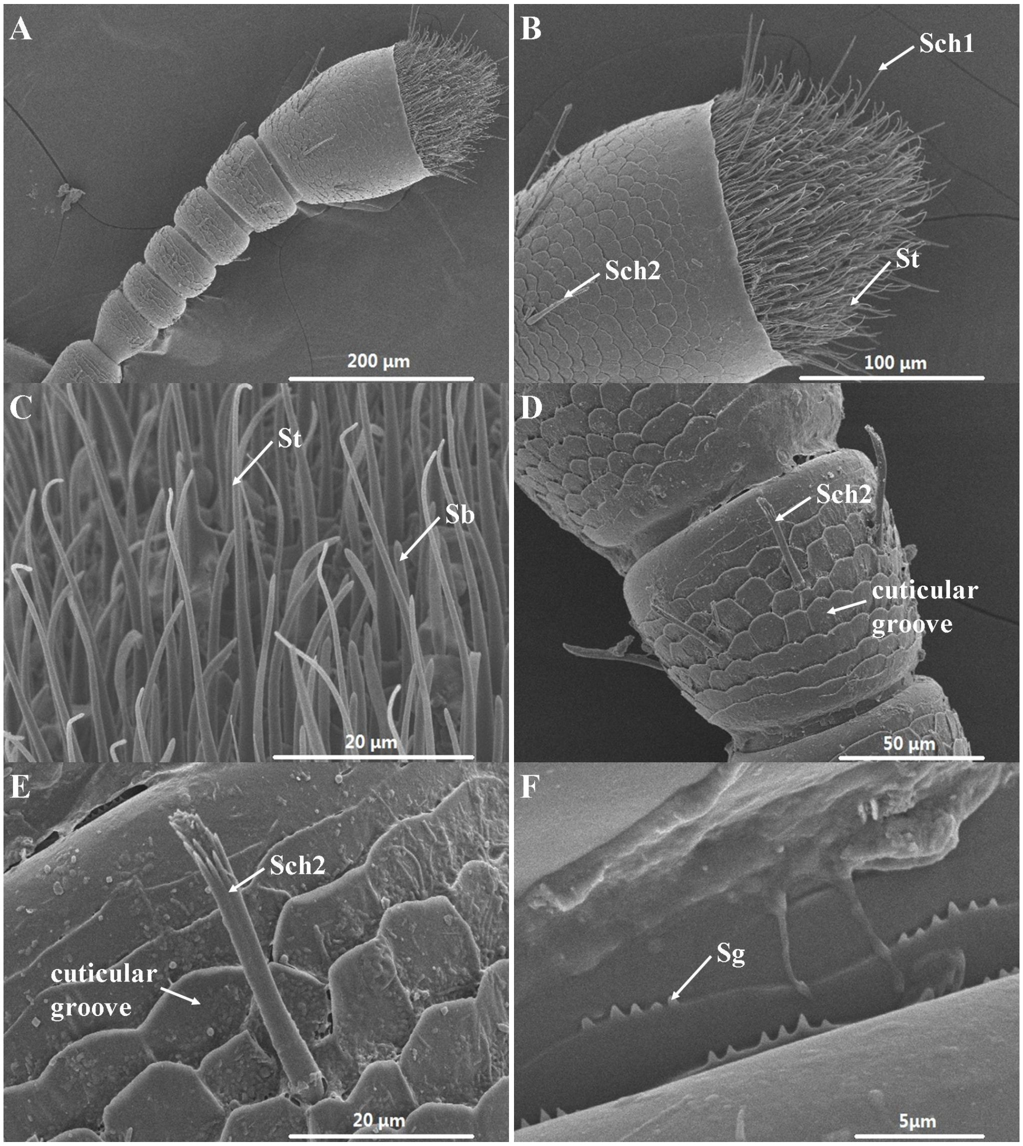

Fig. 1. Scanning electron micrographs of features on the antennal flagellum of *P. mellerborgi*. (A) The whole antennal flagellum of *P. mellerborgi*; (B) the antennal club showing the distribution of sensilla chaetica (Sch1 and Sch2), sensilla trichodea (St); (C) sensilla trichodea (St) and sensilla basiconica (Sb); (D and E) Sch2 on other flagellomeres, and cuticular grooves covering whole flagellum; (F) sensilla gemmiformia (Sg) in the joint between each two neighboring flagellomeres.

2011). The fifth, mechanoreceptor neuron responds to touch (Merivee *et al*., 2008). In Elateridae, three neurons have been electrophysiologically identified in these taste sensilla: the salt, the sugar and the touch sensitive neuron (Tooming *et al*., 2012). Sch2 of *P. mellerborgi* are probably mechanoreceptor located in a wide articular socket and responding to touch.

Sb have been reported on the antennae of several coleopteran species (Dyer and Seabrook, 1975; Alm and Hall, 1986; Dai and Honda, 1990). And they have been shown to function as olfactory chemoreceptors in several species of Coleoptera, such as *Hylobius abietis* L. (Coleoptera: Curculionidae) (Mustaparta, 1975). Lopes *et al*. (2002) reported that Sb in *Phoracantha semipunctata* Fabricius (Coleoptera: Cerambycidae) are structurally adapted to play a functional role in olfactory recognition of plant odours. Although Sg are not common in Coleoptera, which have been not found in Carabidae

(Merivee *et al*., 2000, 2001, 2002) and Elateridae (Merivee *et al*., 1997, 1998, 1999), it means possible that Sg are candidate mechanoreceptors. And Sg reported in here were totally different from *Anoplistes halodendri halodendri* and *A. halodendri ephippium* (Coleoptera: Cerambycidae) (Liu *et al.*, 2012).

In addition, sensilla coeloconic and sensilla styloconicum on most Coleoptera insects (Okada *et al.*, 1992; Zacharuk, 1980) were not found on the antennae of *P. mellerborgi*. And sensilla placodea are usually distributed on the antennae of Curculionidae (Yan *et al.*, 2011), which have not been discovered on the antennae of small banana weevils.

In conclusion, the presence of these sensilla types on the antennae probably suggests the importance of chemoreception in the behavioral ecology of *P. mellerborgi*. These results provide necessary background information on chemical ecology of *P. mellerborgi* although sensilla functions will require further study by electrophysiological and behavioral investigations.

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Conflict of interest declaration

The authors have declared that no conflicts of interest exist.

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