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Effects of the antennal sensilla distribution pattern on the behavioral responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae)

*Suliman A. I. Ali, Mory M. Diakite, Saqib Ali, and Man-Qun Wang**

Abstract

The morphology and distribution of sensilla on the antennal flagella of male and female adults of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), were investigated by using scanning and transmission electron microscopy (SEM and TEM). Behavioral responses of male and female adults were measured with 12 host-plant and leaf-volatile stimuli. No sexual dimorphism was observed in the distribution and types of sensilla. Two types of sensilla, trichodea and basiconica, were observed on the antennae. Sensilla trichodea were distributed on all antennal segments but were most abundant on the last 3 segments. In contrast, sensilla basiconica were found exclusively on the last 3 segments of the antenna. TEM cross sections showed sensilla trichodea to have a thick cuticular wall and regular point-shaped pores around the cuticular wall. Sensilla basiconica possessed a thin wall and continuous pores and dendrites. The pores on the walls of the sensilla indicate that they play a role in sensing chemical stimuli and in olfactory functions. Female and male adults of *T. castaneum* showed broad overlap in their behavioral responses to various odors. Preference for some volatiles was noted, but after removal of the 3 terminal antennal segments, there was no significant preference for any compound that had been previously attractive to the beetles when tested with the complete antennae.

Key Words: antenna; sensillum; scanning electron microscopy; transmission electron microscopy; behavior response

Resumen

Se investigaron la morfología y distribución de las sensilas en los flagelos de las antenas de los adultos machos y hembras de *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) por medio del uso de la microscopía electrónica de barrido y de transmisión (SEM y TEM). Las respuestas de comportamiento de los adultos machos y hembras se midieron con 12 diferentes estímulos de plantas hospederas y volátiles de las hojas. No se observó dimorfismo sexual en la distribución y las clases de sensilas. Se observaron dos clases de sensilas, trichodea y basiconica, en las antenas. Las sensilas trichodeas se distribuyeron en todos los segmentos de la antena, pero fueron más abundantes en los últimos 3 segmentos. En contraste, las sensilas basiconicas se encontraron exclusivamente en los 3 últimos segmentos de la antena. Las secciones transversales de TEM mostró que las sensilas trichodeas tienen una pared cuticular gruesa y poros en forma de puntos fijos alrededor de la pared cuticular. Las sensilas basiconicas poseía una pared delgada y los poros y dendritas continuas. Los poros en las paredes de las sensilas indican que ellos juegan un papel en la detección de estímulos químicos y en las funciones olfativas. Las hembras adultas y machos de *T. castaneum* mostraron amplia superposición en sus respuestas de comportamiento a diferentes olores. Se notó una preferencia para algunas sustancias volátiles, pero después de la eliminación de los 3 segmentos terminales de la antena, no hubo ninguna preferencia significativa por cualquier compuesto que previamente había sido atractivo para los escarabajos cuando fueron evaluados con las antenas completas.

Palabras Clave: antena; sensilas; microscopía electrónica de barrido; microscopía electrónica de transmisión; respuesta de comportamiento

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is an economically important pest of stored products (Kostyukovsky et al. 2000; Campbell 2012). Infestation of stored products by pests could cause major losses because of the consumption of grains and the development of molds (Sauer et al. 1984, Magan et al. 2003). Since the repeated use of pesticides has increased the incidence of resistance in pests, integrated pest management has increasingly focused on the use of semiochemicals to protect agricultural products (Phillips 1997). Insects mainly find hosts and conspecifics using chemosensory signals. These can affect long-range attraction and the ability of individuals to identify and locate mates or hosts; sometimes they act as alarms, induce trail-following, and influence oviposition or aggregation behavior (van der Goes van Naters & Carlson 2006). It has been shown that *T. castaneum* responds to grain-emitted volatiles and an aggregation pheromone created by feeding males that is attractive to males and females (Suzuki et al. 1984; Lu et al. 2011). The beetle is monitored in food facilities using a variety of traps baited with aggregation pheromones and food-based kairomone lures (Barrer 1983; Burkholder 1990; Chambers 1990; Mullen 1992; Cox 2004; Campbell et al. 2009).

Little is known about the physiology of the antennal sensory apparatus of this pest. Insect antennae, which bear sensory sensilla, play an important role in the recognition of various stimuli for finding suitable habitats and food and for locating mates. Several reports have characterized the antennal sensilla of insects including their morphology

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and structure (Roth and Willis 1951; Merivee et al. 1998; Renthal et al. 2003; Sukontason et al. 2007; Abdel-latief 2007; Onagbola & Fadamiro 2008; Rebora et al. 2008; Drilling & Klass 2010; Sun et al. 2011; Galvania et al. 2012; Zhou et al. 2015). However, the antennal sensilla of *T. castaneum* have not been studied despite the important role they play in the life of the insect. Therefore, in this study, the innervation of the funicular sensilla in both sexes of *T. castaneum* was studied, followed by Y-tube bioassays in order to determine whether removal of the last 3 segments of the antennae, which have distinct types of sensilla, had an effect on the behavior of the beetle.

Materials and Methods

INSECT REARING

The *T. castaneum* individuals were obtained from a laboratory culture maintained on wheat grains at 28 °C, 75% RH, and a photoperiod of 8:16 h L:D in the College of Plant Science and Technology at Huazhong Agricultural University, Hubei Province, P.R. China. Alcohol was used to wash the insects before using a binocular microscope for sexing, following the method of Bousquet (1990).

SCANNING ELECTRON MICROSCOPY (SEM)

The antennae of *T. castaneum* were cleaned in an ultrasonic bath (250 W) for 5 s. They were dehydrated through an ethanol series of 30, 45, 60, 75, 90, and 95% ethanol (each for 15 min) and then completely dehydrated twice in 100% ethanol (each for 15 min). After critical point drying, the antenna preparations were mounted on a stub with double-sided adhesive tape and sputter-coated with gold/palladium (40:60). In total, 30 antennae from males and females (15 from each sex) were examined using a Hitachi jsm-6390l (Tokyo, Japan) scanning electron microscope at 20 kV.

TRANSMISSION ELECTRON MICROSCOPY (TEM)

For transmission electron microscopy (TEM), *T. castaneum* antennae from males and females were placed in a mixture of 5% sucrose and 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (PBS, pH 7.4) for 6 h at room temperature. Antennae were then rinsed 10 times with a washing solution containing 0.1 M PBS (pH 7.4) and 6.8% sucrose for 20 min each, followed by dehydration in an ethanol series and 100% acetone. Embedding was done with propylene oxide in Epon 812. Ultra-thin sections were cut with a glass knife on an LKB V Ultra microtome and mounted on Formvar-coated grids. The specimens were examined with a Hitachi H-7500 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

TERMINOLOGY AND DATA PROCESSING

The terms for external morphology used here follow those of Zacharuk (1985). The antennal sensilla length, diameter, and sensilla number per segment were measured directly from the printed scanning and transmission electron micrographs (Sukontason et al. 2007; Hu et al. 2009). Images were improved using Adobe Photoshop 7.0. The data obtained were analyzed to determine the standard error. The numbers and size differences between females and males were analyzed using Student's *t*-tests.

ODOR STIMULI

Synthetic compounds used in this behavioral study were purchased from commercial sources (Sigma-Aldrich Chemical Co; Shanghai, China). They included both host and green tissue compounds. The host compounds were ethyl-1-hexanol (>99%), heptacosane (>95%), (+)-3-carene (>90%), hentriacontane (>95%), cis-3-hexenyl acetate (>97%), acetophenone (>98.5%), tridecanone (>95%), and 1-octen-3-ol (>98%). The plant volatiles were hexanol (>95%), (−)-limonene (>95%), nonacosane (>98%), and ethyl hexanoate (>99%). These chemicals were selected because they are very common semiochemicals produced by wheat panicles and green tissues (Robacker et al. 1992; Bruce & Cork 2001; Birkett et al. 2004; Nawrot et al. 2010), and their electrophysiological response activity has been demonstrated for many insects, including the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) (Birkett et al. 2004; Cha et al. 2011).

A 20 mm diameter glass Y-tube olfactometer was used; this consisted of a 12 cm long stem with 7 cm long arms at 90° from each other. Bioassays were performed in a cabinet in darkness at room temperature. An incandescent light of 25 W was located in front of the olfactometer to encourage the beetles to walk forwards. A 5 µL aliquot of each sample was applied to a piece of Whatman No. 2 filter paper (0.5 ´ 1.5 cm), which was placed inside one arm of the Y-tube olfactometer. A same-sized filter paper treated with water (5 µL) was placed in the other arm as the control. Airflow was driven and purified through a charcoal filter before reaching the glass tube carrying the test stimuli. The airflow through each olfactometer arm was 200 mL/min, determined with a flow meter. In this experiment, beetles were starved for 2 h before each test. Their mobility was checked before each test and beetles not moving within 5 s of introduction were excluded from the test.

A single test insect was released at the downwind entrance of the main tube and allowed to walk through it and choose either of the arms, with maximum observation duration of 10 min per beetle. A direct response was recorded when a beetle entered an arm and crossed a decision line 5.8 cm from the junction, with no return movement. Beetles that did not make a choice within 10 min were recorded as showing no response. The choices made with respect to either source (volatile or blank) and the time taken in making the choices were recorded. Each insect was used only once and discarded after the test. The bioassays were repeated until 40 beetles had responded to each test compound. The test sources were alternated between the olfactometer arms after every 5 runs of each test, and the olfactometer and the sample chambers were washed with ethanol and oven dried between tests.

To understand whether the distribution of antennal sensilla types plays a particular role in the response to volatile compounds, the last 3 segments of the antennae of 40 beetles were removed before a second set of bioassays using the most attractive compounds from the previous test. The beetles were left on wheat grains for 3 d after removal of the last 3 antennal segments before testing. The χ^2 goodness-of-fit test was used to analyze between-group differences in the olfactometer bioassay data. Student's *t*-tests were used to compare the response times within a sex between beetles with intact antennae and beetles with cut antennae.

Results

GENERAL MORPHOLOGY OF THE ANTENNAE

The antennae of both male and female *T. castaneum* had 11 segments, with the last 3 enlarged, forming a loosely segmented club. The length of male antennae (4.35 ± 0.33 mm) was longer than that of female antennae (3.98 ± 0.59 mm) (Fig. 1).

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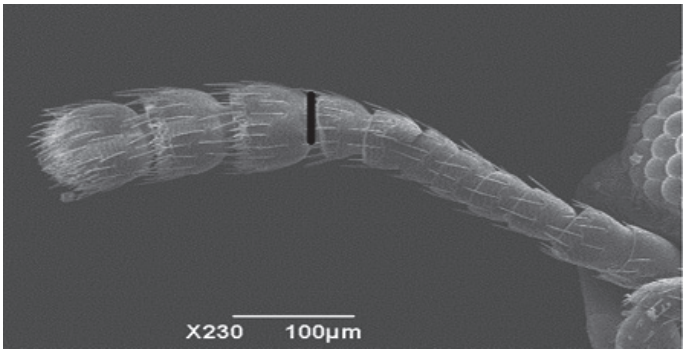


Fig. 1. *Tribolium castaneum* antennal ultrastructure observations. Dorsal view of the whole antenna with 11 antennal segments with a black line indicating where antennae were cut. Sensilla basiconica were found only on the last 3 segments of the antennae and sensilla trichodea on all antennal segments.

ANTENNAL SENSILLA TYPES AND DISTRIBUTION

The 2 main types of sensilla were observed on the antennae of both sexes and included sensilla trichodea with 2 subtypes (Fig. 2) and sensilla basiconica with 6 subtypes (Fig. 3). Sensilla trichodea were the most abundant sensilla. They were found on all antennal segments but were most abundant on the last 3 segments. Sensilla basiconica were found only on the last 3 segments of the antennae.

Sensilla Trichodea

Sensilla trichodea were long, slender, and hair-like becoming smooth at the base**,** and were recessed in wide depressions. Two subtypes were identified based on their external features, sensilla trichodea I and sensilla trichodea II. Sensilla trichodea I were present on all antennal segments of both males and females. These sensilla were characterized by their sharply pointed structures and were slightly

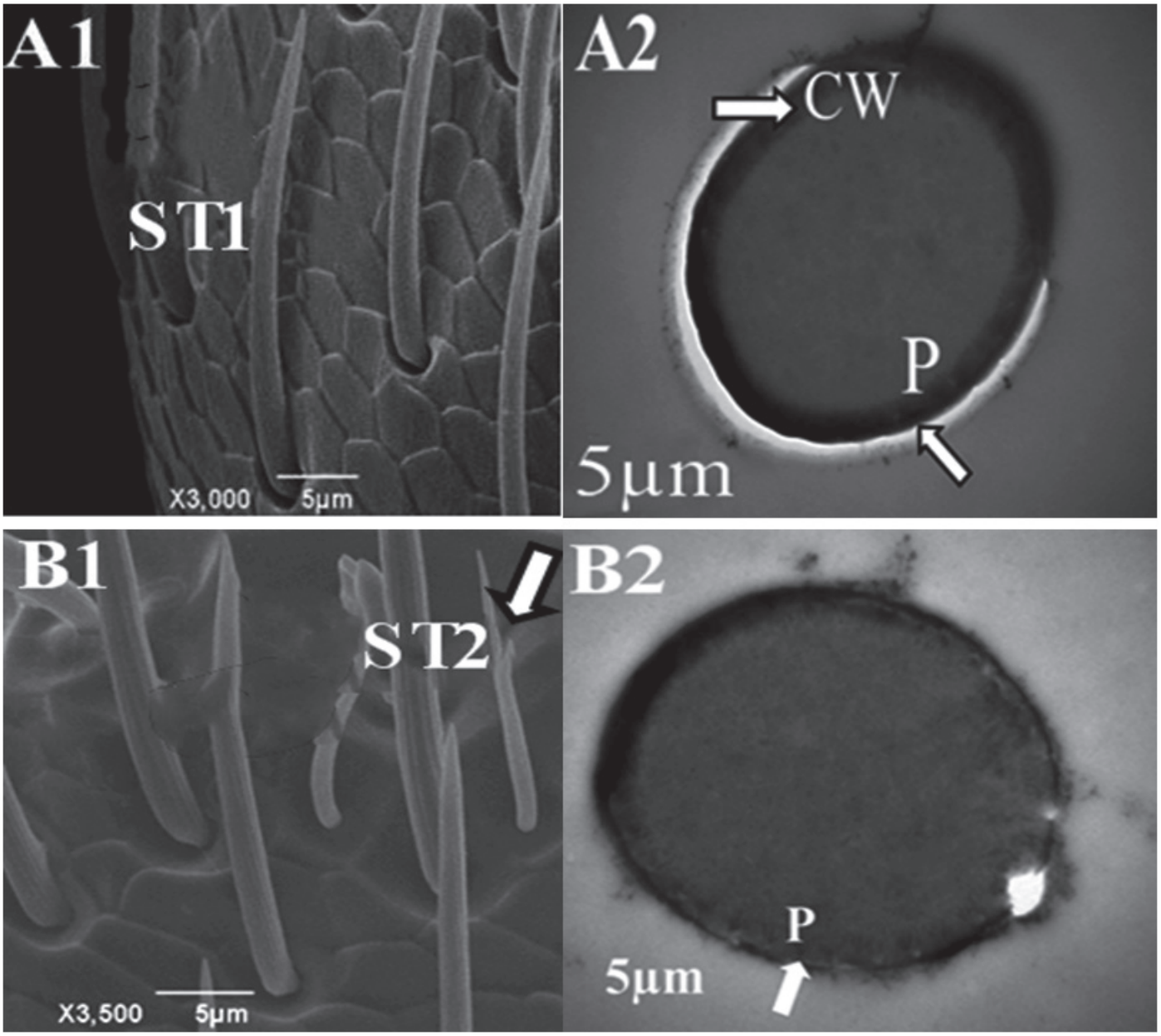


Fig. 2. SEM photomicrographs of 2 types (A1, B1) of sensilla trichodea (ST) on the antennae of *Tribolium castaneum***.** TEM photomicrographs of 2 types (A2, B2) of sensilla trichodea (ST). A2 showed a thick cuticular wall and regular point-shaped pores around the cuticular wall, B2 a thick cuticular wall and pore structures with an irregular pointed shape. CW = cuticle wall, P = pores, STI = sensilla trichodea type I, STII = sensilla trichodea type II.`

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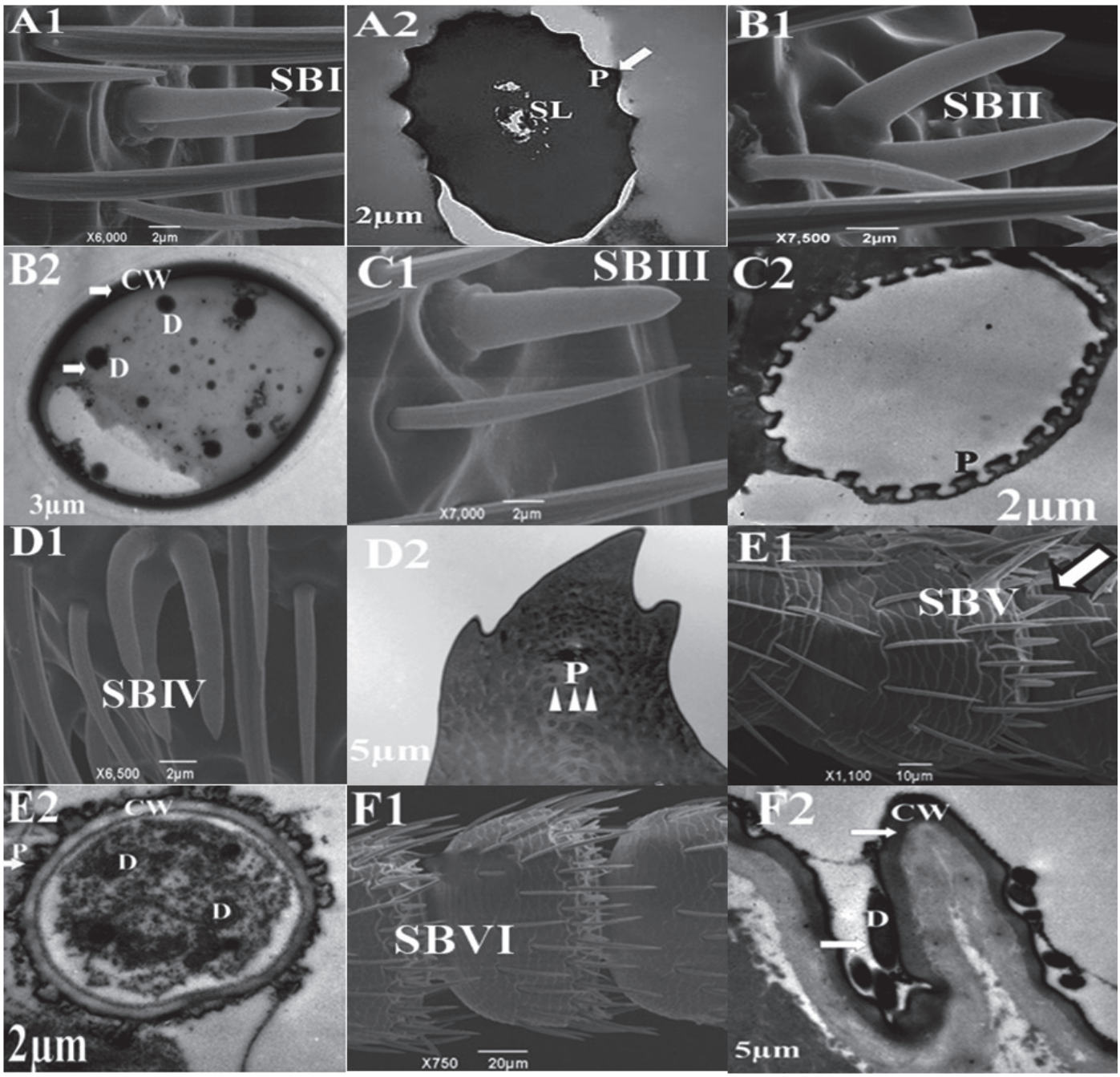


Fig. 3. SEM photomicrographs of 6 types (A1, B1, C1, D1, E1, F1) of sensilla basiconica (SB) showing lateral view of the antennae of *Tribolium castaneum.* TEM photomicrographs 6 types (A2, B2, C2, D2, E2, F2) of sensilla basiconica (SB) showing the thin wall and continuous pores and dendrites. CW = cuticle wall, P = pores, D = dendrites, SBI = sensilla basiconica type 1, SBII = sensilla basiconica type II, SBIII = sensilla basiconica type III, SB IV = sensilla basiconica type IV, SBV = sensilla basiconica type V, SBVI = sensilla basiconica type VI.

curved toward the anterior portion of the segment (Fig. 2A1). The average length and width were 29.14 ± 0.64 µm and 4.31 ± 0.5 µm, respectively. The TEM of their cross-sections showed sensilla trichodea with a thick cuticular wall and regular point-shaped pores around the cuticular wall (Fig. 2A2). Sensilla trichodea II were curved perpendicular to the longitudinal axis of the antenna (Fig. 2B1). The average length and width were 1.68 ± 0.08 µm and 0.43 ± 0.02, respectively. The TEM of their cross-sections showed that sensilla trichodea II had a thick cuticular wall and pore structures with an irregularly pointed shape around the cuticular wall that only covered about one-third of the cuticular wall (Fig. 2B2).

Sensilla Basiconica

Sensilla basiconica were found only on the last 3 segments of the antennae of both sexes. They were characterized by a smooth surface, with a thin or rounded and short apex with no sexual dimorphism. Unlike the sensilla trichodea, each sensillum basiconicum was seated on a

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base and the sensilla basiconica gradually became thinner and shorter towards the tip of the antenna. In terms of their external features, 6 subtypes of sensilla basiconica were identified (Fig. 3). The TEM photomicrographs of these sensilla showed wall pores suggestive of a potential function in olfaction. The average length and width of all 6 subtypes of sensilla basiconica of *T. castaneum* antennae are shown in Table 1.

Sensilla basiconica I were 2 identical joined sensilla basiconica with the same size and thickness that extended from a flexible base that was raised above the cuticle (Fig. 3A1). They had a thick cuticular wall. No dendrites were seen within the sensilla lumen (Fig. 3A2).

Sensilla basiconica II were distinguished from sensilla basonica I by a greater distance between the 2 joined sensilla (Fig. 3B1). The TEM of their cross-sections showed that they had a smooth cuticular wall without a pore structure, and many dendrites were seen within the sensilla lumen (Fig. 3B2).

Sensilla basiconica III were similar to sensilla basiconica I and may be a separate form of the latter. Like the latter, they were sheathed near the cuticular base (Fig. 3C1). The TEM of the sensilla showed that the pores were present within the cuticular wall, and the pore structures were regular around the cuticular wall (Fig. 3C2).

Sensilla basiconica IV included 3 sensilla, 1 of which was shorter than the other 2 (Fig. 3D1). The TEM of the sensilla showed that the pores were present within the cuticular wall (Fig. 3D2).

Sensilla basiconica V differed from the other sensilla basiconica subtypes by having an enlarged shaft (Fig. 3E1). The TEM showed that numerous pores were present within the cuticular wall (Fig. 3E2).

Sensilla basiconica VI were the smallest and shortest sensilla basiconica found in *T. castaneum*. They were distally tapered toward the apex with a pointed tip (Fig. 3F1). The TEM showed that they had a smooth cuticular wall surface with arrowhead-shaped pores around the cuticular wall (Fig. 3F2).

OLFACTORY BIOASSAY

Compared with the blank control, both males and females of *T. castaneum* showed significant preferences for ethyl-1-hexanol and heptacosane. Males also showed a significant preference for nonacosane, (−)-limonene, and octen-3-ol. Males and females showed a significant preference for the blank control over cis-3-hexenyl acetate, acetophenone, ethyl hexanoate, and tridecanone. Moreover, males showed a preference for the blank control over hentriacontane and hexanal, whereas females had a highly negative response to (−)-limonene (Table 2). Based on these results, 5 behavior-affecting compounds were chosen for a new bioassay conducted after removal of the last 3 segments of the antennae in both sexes. The removal of the last 3 segments of the antennae affected the responses of *T. castaneum* to olfactory stimuli; neither male nor female *T. castaneum* showed significant preference for any of the 5 compounds, which previously had been significantly attractive (Table 2). In addition, there was a significant difference in behavior after segment removal in terms of the time taken to make a choice (Table 3).

Table 1. Length and width (mean ± SE) of sensilla basiconica (SB) of *Tribolium castaneum* (*n* = 5).

Sensilla types	Length (μm)	Basal diameter (μm)
SBI	8.33 ± 0.47	1.71 ± 0.08
SBII	29.76 ± 0.87	6.49 ± 0.30
SBIII	19.80 ± 1.11	4.98 ± 0.97
SBIV	10.38 ± 0.76	1.82 ± 0.10
SBV	22.68 ± 4.01	1.80 ± 0.17
SBVI	3.30 ± 0.64	1.00 ± 0.08

Discussion

To our knowledge, this is the first work identifying the antennal sensilla types and their distribution in *T. castaneum*. Based on SEM observations, 2 types of antennal sensilla were found, sensilla trichodea and sensilla basiconica. Sensilla trichodea have been found to play a major role in sensing mechanical stimuli and in recognizing chemical stimuli in insects (Roth & Willis 1951; Amornsak et al. 1998; Roux et al. 2005; Abdel-latief 2007; Onagbola & Fadamiro 2008), whereas sensilla basiconica are considered olfactory receptors in phytophagous insects and are sensitive to host plant chemicals. The morphology of these sensilla suggests that they are sensitive to many kinds of chemical stimuli (Kaissling 1986; Isidoro et al. 1998; Lopes et al. 2002; Onagbola & Fadamiro 2008).

In the current study, sensilla trichodea occurred on all antennal segments in both sexes of *T. castaneum*, and no sexual differences were detected. However, the density was greater on the last 3 than on the first 8 segments. Sensilla basiconica were found only on the last 3 segments of the antennae and were absent on the first 8 segments in both sexes. The majority of the sensilla basiconica subtypes found in *T. castaneum* have been identified previously on the antennae of many other insect species, such as *Pterostichus melanarius* (Illiger) and *Pterostichus niger* (Schaller) (Coleoptera: Carabidae) (Symondson & Williams 1997), *Manduca sexta* L. (Lepidoptera: Sphingidae) (Shields & Hildebrand 1999), *Phoracantha semipunctata* (F.) (Coleoptera: Cerambycidae) (Lopes et al. 2002), *Lophocorona pediasia* Common (Lepidoptera: Lophocoronidae) (Faucheux 2006), and *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae) (Fernandes et al. 2004). No previous study described twin sensilla basiconica similar to those found on antennae of *T. castaneum* (Fig. 3). A similar distally branched type was found in *Coleophora obducta* Meyrick (Lepidoptera: Coleophoridae) and classified as sensilla furcatea by Yang et al. (2009). Furthermore, the small sensilla basiconica type found in *T. castaneum* (Fig. 3F1) was similar to the one found in *Hydrotaea chalcogaster* (Wiedemann) (Diptera: Muscidae) (Sukontason et al. 2007).

The TEM photomicrographs of cross-sections of sensilla trichodea (Fig. 2IA2, IIB2) revealed that they had thick cuticular walls with numerous structural pores, and no dendrites were seen within the sensilla lumen. No sexual dimorphism was observed in these sensilla, and they occurred in equal numbers in both sexes. This suggests that sensilla trichodea play an olfactory role in host or mate location in *T. castaneum*, whereas their longer-than-average length indicates that they also assist in sensing mechanical or chemical stimuli. Sensilla trichodea were the most abundant sensilla on the antennae of *T. castaneum*, and studies on many other insect species have shown that these sensilla could be divided into subtypes according to the number of sensory cells (Hallberg et al. 1994), the presence (or absence) of pores on the sensillum wall (Onagbola & Fadamiro 2008), or the distribution and length of the sensilla (Ren et al. 2014).

The TEM photomicrographs of sensilla basiconica on the antennae of *T. castaneum* showed porous sensilla (Fig. 3A2, B2, C2, and D2) and revealed wall pores suggesting that they are able to detect various chemical compounds (Zacharuk 1985). *Tribolium castaneum* may use the sensilla basiconica to detect sex pheromones, possibly in conjunction with host plant odors, as great numbers of sensilla may indicate high levels of sensitivity (Chapman 1982). Sensilla basiconica are considered to be olfactory receptors to many kinds of chemical stimuli (Kaissling 1986; Isidoro et al. 1998; Onagbola & Fadamiro 2008),

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Table 2. Behavioral responses to compounds in a Y-tube olfactometer of *Tribolium castaneum* before and after removal of the last 3 antennal segments (n = 40).

	Male				Female			
Volatile compound	Before	χ^2	After	χ^2	Before	χ^2	After	χ^2
Ethyl-1-hexanol	26	3.02*	21	0.02ns	27	4.22*	23	0.62ns
Control	14		19		13		17	
Heptacosane	29	7.22**	16	1.22ns	28	5.62**	18	0.22ns
Control	11		24		12		22	
Nonacosane	28	5.62**	22	0.22ns	23	0.62ns	21	0.02ns
Control	12		18		17		19	
(−)-Limonene	29	7.22**	23	0.62ns	8	13.22**	22	0.22ns
Control	11		17		32		18	
1-Octen-3-ol	30	9.02**	18	0.22ns	23	0.62ns	27	4.22*
Control	10		22		17		13	—
Hentriacontane	11	7.22**	—	—	23	9.63**	—	—
Control	29		—	—	17		—	—
Hexanol	14	3.02*	—	—	17	4.03*	—	—
Control	26		—	—	23		—	—
Cis-3-hexenyl acetate	12	5.62*	—	—	14	7.50**	—	—
Control	28		—	—	26		—	—
Acetophenone	9	11.02**	—	—	8	14.70**	—	—
Control	31		—	—	32		—	—
Ethyl hexanoate	11	7.22**	—	—	9	9.63**	—	—
Control	29		—	—	31		—	—
Tridecanone	6	18.22**	—	—	10	24.30**	—	—
Control	34		—	—	30		—	—

Note: The numbers of beetles responding to treatments were compared with the χ^2 goodness-of-fit test; values marked with an asterisk are significantly different between the test compound and the control (*, $P < 0.05$; **, $P < 0.01$; ns, not significant).

though sensilla basiconica were absent in some Noctuidae moths (Flower & Helson 1974; Calatayud et al. 2006; Zheng et al. 2014).

During the first olfactory experiment (before removal of the 3 terminal segments), we noticed that if *T. castaneum* individuals were interested in a given volatile odor, they moved the antennae in various directions immediately after being released. The beetles walked fast, with some sudden stops while still intensely moving the antennae. This movement of the antennae became more intense at the junction of the Y-tube. The results showed that both males and females responded positively to the odor of ethyl-1-hexanol and heptacosane, whereas they responded negatively to the odor of ethyl hexanoate and cis-3-hexenyl acetate. The positive behavioral responses to these green tissue compounds used during this study suggests that the beetles may survive on alternative host plants in the absence of host grains.

Table 3. Comparison of response time (min; mean ± SE) of *Tribolium castaneum* before and after removal of the last 3 antennal segments (n = 40).

	Male		Female	
Volatile compound	Not removed	Removed	Not removed	Removed
Ethyl-1-hexanol	1.24 ± 0.07a	3.53 ± 0.19b	1.22 ± 0.12a	3.08 ± 0.32b
Heptacosane	1.19 ± 0.10a	3.62 ± 0.33b	1.08 ± 0.10a	2.80 ± 0.24b
Nonacosane	1.30 ± 0.13a	3.84 ± 0.41b	1.54 ± 0.18a	3.22 ± 0.20b
(−)-Limonene	1.57 ± 0.16a	3.27 ± 0.26b	1.29 ± 0.12a	1.95 ± 0.23a
1-Octen-3-ol	1.36 ± 0.16a	3.06 ± 0.25b	1.36 ± 0.11a	3.29 ± 0.23b

Note: The values of beetles' response times per sex were compared with Student's t-tests; values within a row and sex followed by different letters are significantly different ($P < 0.01$); values followed by the same letter are not significantly different ($P > 0.05$).

Tribolium castaneum males responded to the odor of (−)-limonene, whereas females did not. There was evidence of sexual preferences for volatiles (Honda et al. 1998; Birkett et al. 2004). Different responses between females and males might be indicative of sex differences in host finding through volatile cues (Light et al. 1988). It has been reported that for many phytophagous insects, although females and males could detect the same range of volatiles, there were differences in the responses of the 2 sexes (Groot et al. 1999; Fraser et al. 2003). Both (*Z*)-3-hexenol (*Z*3-6: OH) and manuka oil emitted by ash foliage were found to be attractive to *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), but the former was more attractive to males, whereas the latter had a greater effect on females (Grant et al. 2010).

The results also showed that the last 3 segments probably contribute to the fast location of food sources because when the segments were removed, there was no significant preference for any of the 5 compounds that previously (before removal of the last 3 segments) had been attractive to the beetles (Table 3). The removal of the last 3 segments increased the time spent in making a choice. An intense excitation towards the preferred volatile odor once the beetles with intact antennae were released was observed. This could be the effect of the sensilla basiconica exclusively distributed on the last 3 segments. The data showed that these sensilla might play a major role in the food source location behavior of the beetles.

Furthermore, when comparing the time spent in making a choice between the 2 sexes, we did not find a significant difference ($P > 0.05$) either before or after cutting the 3 segments except for (−)-limonene, for which males with cut antennae spent significantly more time in choosing than females ($P < 0.01$) (Table 3). Females significantly preferred the control over (−)-limonene (Table 2). Sensilla trichodea may

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also play some role in *T. castaneum* olfactory detection because despite removal of the last 3 segments, which bear both sensilla types (sensilla trichodea and sensilla basiconica), the choice before and after removal was different (Table 2).

In conclusion, the current work has identified and characterized the distribution of 2 sensilla types. The results demonstrated that insect antennae have structures that function in locating mates and host plants. Further research on the functional morphology of the antennal sensilla using TEM joined with electrophysiological recordings will likely verify the roles of the sensilla identified in this work. The results also showed that the last 3 segments should contribute to the fast location of food sources, because when they were removed, there was no significant preference for any of the 5 compounds that had previously been attractive to the beetles. These conclusions help to improve our understanding of insect behavior and to support future research studies on the sensory systems and behavior of pests of stored products.

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References Cited

Abdel-latief M. 2007. A family of chemoreceptors in *Tribolium castaneum* (Tenebrionidae: Coleoptera). PLoS One 2: e1319.

Amornsak W, Cribb B, Gordh G. 1998. External morphology of antennal sensilla of *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae). International Journal of Insect Morphology and Embryology 27: 67–82.

Barrer PM. 1983. A field demonstration of odour-based, host-food finding behaviour in several species of stored grain insects. Journal of Stored Products Research 19: 105–110.

Birkett MA, Bruce TJA, Martin JL, Smart LE, Oakley J, Wadhams LJ. 2004. Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. Journal of Chemical Ecology 30: 1319–1328.

Bousquet Y. 1990. Beetles Associated with Stored Products in Canada: An Identification Guide. Canadian Government Publishing Centre, Ottawa, Canada.

Bruce TJ, Cork A. 2001. Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta*. Journal of Chemical Ecology 27: 1119–1131.

Burkholder WE. 1990. Practical use of pheromones and other attractants for stored-product insects, pp. 497–516 *In* Ridgway RL, Silverstein RM, Inscoe MN [eds.], Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants. Marcel Dekker, Inc., New York, New York.

Calatayud P-A, Chimtawi M, Tauban D, Marion-Poll F, Le Rü B, Silvain J-F, Frérot B. 2006. Sexual dimorphism of antennal, tarsal and ovipositor chemosensilla in the African stemborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). International Journal of Entomology 42: 403–412.

Campbell JF. 2012. Attraction of walking *Tribolium castaneum* adults to traps. Journal of Stored Products Research 51: 11–22.

Campbell JF, Mullen MA, Dowdy AK. 2009. Monitoring stored-product pests in food processing plants with pheromone trapping, contour mapping, and mark–recapture. Journal of Economic Entomology 95: 1089–1101.

Cha DH, Powell TH, Feder JL, Linn Jr CE. 2011. Identification of fruit volatiles from green hawthorn (*Crataegus viridis*) and blueberry hawthorn (*Crataegus brachyacantha*) host plants attractive to different phenotypes of *Rhagoletis pomonella* flies in the southern United States. Journal of Chemical Ecology 37: 974–983.

Chambers J. 1990. Overview on stored-product insect pheromones and food attractants. Journal of the Kansas Entomological Society 63: 490–499.

Chapman RF. 1982. The Insects: Structure and Function. ELBS, Printed Colorcraft Ltd., Hongkong, China.

Cox PD. 2004. Potential for using semiochemicals to protect stored products from insect infestation. Journal of Stored Products Research 40: 1–25.

Drilling K, Klass K-D. 2010. Surface structures of the antenna of Mantophasmatodea (Insecta). Zoologischer Anzeiger—A Journal of Comparative Zoology 249: 121–137.

Faucheux MJ. 2006. Antennal sensilla of male *Lophocorona pediasia* Common 1973 and their phylogenetic implications (Lepidoptera: Lophocoronidae). Annales de la Société entomologique de France 42: 113–118.

Fernandes FF, Pimenta PFP, Linardi PM. 2004. Antennal sensilla of the New World screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae). Journal of Medical Entomology 41: 545–551.

Flower NE, Helson GAH. 1974. Variation in antennal sensilla of some noctuid moths; a scanning electron microscope study. New Zealand Journal of Zoology 1: 59–66.

Fraser AM, Mechaber WL, Hildebrand JG. 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant headspace volatiles. Journal of Chemical Ecology 29: 1813–1833.

Galvania GL, González A, Roig-Alsina AH, Settembrini BP. 2012. Distribution and morphometric studies of flagellar sensilla in Emphorini bees (Hymenoptera, Apoidea). Micron 43: 673–687.

Grant GG, Ryall KL, Lyons DB, Abou-Zaid MM. 2010. Differential response of male and female emerald ash borers (Col., Buprestidae) to (*Z*)-3-hexenol and manuka oil. Journal of Applied Entomology 134: 26–33.

Groot AT, Timmer R, Gort G, Lelyveld GP, Drijfhout FP, van Beek TA, Visser JH. 1999. Sex-related perception of insect and plant volatiles in *Lygocoris pabulinus*. Journal of Chemical Ecology 25: 2357–2371.

Hallberg E, Hansson BS, Steinbrecht RA. 1994. Morphological characteristics of antennal sensilla in the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Tissue Cell 26: 489–502.

Honda K, Ômura H, Hayashi N. 1998. Identification of floral volatiles from *Ligustrum japonicum* that stimulate flower-visiting by cabbage butterfly, *Pieris rapae*. Journal of Chemical Ecology 24: 2167–2180.

Hu F, Zhang G-N, Wang J-J. 2009. Scanning electron microscopy studies of antennal sensilla of bruchid beetles, *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Micron 40: 320–326.

Isidoro N, Bartlet E, Ziesmann J, Williams IH. 1998. Antennal contact chemosensilla in *Psylliodes chrysocephala* responding to cruciferous allelochemicals. Physiological Entomology 23: 131–138.

Kaissling K. 1986. Chemo-electrical transduction in insect olfactory receptors. Annual Review of Neuroscience 9: 121–145.

Kostyukovsky M, Chen B, Atsmi A, Shaaya E. 2000. Biological activity of two juvenoids and two ecdysteroids against three stored product insects. Insect Biochemistry and Molecular Biology 30: 891–897.

Light DM, Jang EB, Dickens JC. 1988. Electroantennogram response of Mediterranean fruit fly, *Ceratitis capitata*, to a spectrum of plant volatiles. Journal of Chemical Ecology 14: 159–180.

Lopes O, Barata EN, Mustaparta H, Araújo J. 2002. Fine structure of antennal sensilla basiconica and their detection of plant volatiles in the eucalyptus woodborer, *Phoracantha semipunctata* Fabricius (Coleopetera: Cerambycidae). Arthropod Structure and Development 31: 1–13.

Lu Y, Beeman RW, Campbell JF, Park Y, Aikins MJ, Mori K, Akasaka K, Tamogami S, Phillips TW. 2011. Anatomical localization and stereoisomeric composition of *Tribolium castaneum* aggregation pheromones. Naturwissenschaften 98: 755–761.

Magan N, Hope R, Cairns V, Aldred D. 2003. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. European Journal of Plant Pathology 109: 723–730.

Merivee E, Rahi M, Bresciani J, Ravn HP, Luik A. 1998. Antennal sensilla of the click beetle *Limonius aeruginosus* (Olivier) (Coleoptera: Elateridae). International Journal of Insect Morphology and Embryology 27: 311–318.

Mullen MA. 1992. Development of a pheromone trap for monitoring *Tribolium castaneum*. Journal of Stored Product Research 28: 245–249.

Nawrot J, Gawlak M, Szafranek J, Szafranek B, Synak E, Warchalewski JR, Piasecka-Kwiatkowska D, Błaszczak W, Jeliński T, Fornal J. 2010. The effect of wheat grain composition, cuticular lipids and kernel surface microstructure on feeding, egg-laying, and the development of the granary weevil, *Sitophilus granarius* (L.). Journal of Stored Products Research 46: 133–141.

Onagbola EO, Fadamiro HY. 2008. Scanning electron microscopy studies of antennal sensilla of *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). Micron 39: 526–535.

Phillips TW. 1997. Semiochemicals of stored-product insects: research and applications. Journal of Stored Products Research 33: 17–30.

Rebora M, Piersanti S, Gaino E. 2008. The antennal sensilla of the adult of *Libellula depressa* (Odonata: Libellulidae). Arthropod Structure and Development 37: 504–510.

Ren L-L, Wu Y, Shi J, Zhang L, Luo Y-Q. 2014. Antenna morphology and sensilla ultrastructure of *Tetrigus lewisi* Candèze (Coleoptera: Elateridae). Micron 60: 29–38.

Renthal R, Velasquez D, Olmos D, Hampton J, Wergin WP. 2003. Structure and distribution of antennal sensilla of the red imported fire ant. Micron 34: 405–413.

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Robacker DC, Warfield WC, Flath RA. 1992. A four-component attractant for the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae), from host fruit. Journal of Chemical Ecology 18: 1239–1254.

Roth LM, Willis ER. 1951. The effects of desiccation and starvation on the humidity behavior and water balance of *Tribolium confusum* and *Tribolium cataneum*. Journal of Experimental Zoology 118: 337–361.

Roux O, van Baaren J, Gers C, Arvanitakis L, Legal L. 2005. Antennal structure and oviposition behavior of the *Plutella xylostella* specialist parasitoid: *Cotesia plutellae*. Microscopy Research and Technique 68: 36–44.

Sauer DB, Storey L, Walker DE. 1984. Fungal populations in U.S. farm-stored grain and their relationship to moisture, storage time, regions, and insect infestation. Phytopathology 74: 1050–1053.

Shields VDC, Hildebrand JG. 1999. Fine structure of antennal sensilla of the female sphinx moth, *Manduca sexta* (Lepidoptera: Sphingidae). I. Trichoid and basiconic sensilla. Canadian Journal of Zoology 77: 290–301.

Sukontason K, Methanitikorn R, Chaiwong T, Kurahashi H, Vogtsberger RC, Sukontason KL. 2007. Sensilla of the antenna and palp of *Hydrotaea chalcogaster* (Diptera: Muscidae). Micron 38: 218–223.

Sun X, Wang M-Q, Zhang G. 2011. Ultrastructural observations on antennal sensilla of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). Microscopy Research and Technique 74: 113–121.

Suzuki T, Kozaki J, Sugawara R, Mori K. 1984. Biological activities of the analogs of the aggregation pheromone of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Applied Entomology and Zoology 19: 15–20.

Symondson WOC, Williams IB. 1997. Low-vacuum electron microscopy of carabid chemoreceptors: a new tool for the identification of live and valuable museum specimens. Entomologia Experimentalis et Applicata 85: 75–82.

van der Goes van Naters W, Carlson JR. 2006. Insects as chemosensors of humans and crops. Nature: 444: 302–307.

Yang H, Yan S-C, Liu D. 2009. Ultrastructural observations on antennal sensilla of *Coleophora obducta* (Meyrick) (Lepidoptera: Coleophoridae). Micron 40: 231–238.

Zacharuk RY. 1985. Antennae and sensilla, pp. 1–69 *In* Kerkut GA, Gilbert LI [eds.], Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Volume 6. Pergamon Press, Oxford, United Kingdom.

Zheng HX, Liu HX, Guo SY, Yan Y, Zong SX, Zhang JT. 2014. Scanning electron microscopy study of the antennal sensilla of *Catocala remissa*. Bulletin of Insectology 67: 63–71.

Zhou C-X, Sun X, Mi F, Chen JY, Wang M-Q. 2015. Antennal sensilla in the parasitoid *Sclerodermus* sp. (Hymenoptera: Bethylidae). Journal of Insect Science 15: article 36.

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