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Antennal Sensory Organs in the Female Millipede *Orthomorphella pekuensis* (Polydesmida: Paradoxosomatidae)

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Abstract: The fine structural characteristics of the antennal sensory organs of a female millipede, *Orthomorphella pekuensis*, were observed with field emission scanning electron microscopy. On the surface of the antenna, four basic types of sensory receptor with the function of either mechanical or olfactory reception are identified in this female millipede. Of these, chaetiform sensilla (CS) and trichoid sensilla (TS) are related to mechanical reception, and four large apical cone sensilla (AS) and three subtypes of basiconic sensilla (BS_1, BS_2, BS_3) are likely to function in olfactory reception, as these receptors have porous structure commonly. Although this millipede also possess a number of primary or secondary sexual characters to improve the efficiency of reproduction, we could not observe their prominent sexually dimorphic characters in the antennal sensilla with the exception of minor structural and numerical differences.

Key words: Antenna, millipede, *Orthomorphella pekuensis*, sensilla

Millipedes are usually found under foliage or stones on the surface of the soil and play an important role in host orientation, food selection and pheromone reception including the decomposition processes of decaying plant (Hopkins and Read, 1992). They usually do not affect on human activities however, there have been some instances of millipedes being of local nuisance to human (Baker, 1985; McKillup, 1988; Niijima and Shinohara, 1988). The true flat-backed millipede, Polydesmida, is the largest order of millipedes, containing more than 2,700 species worldwide (Hopkins and Read, 1992). They are blind and have completely fused sclerites, and most of them have 20 segments (Simonsen, 1990).

Antenna is one of major channels for sensory reception, including receptors for volatile odors and pheromones, contact chemoreception, water vapor, carbon dioxide, sound, proprioception, and touch (Hashimoto, 1991; Steinbrecht, 1997; Kleineidam et al., 2000). Although a lot of papers have suggested possible sensory roles of the antennal sensilla in various insects (Zacharuk, 1980; Okada et al., 1992; Saïd et al., 2003), very little research has been carried out in relation to the sensilla of the millipedes (Nguyen Duy-Jacquemin, 1974, 1989).

There are approximately 10,000 described species of millipedes in the world (Hoffman, 1982). The antennae of the millipede are surrounded in a variety of sensory structures which include mechanical and chemical receptors (Hopkins and Read, 1992). Although biological significance of the sensory receptors on the antenna of the millipede has been widely understood, their structure and function are not clear yet, and more precise analysis is required.

The purpose of the present research was to identify the fine structural characteristics of various sensory organs on the antenna of the millipede *Orthomorphella pekuensis* to better understand their possible behavioral and ecophysiological roles. This paper also focused on the secondary sexual dimorphism on the antennal sensilla of the millipede with the aid of field emission scanning electron microscopy (FESEM).

MATERIALS AND METHODS

Adult female millipedes of *Orthomorphella pekuensis* (Polydesmida: Paradoxosomatidae) were collected in a local area near Cheonan campus of Dankook University,

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Chungnam, Korea. All individuals were maintained under ambient conditions with natural lighting in enclosures with a wooden frame. Specimens were anesthetized with CO_2 and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 mole/L phosphate buffer at pH 7.4. Postfixation was performed with 1% osmium tetroxide in the same buffer.

For scanning electron microscopic examination, the specimens were washed in 0.1 mole/L phosphate buffer following fixation, and put through an ethanol series from 30 to 100% (30 minutes at each concentration, with one repeat with absolute ethanol). The specimens were then transferred to hexamethyldisilazane (HMDS) and allowed to be dried in air. All samples were coated to a thickness of approximately 20 nm with gold-palladium alloy using a sputter coater. The coated specimens were examined on a Hitachi S-4300 field emission scanning electron microscope (Hitachi, Tokyo, Japan) operated with accelerating voltages of 5-20 kV.

Composite number of various sensilla for each segment was counted from the printed images of antennae which were mounted along with their long axis.

RESULTS

The average length of the antenna of adult female millipede *Orthomorphella pekuensis* is shorter than that of the male; length is 3.4 (0.15, n = 5, range = 3.2-3.6) mm, respectively. The antenna has eight distinct articles. The serrate antennal articles of this millipede consist of the scape (1st article), pedicel (2nd article), and six flagellomeres (3rd-8th articles). The articles are all approximately the same length, with the exceptions of the 1th, 7th and 8th articles, which are shorter than the others. Each article is roughly cylindrical but increases in diameter towards the terminal section. The articles develop shorter and wider towards the distal end of the antenna (Fig. 1A).

On the surface of the antenna, there are a variety of sensory receptors, including olfactory and mechanical receptors. According to their morphological and fine structural characteristics, four basic types of antennal sensillum are identified: chaetiform sensilla (CS), trichoid sensilla (TS), basiconic sensilla (BS), and apical cone sensilla (AS). Of these, the BS are divided further into three subtypes: large basiconic sensilla (BS_1) on the 5th and 6th articles, small basiconic sensilla (BS_2) on the 5th article, and a distinct type of the basiconic spiniform sensilla (BS_3) on the 7th article (Figs. 1B, 1C).

Chaetiform sensilla (CS)

The CS are long, sickle-shaped, strong bristles with longitudinal grooves acuminating toward the tip, which are located in an open articulatory socket. According to their external morphology, including microstructural characteristics, the CS were observed on the surfaces of whole segments of the antenna, with the exceptions of the 8th article, which are shorter than the others. They are distributed encircling each segment (Fig. 1D).

The external structures of the CS on the 5th to 7th articles are similar to those observed on the proximal segments (1st to 4th articles). They also appear as long, sickle-shaped, strong bristles with longitudinal grooves with 40 (±5.9, n = 10, range = 30-50) μm in length (Fig. 1E). The CS on the 5th to 7th articles are structurally similar to the TS, but are shorter and more abundant. The lengths of the CS on these distal articles are shorter than those of the proximal articles (Fig. 1F).

Trichoid sensilla (TS)

The TS are long, blunt-tipped, almost straight hairs with deep longitudinal grooves in their lower parts. The TS were also identified on the surfaces of whole segments of the antenna, with the exceptions of the 8th article. They are distributed encircling each segment together with the CS. TS length is mostly 115 (±28.7, n = 10, range = 70-160) μm, in contrast to the length of CS, which is 40 μm (Fig. 1D).

The length of the TS on the 1st to 4th articles is longer than those of the 5th to 7th articles, approximately 120 (±26.3, n = 10, range= 80-160) μm and 95 (±15.2, n = 10, range = 70-120) μm, respectively. The TS of the 5th to 7th articles are straight hairs with deep longitudinal grooves, and are shorter than those on the proximal articles, as the average length of these sensilla gradually decrease towards the distal segment of the antenna (Fig. 1E).

Basiconic sensilla (BS)

The 5th to 7th articles are the most prominent antennal segments in *Orthomorphella pekuensis*. According to their microstructural characteristics, five types of antennal sensilla - the CS, the TS and the three subtypes of BS - are commonly distributed on these articles. There were no BS on the 1st to 4th articles, however, as these articles contain no locally depress areas on their surfaces.

The three subtypes of the BS are distinguished within the cuticular depressed regions of the 5th to 7th articles. They are the large basiconic sensilla (BS_1), small basiconic sensilla (BS_2) and a distinct type of the spiniform basiconic sensilla (BS_3), respectively. The BS_1 can be seen on the 5th article only, while the BS_2 can be seen on the 5th and 6th articles. The BS_3 is present within the depressed region of only the 7th article (Fig. 2A).

Of the eight segments in *O. pekuensis*, the 5th article has the most diverse types of antennal sensillum: two types of BS (BS_1 and BS_2) as well as CS and TS. Each of the BS_1 is a straight, finger-like sensillum with a smooth surface and a dull tip. These sensilla are scattered in the vicinity of the

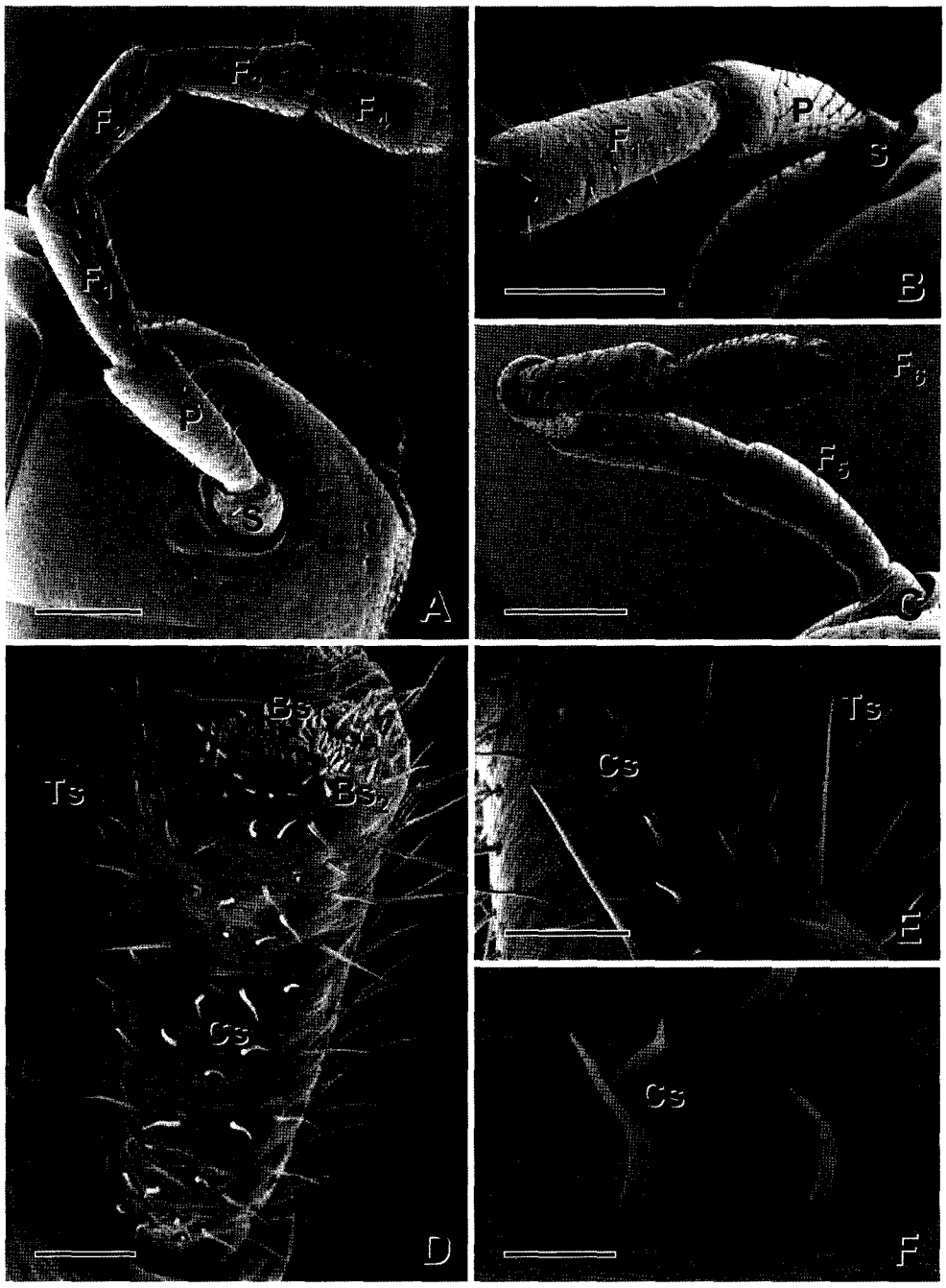

Fig. 1. A-C, The antenna of the female millipede *Orthomorphella pekuensis* has eight distinct articles: a scape (S), a pedicel (P) and 6 flagellomeres (F_1-F_6). The articles are all approximately the same length, with the exceptions of the 1th, 7th and 8th articles, which are shorter than the others. Each article is roughly cylindrical but increases in diameter towards the terminal section. D-F, On the 1st to 4th articles, only two types of antennal sensillum, chaetiform and trichoid, are found. However, on the 5th article, four types of sensillum can be seen: chaetiform (Cs), trichoid (Ts) and two subtypes of basiconic sensilla (BS_1, BS_2). The chaetiform sensilla are long, sickle-shaped strong bristles with deep longitudinal grooves, and the trichoid sensilla are straight hairs. Scale bars, 500 μm (A-C), 100 μm (D-F).

cuticular depressed area of the 5th article. The average size of these sensilla is 33 (±1.7, n = 10, range = 30-36) μm, and the average diameter of the base is 4.0 μm (Fig. 2B). The BS_2 are also straight, finger-like sensilla with a smooth surface, similar to the BS_1. However, they are shorter than the BS_1, with considerably smaller dimensions. The average size of these sensilla is 8.5 (±0.9, n = 10, range = 7-10) μm and the average diameter of the base is 3.0 μm (Fig. 2C).

There is a most distinct and conspicuous cuticular depression on the 6th article, which contains sensilla of type BS_2. The crescent-shaped cuticular depression is located on the dorsal surface of this article. The total

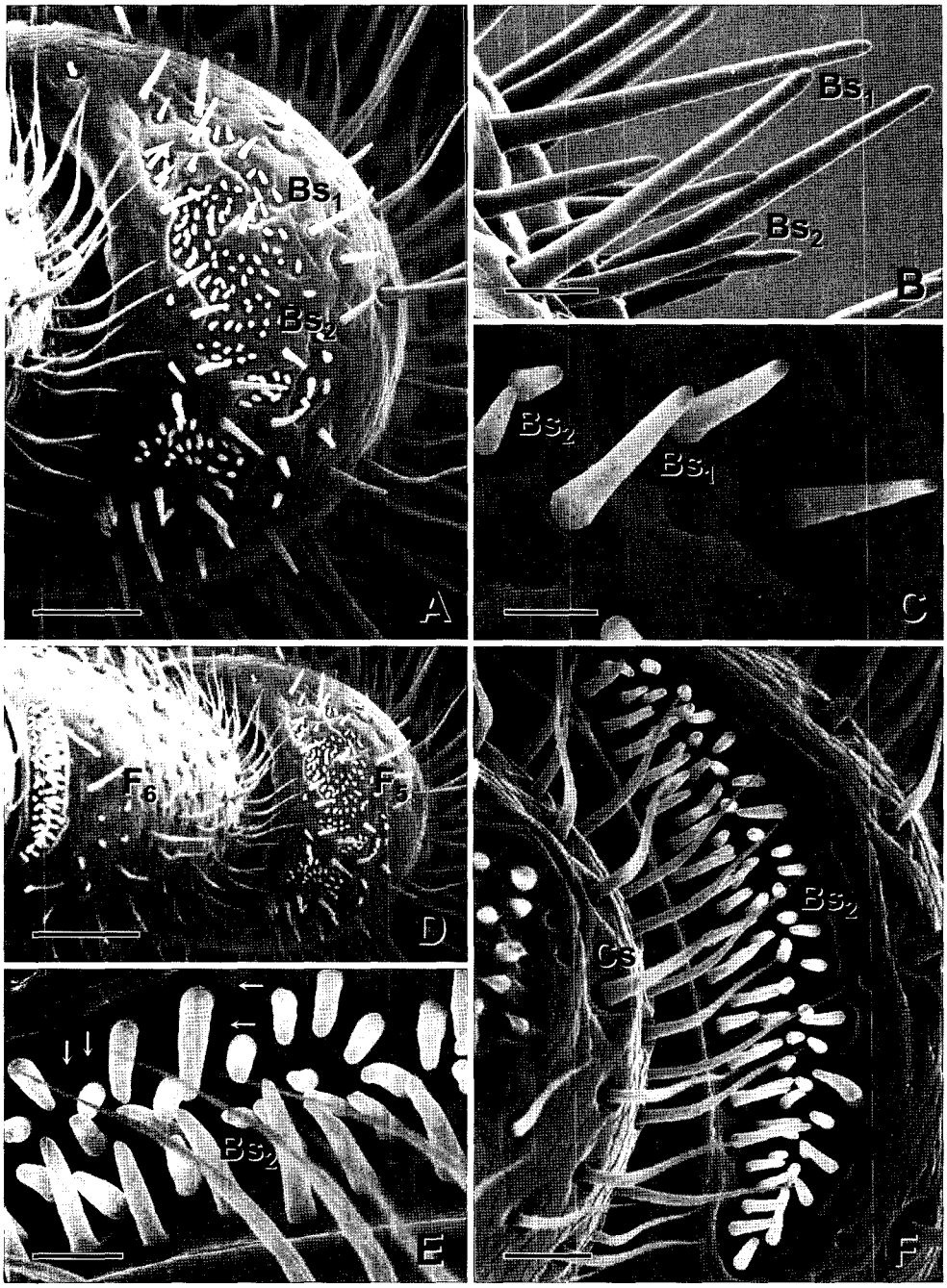

Fig. 2. A-C, Of the eight segments in *Orthomorphella pekuensis*, the 5th article (F_5) has the most diverse types of antennal sensillum. Two subtypes of the basiconic sensillum (BS_1 and BS_2) as well as chatiform sensilla and trichoid sensilla can be seen. These two subtypes are scattered within cuticular depressions of this article, and they commonly have a straight, finger-like appearance. D-F, On the surface of the 6th article (F_6), a subtype of basiconic sensilla, Bs_2, and both types of chatiform sensilla (Cs) and trichoid sensilla can be seen. The small basiconic sensilla are straight, finger-like sensilla with a smooth surface, and each has one or two sensory pores (arrows) around the basal surface. Scale bars, 100 μm (D); 50 μm (A); 20 μm (F); 10 μm (B-C,E).

number of BS_2 found here is approximately 75 (±3.9, n = 5, range = 70-80) in both sexes. They are compactly arranged over the whole surface of the cuticular depressive region (Figs. 2D, 2E). Each BS_2 of this article is a straight and finger-like sensillum with a smooth surface. The length of these sensilla is mostly 12 (±1.1, n = 10, range = 10-14) μm and the average diameter of the base is 3.0 μm. There are one or two sensory pores around the base of each individual BS_2 (Fig. 2F).

The BS_3 are spine-like sensilla with a sharp, narrow tip and a smooth surface. They are found in the depressive region on the 7th article (Fig. 3A). They are markedly different in

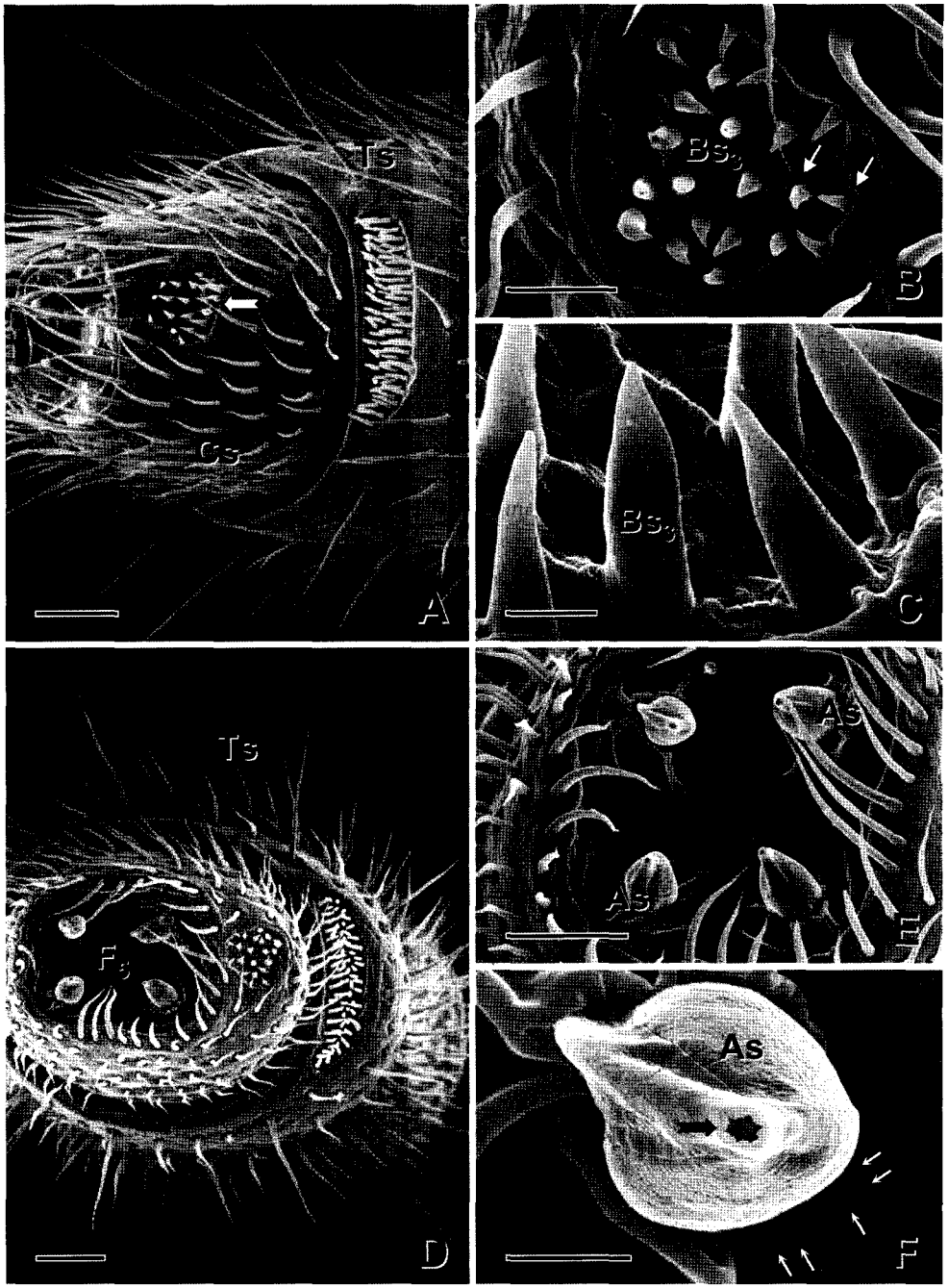

Fig. 3. A-C, The spiniform basiconic sensilla (Bs_3) are spine-like sensilla with a sharp, narrow tip and a smooth surface. They are found in the depressed region (large arrow) on the 7th article. Both chaetiform (Cs) and trichoid sensilla (Ts) appear on this article, with a similar appearance to the sensilla on the proximal articles. Around the base of each sensillum, one or two sensory pores (small arrow) can be seen on the surface of the cuticular depression. D-F, The apical 8th article of the antenna bears four characteristic cone-shaped sensilla (As) distributed in an exact rectangular arrangement. The apical cones on this article are the largest sensilla, and they have several longitudinal grooves along their external surface. Each sensillum has one apical pore (large arrows) and several sensory pores (small arrows). Scale bars, 50 μm (A,D-E); 20 μm (B); 10 μm (F); 5 μm (C).

size and shape from the other types of basiconic sensillum (BS_1 and BS_2). Their average length is 17.0 (±1.8, n = 10, range = 14-20) μm, with a base diameter of 4.0 μm (Fig. 3B). A variety of sensory pits are observed on the surface of the cuticular depression. Around the base of each BS_3, one or two sensory pores are also found (Fig. 3C).

Apical cone sensilla (AS)

The most prominent sensilla are four large AS on the distal tip of the 8th segment. The apical 8th article of the antenna bears four characteristic cone-shaped sensilla. The last segment of this millipede is very small and somewhat difficult to be observed without viewing from the front. At the top view of

the article, four of the apical cone sensilla (AS) are distributed in an exact rectangular arrangement (Figs. 3D, 3E).

The apical cones on this article are the largest sensilla in this millipede, regardless of age or sexes. They have several longitudinal grooves in their external surfaces. These sensilla are 25 (±2.8, n = 10, range = 20-30) μm in length with a basal diameter of 18 (±1.0, n = 10, range = 16-20) μm. Each sensillum has its apical pore at the pointed end (Fig. 3F).

DISCUSSION

Previous work has shown that sensilla on the antenna of the millipede have a variety of functions (Hopkins and Read, 1992), and the external morphology of the antenna is similar to those in other insects (Lavoie and McNeil, 1987; Lopes et al., 2002). Each article is roughly cylindrical but increases in diameter towards the terminal segment. Most antennae of the millipede have been reported to have eight segments (Nguyen Duy-Jacquemin, 1974, 1989). On the basis of microstructural analysis using FESEM techniques, eight distinct segments are identified in the female *O. pekuensis* as well as the male: scape (1st article), pedicel (2nd article), and six flagellomeres (3rd - 8th articles).

Several types of sensory organs have been reported on the antenna of arthropod animals, and various authors have suggested the subdivisions of the particular types into subtypes (Albert and Seabrook, 1973; Wirth and Navai, 1978; Geourge and Nagy, 1984; Saïd et al., 2003). Although these terms were developed using the light microscopic observation, they are still in use, and refer to the appearance of an antennal sensilla (Altner et al., 1983; Altner and Loftus, 1985).

Based on the morphological characteristics of the antenna of *O. pekuensis*, we could identify four types of antennal sensillum: the chaetiform sensilla (CS), the trichoid sensilla (TS), the basiconic sensilla (BS) and the apical cone sensilla (ACS).

The CS are generally referred to as long, sickle-shaped, strong bristles with longitudinal grooves. These sickle-shaped sensilla in *O. pekuensis* can be observed throughout all antennal segments except for the terminal 8th article. They are similar in structure to those reported in other millipedes (Nguyen Duy-Jacquemin, 1974) and other insects (Callahan, 1975; Liu and Liu, 1984; Lopes et al., 2002). Although two or more subtypes have been identified in some insects according to their microstructural differences (Wall, 1978; Lavoie and McNeil, 1987; Renthal et al., 2003; Saïd et al., 2003), our observation was that the CS of most articles in the millipede were morphologically identical, but with size and location in variation. The function of the CS has been suggested to be contact chemoreceptors (Albert and Seabrook, 1973), including a mechanoreceptive function in a moth (Van der Pers and Den Otter, 1978) and a weevil (Saïd et al., 2003).

The straight, blunt-tipped TS in *O. pekuensis* were also identified on the surfaces of whole segments, with the exceptions of the 8th article. Previous work has found sharp-tipped hairs in mosquitoes that respond to a variety of odors (McIver, 1982). A similar type of TS with an olfactory reception function has also been reported in the silk moth (Popov et al., 1994). It is, therefore, likely that the TS of this millipede also respond to various olfactory stimulation.

The BS in *O. pekuensis* are observed within the cuticular depressed regions of the articles from the 5th to the 7th segment. They are divided further into three subtypes according to their relative size, microstructure and location: large basiconic sensilla (BS_1) on the 5th and 6th articles; small basiconic sensilla (BS_2) on the 5th article, and a distinct type of the basiconic spiniform sensilla (BS_3) on the 7th article. The ultrastructures of these sensilla have been reported in millipedes in *P. lagurus* (Nguyen Duy-Jacquemin, 1974) and some moths (Cuperus, 1983; George and Nagy, 1984). The function of the BS without pores in millipedes is not clear yet, however Cuperus (1983) and Faucheux (1991) observed pores in the BS of a Lepidoptera (*Y. vigintipunctatus*) and a moth (*H. nebulella*), and concluded that these pores have an olfactory function, perhaps for the reception of the volatile odors of plants (Van der Pers, 1981; Saïd et al., 2003).

The cone-shaped AS on the 8th article is the largest sensillum in *O. pekuensis* regardless of age or sex. These AS have several apical pores at their pointed end, and the main function of these sensilla is likely to be olfactory reception of the volatile odors of plants (Van der Pers, 1981; Cuperus, 1983; Faucheux, 1991; Hopkins and Read, 1992). Although various authors have suggested a possible role of the sensillum in the sensory stimuli for prey, or mate detection, its exact function is not clear yet and more works are required.

It has been generally reported that the sexual dimorphism of antenna is very common in most arthropod animals. The antenna of the male noctuids has a large number of long TS which are absent in the female (Callahan, 1975). It has been demonstrated in several moths that the TS on the antenna of the male are receptors for the sex pheromone of the female (Van der Pers and Den Otter, 1978; Zacharuk, 1980). We also observe some trivial differences between male and female millipedes in *O. pekuensis* such as some numerical and morphological characteristics. However, we could not observe further characteristics which represent sex-specific dimorphism of the antennal receptors in this millipede.

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