The oriental armyworm, *Mythimna separata* (Walk), is one of the most serious pests of cereals in Asia and Australasia. Although the sex pheromone of *M. separata* (211-16: Ald) has been identified (Zhu et al. 1987, Kou et al. 1992) and monitoring methods have been developed using it as a lure (Jung et al. 2013), there is no information on the physiology of the olfactory sensory apparatus on the antennae of this species.

Insect antennae play an important role in the behavior of insects, such as in finding habitats and mates (Chapman 1998). Also, lots of sensory organs, or sensilla, occur on the antennae of insects, in the form of hairs, pegs, pits or cones which function as chemoreceptors (gustatory and olfactory), mechanoreceptors, thermoreceptors, hygroreceptors, and CO₂ receptors (Keil 1999, Stange and Stowe 1999). Morphology and structure observations of insect sensillum are the basement of the function study, and by now, antennal sensilla have previously been described for many insect species using scanning electron microscopy (SEM) or transmission electron microscopy (TEM) (Onagbola et al. 2008, Rebora et al. 2008, Drilling and Klass 2010, Sun et al. 2011, Galvania et al. 2012, Zhou et al. 2015). According to the morphology, the sensilla were termed as trichoid, chaetica, coeloconica, basiconica, Böhm bristles, etc. (Keil 1999). Sensilla trichoid, basiconic, and coeloconica were thought sensitive to many kinds of chemical stimulus (Kaissling 1986, Isidoro et al. 1998, Onagbola and Fadamiro 2008). Styloconica and one type of coeloconica have been suggested involving in the perception of humidity, temperature, heat, and CO₂ (Altner et al. 1983) and might play a role in preventing desiccation (Kristoffersen et al. 2006). Böhm’s bristles have been thought sensing the position and movements of the antennae (Merivee et al. 2002).

To improve our understanding of their function and of the ways that semichemicals may mediate mate and host location in *M. separata*, the antennal sensory structures of *M. separata* were studied by SEM and TEM.

**Materials and Methods**

*M. separata* larvae were obtained from fields in Yicheng (111° 57’ E; 31° 26’ N), People’s Republic of China, in May 2013 and reared on wheat (Huamai 2152) shoots at 24 ± 1°C under a photoperiod of 12:12 (L:D) h. After emergence, female and male adults were provided with a 10% sucrose solution, and 2–3-d-old adults were used for the observations.

Before SEM and TEM examination, the antennae were cut from the head and cleaned twice in an ultrasonic bath (250 W) for 5 s. SEM and TEM observations were made following the methods of Sun et al. (2011). Briefly, for SEM, the antennae were dehydrated through a graded ethanol series of 30%, 45%, 60%, 75%, 90%, and 95% (for 15 min each) and then fully dehydrated twice in 100% ethanol solution (for 15 min each time). Following drying to the critical point, the antennal preparations were mounted on a holder using double-sided adhesive tape and sputter coated with gold/palladium (40:60). Fifteen antennae from separate males and females were examined in total, using a Hitachi jsm-6390i (Hitachi, Tokyo, Japan) SEM at 20 kV.

For TEM, the antennae were fixed in 2% glutaraldehyde and 1% sucrose in 0.1 M cacodylate buffer for 3 h. The samples were washed in 0.1 M cacodylate buffer and then post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h, all at pH 7.0 and 25°C. The antennae were embedded in epon after dehydration in a graded series of ethanol, 50%, 70%, 80%, and 95% (for 30 min each), and twice at 100% (for 30 min each time). Epon was polymerized at 60°C for 48 h. Serial sections of 80 nm were cut on a Leica Super Nova ultramicrotome with a diamond knife and collected on formvar-coated nickel grids. Sections were contrasted with uranylacetate and stained with 1% toluidine blue. The sample grids were observed using a Hitachi H-7500 TEM (Hitachi, Tokyo, Japan).

**Terminology and Data Processing.** The morphological terms used here follow those defined in the literature (Schneider 1964, Altner 1977, Zacharak 1985). The antennal length, diameter, and sensillum counts for each segment, and the length and diameter of the sensilla were directly
Results

General Description of Antennae of M. separata. Antennae of female and male moths are both thread-like and consist of a scape, a pedicel, and a flagellum. The dorsal surface of each segment is covered by two rows of squamae. The flagellum length of females (10.08 ± 0.68 mm) and males (9.96 ± 0.76 mm) were similar (t = 0.59, P = 0.56), while the number of antennal segments of female flagella (76.8 ± 3.0) were greater than that of the male (73.8 ± 3.8) (t = 3.17, P < 0.05).

Antennal Sensillum Types and Distribution. On the ridge-shaped surface of the antennae (Fig. 2A1), seven different types of sensilla were observed on both male and female antennae: sensilla chaetica, sensilla trichodea (s. trichodea), sensilla basiconica (s. basiconica), sensilla coeloconica (s. coeloconica), sensilla styloconica (s. styloconica), sensilla squamiformia (s. squamiformia), and Boëhm bristles. Based on the presence of pores on the sensillum wall, they could be divided into three groups: uniporous sensilla (s. chaetica), multiporous sensilla (s. trichodea, s. basiconica, s. coeloconica, and s. styloconica), and aporous sensilla (s. squamiformia and Boëhm bristles).

S. chaetica/SC. S. chaetica arose from a doughnut-shaped base, grooved longitudinally when observed at high magnification (Fig. 1A1) and were significantly longer and wider than the other sensilla (Table 2). The TEM micrographs of s. chaetica showed a thick wall and no pores (Fig. 1B), except with a terminal pore (Fig. 1C). Both of female and male antennae had six s. chaetica surrounding each segment except that 12 s. Chaetica were found on the last segment of the antennae (Table 1), and the length of s. chaetica distributed on the latero-ventral was longer than that of those on the medio-dorsal or on the medio-ventral (Fig. 1A2; Table 2).

S. trichodea/ST. S. trichodea-like hairs filled the surface socket opening and obvious ring-like striations occurred from the middle part to the apex, becoming smooth at the base (Fig. 2A1). The TEM micrographs of their cross-sections showed they have thick cuticle walls and a few pores (Fig. 2B1 and B2). Three different types were identified based on their external features.

S. trichodea I occurred on the lateral regions of the proximal and median segments and were “S” curved, usually stood perpendicular to the antennae, and had grooves on the surface (Table 1). In male antennae, s. trichodea I clustered in three or four rows (Fig. 2A2 and A3) and decreased gradually in length from the lateral edge toward the center of the segment within each row. The number of s. trichodea I decreased after about 40 segments. However, in female antennae, s. trichodea were dispersed (Fig. 2A4) and only occurred on the first 40 segments of the antennae (Table 1). S. trichodea II, which were shorter than s. trichodea I (Table 2), curved from the base in a “C-shape” with grooves on the surface, and were not arranged in rows (Fig. 2A2; Table 1). S. trichodea III had grooves on the surface, were either slightly curved or not, and lay almost parallel to the antennal flagellum, usually appearing in the ventral region of each segment (Figs. 2A5, 3A2, and 4A2; Table 1).

The total number of s. trichodea I of males was significantly higher than that of females (t = 72.85, P < 0.05), while the numbers of s. trichodea II and III of females were significantly higher than those of males (t = 21.85, P < 0.05; t = 35.30, P < 0.05) (Table 1).

The length of s. trichodea I was similar between females and males, while the width of females was larger than that of males. The length of s. trichodea II of males was longer than that of females, while those in females were wider than in males. S. trichodea III were longer in...
females than in males, and they were wider in males than in females (Table 2).

S. basiconica/SB. The bases of s. basiconica were flat, arose from round pits, and did not totally fill the insertion socket, which had faint herringbone striations (Fig. 3A1 and A2). TEM photomicrographs of these sensilla showed thin cuticle walls and conspicuous pores and dendrites (Fig. 3B). S. basiconica were randomly located on the lateral and ventral surfaces of the segment (Table 1). Based on the morphology, there are two types of s. basiconica.

S. basiconica I arose from the socket and were slightly curved or not (Fig. 3A1). The number of s. basiconica I of males was significantly higher than that of females ($t = 28.61$, $P < 0.05$) (Table 1).

S. basiconica II had bases vertical to the flagellum and were sharply curved (Fig. 3A2). The number of s. basiconica II of females...
Table 1. Average number of sensilla on different parts of *M. separata* antennal segments

| Parts of antennal segment | Sex | *s. chaetica* | *s. trichodea* | *s. trichodea* II | *s. trichodea* III | *s. basiconica* I | *s. basiconica* II | *s. coeloconica* I | *s. coeloconica* II | *s. styloconica* | *s. squamiformia* |
|--------------------------|-----|---------------|----------------|-------------------|-------------------|------------------|------------------|-------------------|------------------|----------------|----------------|
| Flagellum | 1–10 | 1.5 | 0.0 | 17.6 | 6.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 64.4 |
| | 11–20 | 0.0 | 0.0 | 1.4 | 84.8 | 1.6 | 15.2 | 2.3 | 1.3 | 4.2 | 1.3 |
| | 31–40 | 0.0 | 0.0 | 6.0 | 0.0 | 0.0 | 0.0 | 3.6 | 7.3 | 3.2 | 0.0 |
| | 51–60 | 0.0 | 0.0 | 11.0 | 4.3 | 1.6 | 2.2 | 4.2 | 1.3 | 4.2 | 1.3 |
| | 61–70 | 0.0 | 0.0 | 6.0 | 0.0 | 0.0 | 0.0 | 3.6 | 7.3 | 3.2 | 0.0 |
| Apical | 150–200 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 4.2 | 1.3 | 0.0 |

Discussion

Seven sensillum types identified in *M. separata* were similar to those reported in most Lepidoptera, including multisensorial s. trichodea, *s. basiconica* and *s. coeloconica*, and *s. styloconica*; uniporous *s. chaetica*; and aporous *s. squamiformia* and Böhm bristles (Jefferson et al. 1970, Flower and Helson 1974, Liu and Liu 1984, Lavoie-Dornik and McNeil 1987, Castrejón-Gómez et al. 1999, Zohry 2008, Diongue et al. 2013).

*S. chaetica* were found distributed around each antennal segment of *M. separata* except the last segment which had a higher number of *s. chaetica*, and the similar distribution of this sensilla have been reported in *Pseudaletia unipuncta* (Flower and Helson 1974), *Helicoverpa armigera* (Diongue et al., 2013), and *Spodoptera littoralis* (Seada 2015). *S. chaetica* were in general recognized as mechnoreceptors (Keil and Steinbrecht 1984), while as uniporous (terminal pore) sensilla, it also have been suggested to have contact/chemoreceptor functions (Seada 2015). And the characteristic that *s. chaetica* were significantly larger and wider than other sensilla suggests that this sensilla might have a protection role for other sensilla except as mechnoreceptors or contact/chemoreceptors.

Pores, or even continuous pores, were found on the walls of s. trichodea and basiconica, on the central peg of s. coeloconica, and on the apices of s. styloconica. Pores on the walls of sensilla indicate that they play a role in sensing chemical stimuli and in olfactory functions (Keil and Steinbrecht 1984). S. trichodea are the most abundant sensilla on the antennae of *M. separata*, and studies of many other lepidopteran
Fig. 3. SEM micrographs two types of s. basiconica (A1, A2). TEM micrographs of s. basiconica (B), showing its thin wall and continuous pores and dendrites. D, dendrites; CW, cuticle wall; P, pores; SB I, s. basiconica type I; SB II, s. basiconica type II.

Fig. 4. SEM micrographs of s. coeloconica (A1, A2). (B) The entire transverse section of s. coeloconica central peg. D, dendrites; CW, cuticle wall; P, pores; SCo I, s. coeloconica type I; SCo II, s. coeloconica type II.

Fig. 5. SEM (A) and TEM (B) micrographs of s. styloconica. CW, cuticle wall; P, pores; SST, s. styloconica.

Table 2. Mean ± SD sizes of the main antennal sensilla of *M. separata* antennal segments

| Sensilla Type          | Length (μm) | Width (μm) |
|------------------------|-------------|------------|
|                        | Female      | Male       | Female      | Male       |
| Medio-dorsal s. chaetica | 83.41 ± 8.68 | 86.54 ± 8.47 | 3.78 ± 0.42 | 3.35 ± 0.41 |
| Latero-ventral s. chaetica | 98.46 ± 12.23 | 106.47 ± 16.07 | 5.77 ± 0.65 | 6.88 ± 0.67 |
| Medio-ventral s. chaetica | 53.73 ± 15.18 | 57.08 ± 16.91 | 5.05 ± 0.77 | 5.64 ± 0.53 |
| s. trichodea I          | 44.69 ± 0.77 | 44.11 ± 7.49 | 1.78 ± 0.45 | 2.05 ± 0.38 |
| s. trichodea II         | 30.97 ± 4.63 | 34.21 ± 7.34 | 1.95 ± 0.32 | 1.65 ± 0.32 |
| s. trichodea III        | 31.69 ± 6.05 | 28.54 ± 4.79 | 1.57 ± 0.27 | 1.59 ± 0.27 |
| s. basiconica I         | 17.42 ± 2.57 | 16.29 ± 3.50 | 1.30 ± 0.23 | 1.35 ± 0.38 |
| s. basiconica II        | 7.38 ± 2.38  | 8.95 ± 2.39  | 1.84 ± 0.42 | 1.82 ± 0.29 |
| s. coeloconica I        | 8.28 ± 0.80  | 8.55 ± 0.66  | 1.23 ± 0.26 | 1.00 ± 0.30 |
| s. coeloconica II       | 5.85 ± 0.74  | 5.32 ± 0.69  | 1.54 ± 0.14 | 0.1423     |
| s. styloconica          | 20.69 ± 3.94 | 17.31 ± 2.59 | 1.54 ± 0.14 | 0.1423     |
| s. squamiformia         | 34.03 ± 2.17 | 34.24 ± 1.64 | 0.23 ± 0.32 | 3.02 ± 0.30 |
| Böhm bristles I         | 3.00 ± 1.50  | 3.00 ± 1.20  | 0.16 ± 0.20 | 0.16 ± 0.20 |
| s. squamiformia         | 34.03 ± 2.17 | 34.24 ± 1.64 | 0.23 ± 0.32 | 3.02 ± 0.30 |
| Böhm bristles II        | 14.81 ± 2.56 | 14.63 ± 3.11 | 1.21 ± 0.20 | 2.15 ± 0.20 |

*The mean length of pit diameter.

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species have shown that these sensilla could be divided into more subtypes according to the number of sensory cells (Hallberg et al. 1994) or the presence (or absence) of pores on the sensillum wall (Onagbola et al. 2008), or depended on the distribution and length (Ren et al. 2014). In our study, it is difficult to pair SEM photos of three subtypes of s. trichodea with TEM photos, respectively, and s. trichodea were divided into three subtypes according the shape of the sensilla. In our study, sexual dimorphism of s. trichodea was found, the number of s. trichodea I were higher than that of females. Many studies on noctuid moths have reported that only males have s. trichodea I (“long s. trichodea”) (Flower and Helson 1974), but s. trichodea I are actually seen in female M. separata (Fig. 2A4). Similar with other insect species, s. trichodea I, which called long s. trichodea in other papers, might associate with olfactory reception of sex pheromones (Kaissling 1979, Zacharuk 1985, Steinbrecht et al. 1992).

S. basiconica are considered to be olfactory receptors to many kinds of chemical stimulus (Kaissling 1986, Isidoro et al. 1998, Onagbola and Fadamiro 2008), though s. basiconica were neglected in some
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