Scanning Electron Microscope Observations on the Antennal Sensilla of Two Stored Grain Pests Trogoderma granarium and Trogoderma variabile (Coleoptera: Dermestidae)

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Scanning electron microscope observations on the antennal sensilla of two stored grain pests

*Trogoderma granarium* and *Trogoderma variabile* (Coleoptera: Dermestidae)

Chunyan Wei, Bingzhong Ren, Xin Chen, Xiao Zhou, Weili Wang and Zhenguo Wang

Abstract

In this study both adult male and female antennal sensilla of *Trogoderma granarium* Everts, 1896 and *Trogoderma variabile* Ballion, 1878 (Coleoptera: Dermestidae) were observed by scanning electron microscope. The antennae of both species were found to consist of a scape, a pedicel and a flagellum with 9 subsegments, i.e., flagellomeres. Four categories of antennal sensilla including 10 types were found in these 2 species. The sensilla were designated, sensilla chaetica I, sensilla chaetica II, sensilla chaetica III, sensilla basiconica I, sensilla basiconica II, sensilla basiconica III, sensilla basiconica IV, sensilla basiconica V, sensilla coeloconica and Böhm bristles. The characteristics and distribution of these antennal sensilla were described, and relevant differences between the male and the female were compared. Finally, the probable functions and their applications in taxonomy were briefly discussed. These findings provide an improved understanding of the morphology of the antennae in these 2 species and can help to distinguish them clearly. Besides, these results also will support investigations into adaptations of these *Trogoderma* species to storage environments.

Key Words: adaptation; flagellomere; sensilla queticas; sensilla basiconicas; sensilla coeloconica; Böhm bristles; scanning electron microscopy (SEM)

Resumen

En este estudio, se observaron por microscopía electrónica de barrido, las sensilas de las antenas de los machos y hembras de *Trogoderma granarium* Vuelca, 1896 y *Trogoderma variabile* Ballion, 1878 (Coleoptera: Dermestidae). Se encontró que las antenas de ambas especies consisten en un escapo, un pedicelo y un flagelo con 9 subsegmentos, los que se llaman flagelómeros. Se encontraron tres categorías, incluyendo 10 tipos de sensilas antenales en estas 2 especies. Las sensilas fueron designadas, Sensila queticas I, Sensila queticas II, Sensila queticas III, Sensila basiconica I, Sensila basiconica II, Sensila basiconica III, Sensila basiconica IV, Sensila basiconica V, Sensila coeloconica y setas Böhm. Se describen las características y la distribución de estas sensilas antenales y se comparan las diferencias relevantes entre las del macho y la hembra. Por último, se discuten brevemente las funciones probables y sus aplicaciones en la taxonomía. Estos resultados aportan una mejor comprensión de la morfología de las antenas en estas 2 especies y su apoyo en la investigación de las adaptaciones de estas especies *Trogoderma* a los ambientes de almacenaje.

Palabras Clave: adaptación; flagelómero; sensilas queticas; sensilas basiconicas; setas Böhm; microscopía electrónica de barrido (SEM)

*Trogoderma granarium* Everts, 1898 and *Trogoderma variabile* Ballion, 1878 (Coleoptera: Dermestidae) are the most widespread storage pests around the world. They can tremendously damage animal and vegetable products including grains and grain products, seeds, furs, leathers, silks, etc. (Campbell et al. 2002; Bell & Wilson 1995). *Trogoderma granarium* is even a more serious pest than *T. variabile*, and it is one of the most concerning quarantine pests in the world (Gomah 2014). Larvae of *T. granarium* can indirectly cause stored commodities to heat up and rot, and thereby cause great economic losses every year. In addition, *Trogoderma* infestations may pose a human health hazard because of the larval exuviae, which can contaminate food, may be allergenic. *Trogoderma variabile* has adapted to a large variety of food materials (Burges 1960), and dense populations of the larvae are often found in stored food materials, where they cause great damage. These insects are difficult to control because they tolerate fairly high and low temperatures, survive in very dry environments and are resistant to many insecticides (Bell & Wilson 1995). When they are in a favorable environment, they reproduce rapidly and readily destroy 20% of the stored materials. These dermestid species have similar in morphologies, share similar habitats, exhibit similar behaviors, so it is difficult to distinguish between them (Zhao 1966).
Many adaptations have occurred during the evolution of insects, which are manifested in food selection, foraging, courtship, mating, reproduction, rest, defense, migration, etc. For that they lived in partially or completely dark environments, so mechanical signals are important for the species in this genus and they are also affected by many chemicals including their sex pheromone, insecticides, repellents, food attractants, etc. (Levinson & Ilan 1970; Sattar et al. 2010; Ahmad et al. 2013; Olson 2013). Thus, detailed knowledge of the morphologies of the major sense organs – especially the antenna and the antennal sensilla – is critically important. Recent publications on coleopteran antennal sensilla are fairly numerous and they include studies on Lasioderma serricorne F. (Anobiidae) (An et al. 2009), Callosobruchus maculatus (F.), Callosobruchus chinensis Laterelle (Chrysomelidae) (Hu et al. 2009), Odoiporus longicollis Oliver (Curculionidae) (Gao et al. 2011), Scolytus schevyrewi Semenov (Curculionidae) (Fan et al. 2011), Agriotes obscurus (L.) (Elateridae) (Merivee et al. 1997) and Psylliodes chrysocephala L. (Chrysomelidae) (Isidoro 1998).

Different sensillum type has different functions and different species have different types of sensillum. Alabi (2014) showed that they found five sensillum types on the club of the antenna of both sexes of Tribolium brevicornis, represent the most prominent stored food product pests worldwide. While, until now, no detailed reports on the antennal sensilla of T. granarium and T. variabile antennae have been published. In this study, the antennal sensilla of T. granarium and T. variabile were observed by scanning electron microscopy (SEM). Detailed observations were performed on the quantity, type, distribution and gender variations of the antennal sensilla. The results provide useful information for future functional studies on clarifying the relationship between chemical receptors and behavior, and assist in the overall classification of the sensilla.

Material and Methods

STUDIED MATERIAL

*Trogoderma granarium* specimens were captured from imports of mung from Muse, Burma (N 23° 58' 45" E 97° 54' 17"), and *T. variabile* specimens were collected from storage facilities in Dandong (N 40° 07' E 124° 23'3), Liaoning Province, China. The sample size of each of these 2 species was 20 (10 males and 10 females).

SCANNING ELECTRON MICROSCOPE (SEM) OBSERVATION

Antennae were carefully excised from the antennal sockets with fine forceps under a stereomicroscope. The antennae were first stored in a 70% ethanol solution. They were then cleaned for 3 min by ultrasonic waves and then dehydrated by an ethanol serial solutions.
(75%, 80%, 85%, 90% to 100%), with a 10 min interval between solutions. Five pairs of antennae of each species were mounted on the ventral or dorsal side on a sticky tape, and then sputter coated with gold/palladium. The specimens were examined by a Hitachi S-570 SEM set at 20 kV. Micrographs of the antennae, antennomeres and sensilla were taken.

**DATA ANALYSIS**

Photoshop 7.0 image processing software was used, and each part of antennae sensilla was measured using Smile View (Ver. 2.71) software. SPSS17.0 was used to produce the statistical results expressed as mean ± SE.

**Fig. II (1–9).** Antennal sensilla of *T. granarium*. 1. SC1: sensilla chaetica 1 bar = 5.0 μm; 2. SC2: sensilla chaetica 2, bar = 15.0 μm; 3. SC3: sensilla chaetica 3, bar = 17.2 μm; 4. SB1: sensilla basiconica 1, bar = 2.0 μm; 5. SB2: sensilla basiconica 2, bar = 3.0 μm; 6. SB3: sensilla basiconica 3, bar = 3.0 μm; 7. SB4: sensilla basiconica 4, bar = 2.0 μm; 8. SB5: sensilla basiconica 5, bar = 2.5 μm; and 9. BB: Böhm bristles, bar = 2.3 μm.
SENSILLA NOMENCLATURE

Sensilla were named according to Schneider (1964), Altner (1977) and Zacharuk (1985). The morphology and distribution of sensilla were observed, and the number of sensilla was determined on both the ventral and the dorsal side. Four groups totaling 20 antennae were observed ventrally (5 females and 5 males) and dorsally (5 females and 5 males). The types of antennae sensilla were named according to Schneider (1964) and the nomenclature of Zacharuk (1980, 1985), then compared with the sensilla of other coleopterans.

**Fig. III (1–9).** Antennal sensilla of *T. variabile*. 1. SC1: sensilla chaetica 1, bar = 3.0 μm; 2. SC2: sensilla chaetica 2; SC3: sensilla chaetica 3, bar = 8.6 μm; 3. SC2: sensilla chaetica 2, bar = 15 μm; 4. SC3: sensilla chaetica 3, bar = 15.0 μm; 5. SB1: sensilla basiconica 1, bar = 3.0 μm; 6. SB2: sensilla basiconica 2, bar = 4.3 μm; 7. SB5: sensilla basiconica 5, bar = 4.3 μm; 8. SCo: sensilla coeloconica, bar = 1.5 μm; and 9. BB: Böhm bristle, bar = 6.0 μm.
Results

GENERAL STRUCTURE OF THE ANTENNAE

Most antennae of both species had a hammerhead shape with 11 segments, but some had only 9 or 10 segments. Antennae extended from between the compound eyes. The antennae consisted of 3 parts: a proximal scape, a pedicel, and a distal flagellum, with the latter composed of 9 flagellomeres (Fig. I). The antenna of males was longer than those of females. Antennal fossae were deep (Fig. II).

The length of the distal flagellomere (flagellomere 9) was approximately equal to the sum of lengths of the ninth and tenth antennal segments (flagellomeres 7 + 8). The male antenna was 492.925 μm ± 15.854 μm long, while the female’s antenna was 403.666 μm ± 48.787 μm long (Table 1).

The *T. variabile* male had 7–8 flagellomeres in his antennal club and the length of the proximal flagellomeres (flagellomeres 2–8) was about 2 times the length the distal flagellomere (flagellomere 9). In females, the length of the distal flagellomere (flagomere 9) was almost equal to the length of the 3 proximal flagellomeres (flagellomeres 6–8). *Trogoderma variabile* male and female antennae were 533.500 μm ± 33.348 μm and 801.425 μm ± 6.097 μm in length, respectively (Table 1).

ANTENNAL SENSILLA OF TWO SPECIES IN GENUS TROGODERMA

Based on morphology, surface characteristics and growth position, 10 types of sensilla on the antennae of both female and male were recognized including 4 types of sensilla chaetica (SC1, SC2 and SC3), 5 types of sensilla basiconica (SB1, SB2, SB3, SB4 and SB5), 1 type of sensilla coeloconica (SCo) and Böhm bristles (BB) (Table 2). The approximate number and distribution of various sensilla types on each antennal segment of the 2 sexes are listed in Table 3 and Table 4, and elaborated below.

SENSILLUM CHAETICUM I [SC1, FIGS. II (1) AND III (1)]

The SC1 presented the form of comparatively small straight bristle with longitudinal grooves wider at the base and tapering toward the apex. The top of SC1 is blunt and the bristle leans along the antenna axis toward the apex. These sensilla are located on the scape and the pedicel of the antennal surface in both males and females, being on flagellomeres 6–9 in males and on flagellomeres 2–9 in females (Fig. II (1) & III (1)). SC1 are 11.230 μm ± 0.453 μm long and 1.435 μm ± 0.062 μm wide at the base.

SENSILLUM CHAETICUM II [SC2, FIGS. II (2) AND III (2 & 3)]

SC2 are the most widespread sensilla, and are present in the largest numbers, being found on each part of the antenna in both the female and the male. However, the arrangement of SC2 is a circular permutation on the last 6 flagellomeres. They are set in an open socket, and present obvious longitudinal grooves on the cuticular surface. The sensilla are very close to the surface and point toward the tip of the antenna (Figs. II (2) and III (2 & 3)). SC2 are 23.620 μm ± 1.137 μm long and 1.435 μm ± 0.062 μm wide at the base.

SENSILLUM CHAETICUM III [SC3, FIGS. II (3) AND III (2 & 4)]

The SC3 look like long sickle-shaped bristles that exist only on the scape and the pedicel (Fig. II (3) & III (2 & 4)). They are longer than...
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other sensilla with a mean length of 45.094 μm ± 1.630 μm and a mean base width of 2.740 μm ± 0.093 μm. SC3 are located in an open articulating socket. The angle between the sensillum and antenna ranges from 60° to 80°. They are characterized by a longitudinally grooved ridge that narrows toward the tip.

SENSILLUM BASICONICUM I [SB1, FIG. II (4) AND III (5)]

SB1 are straight, conical, smooth-walled without longitudinal grooves, and blunt-tipped with a distinctive droplet shape at the tip. There are pores on the surface of this type of sensillum (Fig. II (4) & III (5)). This sensillum is 6.295 μm ± 0.395 μm long and 1.610 μm ± 0.084 μm wide at the base. They are situated as a dense group at the joints of flagellomeres 6 – 9 and on the foreside of flagellomere 9.

SENSILLUM BASICONICUM II [SB2, FIG. II (5) AND III (6)]

Shaped like curved fingers, SB2 sensilla are gradually curved toward the apex and insert into a broaden pedestal, which is raised slightly above the cuticle and is rather thick with a conical tip. They are located in different areas of the flagellum of females and males (Fig. II (5) & III (6)). In T. granarium SB2 sensilla are dispersed on segments 6 – 9 in males, but on the segments 8–9 in females. In T. variabile they are distributed on segments 2–9 in males, but these sensilla only appear on segment 9 in females. SB2 sensilla were 7.165 μm ± 1.360 μm long and 1.360 μm ± 0.042 μm wide at the base.

SENSILLUM BASICONICUM III [SB3, FIG. II (6)]

The SB3 sensillum has an appearance similar to the Latin letter “Y”. Both tips are cone-shaped, short, and blunt (Fig. II (6)). This type of

<table>
<thead>
<tr>
<th>Types of sensilla</th>
<th>Length (μm)</th>
<th>Diameter(μm)</th>
<th>Tip</th>
<th>Wall</th>
<th>Shape</th>
<th>Socket</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>4.080 ± 0.221</td>
<td>1.035 ± 0.043</td>
<td>Sharp</td>
<td>Smooth</td>
<td>Straight</td>
<td>Wide</td>
</tr>
<tr>
<td>SC1</td>
<td>11.230 ± 0.453</td>
<td>1.435 ± 0.062</td>
<td>Blunt</td>
<td>Grooved</td>
<td>Straight</td>
<td>Wide</td>
</tr>
<tr>
<td>SC2</td>
<td>23.620 ± 1.137</td>
<td>1.905 ± 0.090</td>
<td>Blunt</td>
<td>Grooved</td>
<td>Straight</td>
<td>Wide</td>
</tr>
<tr>
<td>SC3</td>
<td>45.094 ± 1.630</td>
<td>2.740 ± 0.093</td>
<td>Blunt</td>
<td>Grooved</td>
<td>Straight</td>
<td>Wide</td>
</tr>
<tr>
<td>SB1</td>
<td>6.295 ± 0.395</td>
<td>1.610 ± 0.084</td>
<td>Blunt</td>
<td>Smooth</td>
<td>Straight</td>
<td>Tight</td>
</tr>
<tr>
<td>SB2</td>
<td>7.165 ± 1.360</td>
<td>1.360 ± 0.042</td>
<td>Blunt</td>
<td>Smooth</td>
<td>Straight</td>
<td>Tight</td>
</tr>
<tr>
<td>SB5</td>
<td>15.658 ± 1.575</td>
<td>1.575 ± 0.065</td>
<td>Blunt</td>
<td>Smooth</td>
<td>Curved</td>
<td>Tight</td>
</tr>
</tbody>
</table>

Measurements: [mean ± SE] obtained from a total of 20 sensilla per type from antennae of males and females (10 per sex).

BB: Böhm bristles; SC1: Sensilla chaetica 1; SC2: Sensilla chaetica 2; SC3: Sensilla chaetica 3; SB1: Sensilla basiconica 1; SB2: Sensilla basiconica 2; SB5: Sensilla basiconica 5.

SENSILLUM BASICONICUM IV [SB4, FIG. II (7)]

The SB4 sensillum has a bifurcated tip, which looks like the Latin letter “V” (Fig. II (7)). SB4 is observed only on flagellomeres 6–9 of *T. granarium* males. The length from the highest point to the base is 4.580 μm ± 0.199 μm, and the distance of the second highest point to the base is 3.834 μm ± 0.156 μm and that of the lowest point to the base is 3.150 μm ± 0.233 μm. The base is 1.510 μm ± 0.091 μm wide.

SENSILLUM BASICONICUM V [SB5, FIGS. II (8) AND III (7)]

The SB5 sensillum has surface properties similar to those of SB2, but it stands up right like an erect finger (Fig. II (8) & III (7)). SB5 sensilla are 15.658 μm ± 1.575 μm long and 1.575 μm ± 0.065 μm wide at the base. SB5 sensilla are located on the flagellomeres 8–9 of the female and on flagellomeres 6–9 of *T. granarium* males, and they also present on flagellomeres 2–9 of the male of *T. variabile* males.

SENSILLUM COELOCONICA [SCO, FIG. III (8)]

Sensilla coeloconica were found on dense areas of sensilla and their tips gathered like flower buds (Fig. III (8)). They can only be found on the ninth flagellum in the female of *T. variabile*. These sensilla are the shortest of all the types, with a length of 2.574 μm ± 0.113 μm and a width of 1.178 μm ± 0.661 μm.

BÖHM BRISTLES [BB, FIGS. II (9) AND III (9)]

Böhm bristles (BB) are shorter than sensilla trichodea and thinner than sensilla basiconica, mainly occurring in dense clusters on the bases of the antennal joints between the scape and the head and between the scape and the pedicel of female and male *T. granarium* and *T. variabile*. They are surrounded by a shallow cuticular socket with obtuse tops and smooth cuticles, standing almost perpendicular to the antennal surface (Fig. II (9) & III (9)). BB is 4.080 μm ± 0.221 μm in length and 1.035 μm ± 0.0428 μm in width.

COMPARISONS OF THE TYPES AND NUMBERS OF SENSIlla BETWEEN SPECIES AND SEXUAL GENDERS

The SC1 sensillum is the most abundant sensillum type in the studies (with the exception in female *T. granarium* in which SC2 is the most abundant), the number of SC1 sensilla in *T. variabile* was much greater than in *T. granarium*. Males had more sensilla of most types than females. It is noteworthy that the male of *T. variabile* had an extremely large number of SB2 type sensilla. Comparison data of the sensilla types between *T. granarium* and *T. variabile* species and between males and females are displayed in Fig. IV.

**Discussion**

**SEXUAL DIMORPHISM**

Our results showed total of 10 types of sensilla on the antennae of adult *T. granarium* and *T. variabile*, and the amount and distribution of sensilla varied with different segments. *T. granarium* had 9 types of sensilla, and lacked SCo. *T. variabile* had 8 types of sensilla, and lacked SB3 and SB5. Generally speaking, the male has a greater number of sensilla than the female in both *T. granarium* and *T. variabile*. A reason for this gender difference is that in these species the male’s antenna is much longer than female’s. For example, the number of SC1 sensilla in male was extremely large compared with the female, which suggested these sensilla serve some important functions, such as mechanical reception, in male of these species. However, some types of sensilla, like SB4 and SCo, are very much more numerous in females than males, so that the length of the antenna seems not to be the only determinant of the number of sensilla. The SC was commonly identified as mechanosensory/gustatory sensilla by Rüth (1976). Chemical signals are very important and affect foraging and many other biological functions in these *Trogoderma* species (Cohen et al. 1974). Also, a highly developed mechanosensory sensilla benefited species that lived in near darkness with very weak visual signals. Thus the males in these 2 *Trogoderma* species might possess a more sensitive mechanosensory/gustatory sense than the females. Also the *T. variabile* males possessed more SB2 sensilla than females. The SB2 sensilla are “poreless sensilla with inflexible sockets”, and are thought to be thermo-hygroreceptive (Altner& Loftus 1985; Altner & Prillinger 1980; Altner et al. 1983), but we still do not know why the number of SB2 is so great in *T. variabile*.

There are no big differences in the numbers of Böhm bristles between males and females of both species. Many studies have demonstrated that Böhm bristles exist in the same anatomical location on many insects, so this is considered to be a separate type of sensilla. This sensillum is often found at the junction of the head and the scape, or at the junction of the scape and the pedicel. BB were deduced to be proprioceptors to perceive antennal movement and position by Ochieng et al. (2000) and Onagbola & Fadamiro (2008). Previous studies also shown that the BB of *T. granarium* and *T. variabile* had the functions of sensing mechanical stimulations, and that they induced a cushioning action when stimulated, and control the speed of antennal movement. As a result, there is no sexual dimorphism with respect to BB has been observed.

**INTERSPECIES DIFFERENTIATION**

The results demonstrate some quantitative differences concerning the sensilla between the two species. Thus, *T. variabile* males had many more SC1 and SB2 sensilla than *T. granarium* males, but in the female, *T. granarium* had more SC2 sensilla than *T. variabile*. More importantly, our results indicate the location of each type of sensilla was also somewhat different between the 2 species. SC and BB had the same anatomical location; SB other than SB1, all had different locations on the 2 species. In *T. granarium* SB2 and SB5 were mainly distributed on segments 6–9, but they were distributed on segments 2–9 in *T. variabile*; SB3 and SB4 were found only on *T. granarium*, and SCo only on *T. variabile*. As a result, these differences are sufficient to allow the correct identification of these species, and this overcomes the disadvantage of having to identify these 2 species based on other morphological characters, which differ very little. Some other reports also showed using micro structure characters of antenna in taxonomy was accurate and efficient, especially in some closely related species which were very similar in morphologies (Tan et al. 2012; González & Zaballos 2013).

The functions of sensilla and their significance to taxonomy described here are merely a starting point. More evidences need to be provided by transmission electron microscopy (TEM), single sensillum records (SSR) and other advanced techniques to confirm and expand on these findings.
Table 3. Abundance and distribution of different sensilla on the antenna of female and male *Trogoderma granarium*.

<table>
<thead>
<tr>
<th>T. granarium</th>
<th>BB</th>
<th>SC1</th>
<th>SC2</th>
<th>SC3</th>
<th>SB1</th>
<th>SB2</th>
<th>SB3</th>
<th>SB4</th>
<th>SB5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Scape</td>
<td>9 ± 5</td>
<td>11 ± 2</td>
<td>12 ± 4</td>
<td>16 ± 4</td>
<td>15 ± 4</td>
<td>9 ± 1</td>
<td>4 ± 1</td>
<td>7 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>Pedicel</td>
<td>7 ± 1</td>
<td>11 ± 1</td>
<td>12 ± 7</td>
<td>17 ± 2</td>
<td>14 ± 2</td>
<td>16 ± 1</td>
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<td>2</td>
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<td>0</td>
<td>1 ± 1</td>
<td>8 ± 1</td>
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<td>3 ± 2</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
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<tr>
<td>4</td>
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<td>0</td>
<td>0</td>
<td>10 ± 7</td>
<td>7 ± 1</td>
<td>11 ± 3</td>
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<td>29 ± 9</td>
<td>12 ± 2</td>
<td>10 ± 1</td>
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<td>6</td>
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<td>26 ± 8.4</td>
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<td>0</td>
<td>46 ± 12</td>
<td>70 ± 14</td>
<td>21 ± 10</td>
<td>11 ± 2</td>
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<td>0</td>
<td>63 ± 8</td>
<td>76 ± 24</td>
<td>21 ± 6</td>
<td>29 ± 11</td>
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<td>0</td>
<td>96 ± 13</td>
<td>208 ± 16</td>
<td>8 ± 2</td>
<td>9 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: F means female; M means male.

Table 4. Abundance and distribution of different sensilla on the antenna of female and male *Trogoderma variabile*.

<table>
<thead>
<tr>
<th>T. variabile</th>
<th>BB</th>
<th>SC1</th>
<th>SC2</th>
<th>SC3</th>
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Note: F means female; M means male.
Acknowledgments

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References Cited


