Short Communication

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Descriptions of the antennal structures of the millipede *Trigoniulus macropygus* Silvestri, 1897 (Spirobolida: Pachybolidae) and centipede *Scolopendra subspinipes* Leach, 1816 (Scolopendromorpha: Scolopendridae) using scanning electron microscopy

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Abstract

The antenna is one of the major channels for sensory reception vital for gathering environmental information. An antenna mainly consists of a scape, pedicel, and repeating segments of flagellomeres which collectively constitute the antennomere. These are covered with tiny hair-like projections called sensilla or sensory bristles which greatly increase the surface area of sensory reception. Structural imaging of the antennal features of *Trigoniulus macropygus* and *Scolopendra subspinipes* using scanning electron microscopy (SEM) revealed the presence of two types of sensilla: sensilla trichodea and sensilla microtrichodea. Electron micrographs also revealed that the antennal structures vary significantly between organisms in terms of segmentation, sensillum density, length, and number of flagellomeres. It was also observed that sensillum density increases from the proximal end to the distal end of the antenna with reference to the head. The study also incorporated possible functions of these sensilla in both organisms.

Keywords: *Antenna, morphology, scanning electron microscopy, segmentation, sensilla, sensory reception*

Sensory reception is a key characteristic of organisms, inherent in all living things and vital for survival. The antenna is one of major channels for sensory reception, including receptors for volatile odors and pheromones, moisture, gases, sound, and touch (Sombke & Ernst, 2014; Kleineidam *et al*., 2000; Steinbrecht, 1997; Hashimoto, 1991). Myriapods, in general, tap their antennae on the substrate, probably to gather environmental information. The function of the antenna as receptors and signaling centers have been well documented in entomological, however, accounts on Myriapodan (an artificial group which includes millipedes and centipedes) antennal structures are still limitedly explored. In their book on 'The Biology of Millipedes' Hopkin and Read (1992) stated that "despite being one of the better-studied areas of millipede biology, there are still considerable areas for discovery until structure and function of the sensory receptors and organs become well-understood as those of insects". Moreover, detailed information on sensory organs of these species, especially on antennal sensilla, are still sparse and fragmentary (Sombke & Ernst, 2014; Chung & Moon, 2006).

The present research note aimed to document differences and similarities in the external morphological characteristics of the antenna of a representative millipede specimen *Trigoniulus macropygus* and centipede specimen *Scolopendra subspinipes* through structural analysis of their antennal sensory organs using scanning electron microscopy.

In obtaining the samples, several adult millipede and centipede specimens tentatively identified as *Trigoniulus macropygus* and *Scolopendra subspinipes*, respectively (Figure 1A-B) regardless of sex, were collected in the vicinity of Bicol University College of Agriculture and Forestry, Guinobatan, Albay, mainly found under foliage or stones using pinning forceps. If not fixed directly after capture, live organisms were kept in separate polypropylene containers for temporary storage. Subsequently, photographs were

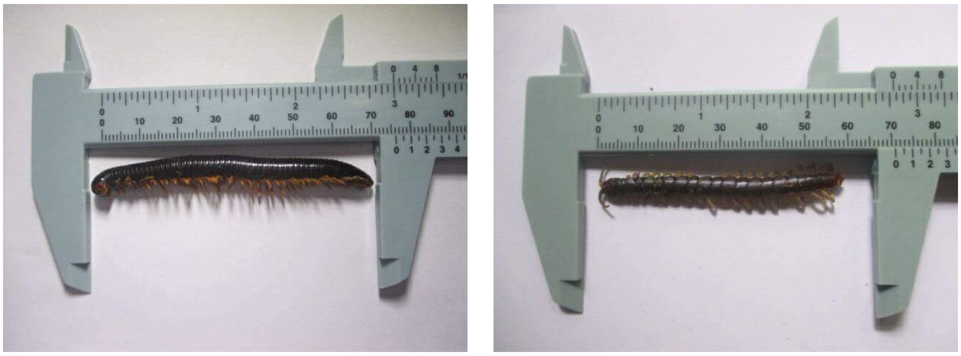

Figure 1. (A) *Trigoniulus macropygus* and (B) *Scolopendra subspinipes* specimens measuring ~70cm and ~60cm in length, respectively.

also taken to properly document the specimens with its original colour, texture, and structures. Species identification was based on its gross morphology with the aid of taxonomic keys adopted from Lewis (2010) and Wesener and colleagues (2009) along with other similar articles containing qualitative descriptions of the specimens to supplement the identification process. After identification, samples were submitted to the Analytical Services Laboratory of the University of Santo Tomas for imaging. Briefly, live specimens were terminated in the laboratory prior to imaging, the antennae of both specimens were then dissected from the individuals and then freshly mounted on the platform before subsequent examinations using a Scanning Electron Microscope (SEM) (Hitachi TM3000) were done. Samples were deposited and stored accordingly using standard entomological fluid preservation method. Briefly, voucher specimens were stored in an airtight glass container with 70% ethanol, which was replaced several times in a week. The glass containers were labeled accordingly with all the necessary collection data.

In terms of the antennal structures, microscopical observations revealed that both *T. macropygus* and *S. subspinipes* antennae consisted of a scape, pedicel, and flagellum. Consequently, *T. macropygus* and *S. subspinipes* antennal articles (or antennomeres) are composed of five (5) and nineteen (19) segments, respectively, that are connected by thin and moveable cuticular joint membranes. Hence, diplopodan antenna is shorter in terms of length as compared to the chilopodan antenna. The former was perceived to have antennomeres that are relatively uniform in terms of its size and diameter and exhibits asymmetrical contortions (Figure 2A & 2B). On the other hand, the latter has a long flagellum consisting of round, small antennomeres (or antennal articles, annuli; Figure 2D & 2E) that tapers distally with reference to the head, that is, these antennal segments diminish in size and diameter along the length of the flagellum from its base to the apex. Moreover, in terms of the number of flagellomere segmentation, *S. subspinipes* consists of seventeen (17) as compared to *T. macropygus* which only consists of three (3). A brief comparison of these structures are summarized in Table 1.

Subsequently, in terms of antennal microstructures, these antennal segments are covered with rows of sensilla (or touch bristles, trichomes; Figure 2) and the density of these sensory bristles increases from basal to the terminal article which are highly numerous in *S. subspinipes* (Figure 2D & 2E) and are very prominent in the apical region of its antenna (Figure 2F). Accordingly, these are minimally distributed in *T. macropygus* (Figure 2A & 2B) and the bases of its sensilla consist of cylindrical shafts and are firmly inserted to the antennal cuticle which surface shows a characteristic texture, and the apices often appear slightly hooked (Figure 2C & 2F). Additionally, two types of antennal sensilla were distinguished by SEM: sensilla trichodea were observed in both organisms (Figure 2B & 2F), while, sensilla microtrichodea was only observed in *S. subspinipes* (Figure 2G).

Sensilla trichodea or trichoid sensilla (sing. sensillum trichodeum) generally are long, almost straight hairs with deep longitudinal grooves in its lower parts and the most common type of sensilla. In *T. macropygus*, sensillum trichodeum consists of a slender cylindrical shaft with a pointed tip and a helically ribbed surface or a collar-like cavity (Figure 2C) in which the movable hair shaft is inserted. These sensilla are present on every article but are sparsely distributed to the lateral regions of the antennomere (Figure 2A &

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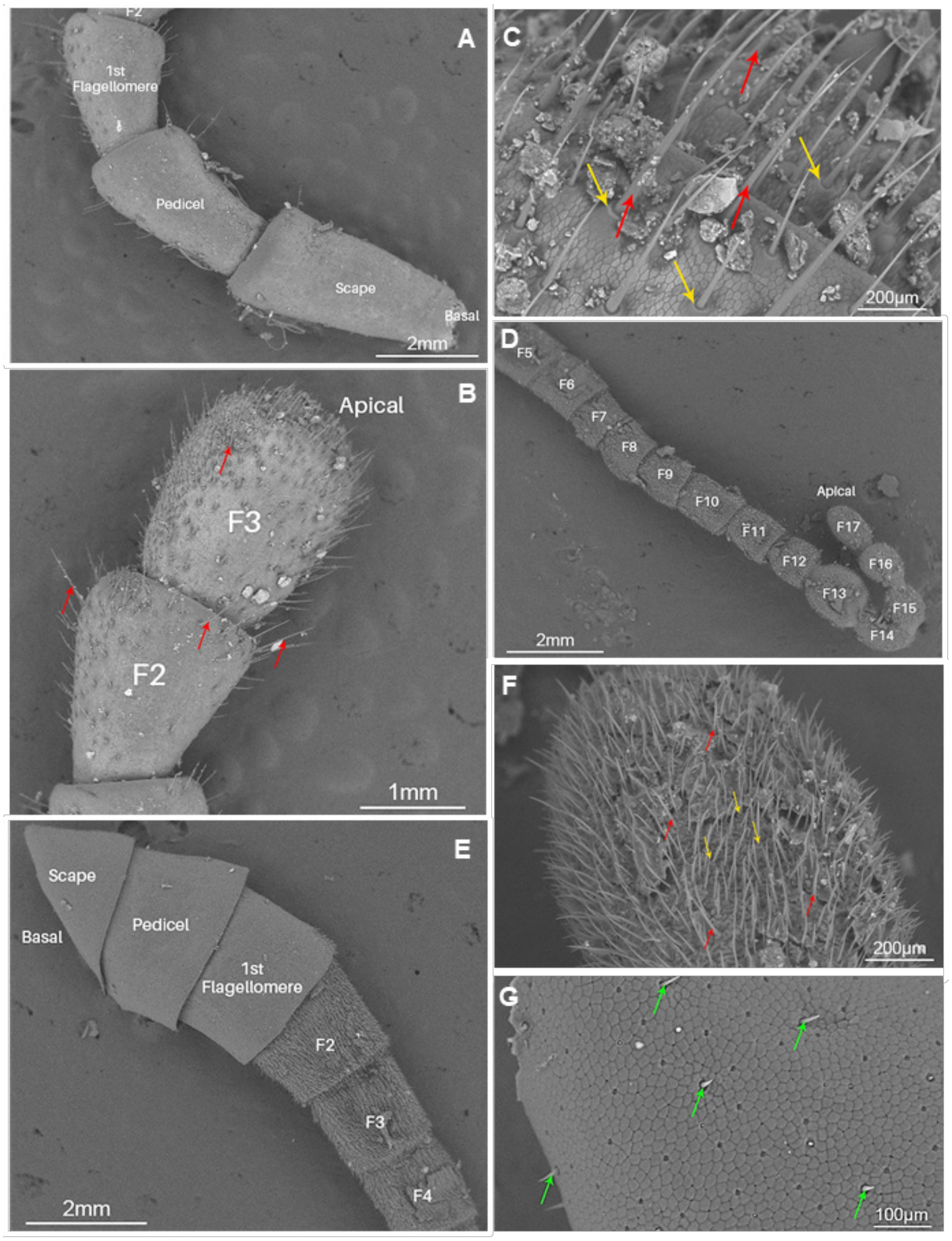

Figure 2. Electron micrograph of the surface ultrastructure of a myriapodan antennae showing the different structures throughout its antennal segment. A-B show the diplopodan antenna of the millipede *T. macropygus* which features five (5) individual articles of the antennomere and its asymmetrical morphology. C shows the diplopodan terminal article with rows of sensilla. Whereas, D-E show the chilopodan antenna of the centipede *S. subspinipes* consisting of nineteen (19) segments which tapers apically. F shows the chilopodan terminal article and G shows the antennal scape displaying the presence of sensilla microtrichodea. Surface details of the antennae are also indicated by colored arrows: (red) sensilla trichodea, (green) sensilla microtrichodea and (yellow) the cylindrical shaft or collar-like cavity to which these sensilla articulate.

Table 1. Summary of the gross antennal morphological structures of *T. macropygus* and *S. subspinipes*.

Antennal Structures	*Trigoniulus macropygus*	*Scolopendra subspinipes*
Scape	Present	Present
Pedicel	Present	Present
Antennomeres or antennal segmentation	5 segments	19 segments
Flagellomeres	3 segments	17 segments
Antennal length	Short	Long
Sensory bristles in the apical region of antenna	Minimally distributed	Highly numerous
Antennomere segment structures	Asymmetrical	Tapered
Base morphology of sensilla	Cylindrical	Cylindrical
Apical morphology	Slightly hooked	Slightly hooked

2B) and becomes concentrated in its terminal articles (Figure 2B). In *S. subspinipes*, however, these are densely distributed throughout its entire antennal articles (Figure 2D-E).

This sensillum serves as chemoreceptive and mechanoreceptive elements for chilopodan species responsible for the detection of chemical signals. The innervation of the biciliated sensory cells end at the base of the sensillum and possess a tubular body that is involved in the stimulus transformation by mechanical bending of the hair shaft therefore exhibiting another function as a mechanoreceptor (Thurm, 1964 as cited by Ernst *et al*., 2009). As for diplopodans, the sensilla primarily functions as a chemoreceptor, which interacts with various chemicals that corresponds for olfactory and gustatory stimuli. Its function as a chemoreceptor is due to the presence of tubular bodies and elongated dendritic segments that project into the sensillar shaft (Sombke & Ernst, 2014).

Sensilla microtrichodea (sing. sensillum microtrichodeum) are miniaturized trichoid sensilla. They show a short, slender, hair-like form with slightly curved tips. They are located at the joint membranes of most antennal articles mostly on the basal articles of the antenna. In this study, sensilla microtrichodea were observed only on the centipede antenna resembling short and minute hair projections and observed to be sparsely distributed within its antennal scape (Figure 2G). It was unfortunately difficult to distinguish the sensilla in the millipede specimen due to the presence of debris attached to the antennae and the microscope's relatively low magnification. Accordingly, extensive literature search provides no evidence of the presence of this type in diplopods until Sombke and Ernst (2014) were the very first to report its presence at the base of the second antennal article in the diplopod *Oranmorpha guerinii*. To date, this is the only study that roughly provides basis for the occurrence of this sensillar type in diplopods. We recommend therefore, to further explore this area to provide supplementary basis for its specific ubiquity.

Moreover, the movable hair shafts of this sensilla were reported to be housed by mechanoreceptive (proprioreceptors) and chemoreceptive sensory cells. The dendritic outer segments of the chemoreceptive sensory cells establish contact with the environment by means of a terminal pore, providing basis for the assumption that these sensilla function as contact-chemoreceptors (Ernst *et al*., 2009). While the sensilla microtrichodea function mainly as proprioreceptors to give information about the position of the antenna, they are also reported to respond to distortions in the cuticle resulting from pressure, and feedback information to its central nervous system. Summing up, the slender and ribbed shaft and the presence of a terminal pore has led to the presumption that the sensillum microtrichodeum might be a miniaturized sensillum trichodeum (Lucas, 1846 as cited by Ernst *et al*., 2013).

In order to supplement discussions on structural comparisons between organisms that was not captured in our results, we summarized several studies conducted by Sombke and Ernst (2014), Erns and colleagues. (2013), Sombke and colleagues (2011) and Chung and Moon (2011, 2006), which reported more types of sensilla other than the types mentioned in this study. Their findings are presented and categorized according to structure and function in Table 2. These sensilla have a variety of functions suited to the unique sensory complexes for both diplopodan and chilopodan species. Exploring the juxtapositions where these group

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Table 2. Presence and types of sensillial structures of both Diplopod and Chilopod species with possible functions as mentioned on several related literatures.

Types of sensilla	Diplopods	Chilopods	Possible functions
Sensilla trichodea	Present	Present	Contact chemoreception
Sensilla microtrichodea	Present*	Present	Contact chemo- & proprioreception
Sensilla brachyconica	Absent	Present	Contact chemo- & hygroreception
Sensilla basiconica (and subtypes)	Present	Present	Olfaction & gustation
Sensilla chaetica	Present	Absent	Mechano- & chemoreception
Contorted sensilla	Absent	Present	Contact chemoreception
Club-shaped sensilla	Absent	Present	Chemoreception
Hat-shaped sensilla	Absent	Present	Chemoreception
Sensory cones	Absent	Present	Chemoreception
Apical cones	Present	Absent	Olfaction, chemo-mechanoreception, gustation & prey/mate detection (?)

**Reported by Sombke & Ernst (2014)*

of animals share a common characteristic that enables them to become closely associated with each other may shed light to unknown or unexplored but shared evolutionary relationships that can elucidate more robust information about this less explored group of animals. The organisms which use their antenna to react to their environment to survive in the most basic sense may facilitate our understanding of their ability to adapt. Further, the elucidation of their antennal morphology may provide clarity on how they will acclimatize in the current hostile climate situation.

In an age marked by fast-paced and extraordinary developments in molecular biology, there is often the danger of underestimating the importance of morphology. The science of form also needs constant refinement and updating, as with other more dynamic fields within biology. This necessary advancement in morphology must correspond both as a revision of its conceptual foundations and as a continuous empirical enquiry in order to update its existing obsolete concepts (Minelli, 1992). Contributing a basis for subsequent homology analyses, which are necessary for proper phylogenetic reconstruction and, in particular, for understanding the evolutionary transformations of the antennal sensillar equipment in this group as part of its conservation efforts has been the core motivations of this study. However, fine structural and electrophysiological investigations are still vital to fully describe the functional modalities of these prolific sensory organs.

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