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A scanning electron microscope study of the sensilla on antenna and mouthparts in *Eurygaster testudinaria* (Geoffroy, 1785) (Hemiptera, Heteroptera, Scutelleridae)

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ABSTRACT: In insects, there are many sensilla showing different structural features on the mouthparts and antennae. These sensilla act as the sensory organs of insects. Main functions of the sensilla in insects are chemoreception, mechanoreception, and thermo-hygrosensory properties. *Eurygaster testudinaria* (Geoffroy) (Hemiptera, Heteroptera, Scutelleridae) is a widespread species that is a perilous pest for agricultural areas. In this study, the sensilla on the mouth parts and antennae of *E. testudinaria* were investigated by using scanning electron microscope technique. In our results we obtained, we were identifed four types of sensilla such as sensilla basiconica, peg-like sensilla, sensilla trichodea, sensilla campaniformia. Each sensilla type were divided into subtypes and numbered. We hope to contribute to similar studies in the future to be carried out with this morphological study.

KEYWORDS: Insect, Heteroptera, chemoreceptor, mechanoreceptor, morphology, systematic, taxonomy

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INTRODUCTION

In insects, sensilla, found in the mouth and antennae parts, plays an important role in vital functions such as mating by identification of sex pheromones, feeding and finding a host alive (Isidoro et al., 2001; Fu et al., 2012; Cao and Huang, 2016; Faucheux et al., 2020). The mouthparts and antenna in insects host many different types of sensilla that act as chemoreceptors and mechanoreceptors (Li et al., 2016). Most of these receptors are chemoreceptors (Brozek and Chlond, 2010). The researchers have been classified the sensilla in insects into four major groups according to the sensory modality: gustatory, olfactory, mechanosensory and thermohygrosensory (Fernandes et al., 2008; Nowinskal and Brozek, 2017; Li et al., 2018). Some researchers classify olfactory sensilla and gustatory sensilla under the name of chemoreceptors (Brozek and Chlond, 2010). In addition, there are also varieties of sensilla such as trichoid, basiconic, plate-like, placoid, long hair-like and coeloconic when looking at external morphology (Slifer 1970; Altner and Prillinger 1980; Hallberg and Hansson 1999; Shields 2010; Nowinskal and Brozek, 2017). Besides, sensilla are divided into 3 major groups according to the presence of pores each with different functions: aporous, uniporous (terminal pores) and multiparous (wall pores) (Nowinskal and Brozek, 2017).

In the species belonging to the ordo Heteroptera (Hemiptera), the labial sensilla track the surfaces of the food sources such as plant and animals (Chapman, 1998; Brozek and Zettel, 2014; Parveen et al., 2015). The outer structure of the sensilla of insects show variation among Hemiptera species (Brozek and Bourgoin, 2013; Nowinskal and Brozek, 2017; Taszakowski et al., 2019).

Insects perceive volatile chemicals in the air with their antennae (Carey and Carlson, 2011). Antennas are the primary sensory organs of insects and have a large number of different types of sensilas on them. These sensillas act as thermohygrorecertors, chemoreceptors and mechanoreceptors (Akent'eva, 2008; Fu et al., 2012; Brozek and Bourgoin, 2013; Freitas et al, 2020; Zhang et al., 2021). In species belonging to the order Hemiptera, antenna sensilla are used by the insect to recognize plants at a distance by olfactory ability (Brozek, 2013).

Hemiptera is a very large order that includes a wide variety of species. The piercing-sucking mouthparts of these Hemiptera species are a feature that allows them to be fed with plant sap (Kanturski et al., 2017). Therefore, insects belonging to this ordo are generally known as plant pests (Hao et al., 2016).

Eurygaster is a holarctic genus of ordo Heteroptera (Hemiptera) which has 15 species (Kaplin and Burlaka, 2019). *Eurygaster testudinaria* (Geoffroy) (Heteroptera, Scutelleridae) is a species that belongs to this genus and has a wide distribution area. They have trans-Palaearctic distribution and have been recorded in Norway, Ireland, Finland, Great Britain, and Turkey in Europe, Tunisia and Morocco in Africa, Tajikistan, Kyrgyzstan, Kazakhstan, Uzbekistan, Japan, and Korea in Asia (Syromyatnikov et al., 2017; Kaplin and Burlaka, 2019). *E. testudinaria* has been recorded in meadows and on the species belonging the Cyperaceae family. Besides, it is also known to be a very dangerous pest for cereals (Linnavuori, 2008; Syromyatnikov et al., 2017).

The aim of this study is to divulge the morphological features of the sensilla of the mouth and the antenna parts according to their cuticular structures and to make the classification of them in *E. testudinaria*, an agricultural pest.

MATERIAL AND METHODS

The adult individuals of *Eurygaster testudinaria* were taken from field survey in Ayaş and Haymana in Ankara province in July, 2018 and carried to the laboratory in 2,5 L plastic bottles. The external structures of specimens were cleaned. The cleaned specimens were attached to SEM stubs after they were dried in air. Subsequently, the SEM stubs with specimens were coated with gold and observed in SEM (JEOL JSM 6060 LV). The micrographs were taken at 10kV accelerating voltage in different magnifications. All studies were carried out at Gazi University, Faculty of Science, Prof. Dr. Zekiye Suludere Electron Microscope Center.

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RESULTS AND DISCUSSION

There are many sensory organs that determine different chemical substances and mechanical actions on the outer surface of the insects. Most of them are found on the mouthparts surface and they can perform the finding food for feeding with these various sensory organs. The others are found on the antenna surface and they can serve as the functions of both smell and touch (Blaney & Chapman, 1969; Cao & Huang, 2016). In this study, we are revealed the sensilla morphology of the head including mouthparts, antenna and surface of the head in adult male and female *E. testudinaria* with scanning electron microscope (SEM). Different types of sensilla were observed on the surface of the mouthparts, the antenna, and head. Each region of the mouthparts and antenna was separately described and discussed those with other previous studies. No obvious differences were noted between the mouthpart, antenna and head structure of female and male individuals.

The mouthparts in hemipteran species are composed of the labrum which has a short and conical in shape, the labium which is long and segmented, and a labial groove within which lie the mandibular and maxillary stylets, respectively (Wang et al., 2020). In the insect being studied (*E. testudinaria*), dorsal view of the species is shown that there are three-segmented labium, labial groove, labrum, and stylet fascicle in mouthparts. The defining feature of hemipterans is being a "stylet" which is sheathed within a modified labium (Figure 1A, 1B). In some species belongs to Hemiptera order, Heteroptera suborder such as *Dolycoris indicus*, *Plautia crossota*, *Piezodorus hybneri*, *Eocanthecona furcellata*, *Perillus bioculatus* (Parveen et al., 2015), *Cheilocapsus nigrescens* (Wang et al., 2019), *Macrocheraia grandis*, *Physopelta quadriguttata*, *Physopelta cincticollis*, and *Physopelta gutta* (Wang et al., 2020) the mouthparts have four-segmented labium.

In different Heteroptera species, while mouth parts are specified, the sensilla types on them are also shown. Various types of sensilla are determined unsymmetrically in each part, on either sides of the labial groove or positioned on the distal end of the labium in *E. testudinaria* (Figure 2).

The labrum (Lm) attaches to the anterior margin of the head and extends to the junction of the head and thorax in both sexes (Figures 1A, 1B). The region (proximal region) where it attaches to the head is wide and the free distal end is thinner than the proximal region (Figures 3A, 3B). While the proximal surface of the labrum has short dome-shaped protrusions (Figures 3C, 3D), the other surface of it is almost smooth and also found light and transverse pits (Figures 3E, 3F). Plate-shaped structures are noticeable at the end edges of the labrum (Figures 3G, 3H). Sensilla have not been found in this area. The similar structures related to the labrum are reported in *M. grandis* (Heteroptera), *P. quadriguttata* (Heteroptera), *P. cincticollis* (Heteroptera), and *P. gutta* (Heteroptera) (Wang et al., 2020).

The labium (Lb) is long, slender, and three segmented. Its anterior surface is deeply concave to form a longitudinal channel due to containing the mandibular and maxillary stylets. Each segment of three-segmented labium varies widely in morphologically. The middle of the first segment is concave, and the labrum extends into this area (Figures 4A, 4B). Although the apex of the first segment is smooth and no sensilla (Figures 4A, 4B), there are many and different size sensilla in the middle part of it, and great number of small protrusions (Figures 4C, 4D). Sensilla are in form as sensilla basiconica (Sb) and sensilla trichodea (St). Sensilla basiconica and sensilla trichodea are numbered according to diagram in Figure 2. The last part of the first segment in labium is smooth in both sexes like in the apex. In the male individual, that area appears to be more swollen (Figures 4D, 4E).

The second and third segments of the

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labium have similar morphology along their length, but the second segment is narrower in contrast to the first and third segment. The surface of the area where is junctions of longitudinal channel in the second segment is differentiated as a plate (Figures 5A, 5B). The other surface is smooth and various sensilla are seen (Figures 5C-5F). There are four types of sensilla such as sensilla basiconica, sensilla trichodea, sensilla campaniformia, and peg-like sensilla in the second segment in both sexes (Figures 5C-5F).

A small canal structure was seen on the right and left edges of the junction of the second and third and the last segments in both sexes (Figures 6A, 6B). One sensilla campaniformia I (Sca1) and one sensilla basiconica V (Sb5) type sensilla were observed at the edges of both channels (Figures 6C, 6D).

In the third segment of the labium, a large number of sensilla basiconica I (Sb1), sensilla basiconica II (Sb2), sensilla basiconica III (Sb3), and basiconica V (Sb5) are interlaced on the surface (Figures 7A, 7B). They are quite straight, with smooth surfaces. Apart from sensilla basiconica (Sb), sensilla trichodea III (St3) and sensilla campaniformia II (Sca2) type sensilla was also located in the third segment (Figures 7C-7F). The last part of the third segment is symmetrically divided into two lateral lobes (Figures 8A, 8B). There are many sensilla trichodea III (St3), sensilla trichodea IV (St4), and sensilla basiconica III (Sb3) type sensilla located on it (Figures 8C, 8D).

The antenna of *E. testudinaria* composed of five segments in both sexes (Figure 9A, 9B). There were no significant differences in each segment between females and males. Four types of antennal sensilla, including four subtypes of sensilla basiconica (Sb), three subtypes of sensilla trichodea (ST), one type each of peg-like sensilla (Ps), and one type each of sensilla campaniformia (Sca) are observed along the its surface (Figures 10-14).

The surface of the head in *E. testudinaria* have three type of sensilla such as St1, sensilla trichodea I; St2, sensilla trichodea II; Sca2, sensilla campaniformia II. These types of sensilla are shown in Figure 15. St1, sensilla trichodea I type sensilla is the most common type of sensilla on the surface of the head.

The labium of hemipterans plays the important roles of recognizing the foods using the sensory organs present on its surface (Backus, 1988; Wang et al., 2019). Four types of sensilla on the tip and surface were observed on the labium of *E. testudinaria*. Each group of sensilla have different length and thickness, therefore, they were numbered in themselves. The most abundant sensilla on the labium are sensilla trichodea and sensilla basiconica. However, only sensilla trichodea on the labium was observed in *C. nigrescens* (Heteroptera, Miridae) (Wheeler, 2001; Wang et al., 2019). It has been stated that sensilla trichodea act as mechanoreceptors to find nutrients, whereas sensilla basiconica type sensilla are involved in the movement of mouth parts (Liang et al., 2013; Gullan & Cranston, 2014; Wang et al., 2019). When we look at the sensors on the antenna, we were observed four types of sensilla. These sensilla help insects to understand on own environment. The feeding mechanism may be understood from the mouthpart morphology of insect species. The insect can choose the food with the sensilla on the surface of the mouthparts. The diverse type, number and distribution of labial sensilla appear much more important because of being used as the morphological characteristics, and presenting taxonomic and phylogenic data. In the light of this data we have obtained, we hope to contribute to next studies to be carried out about the insect mouthparts.

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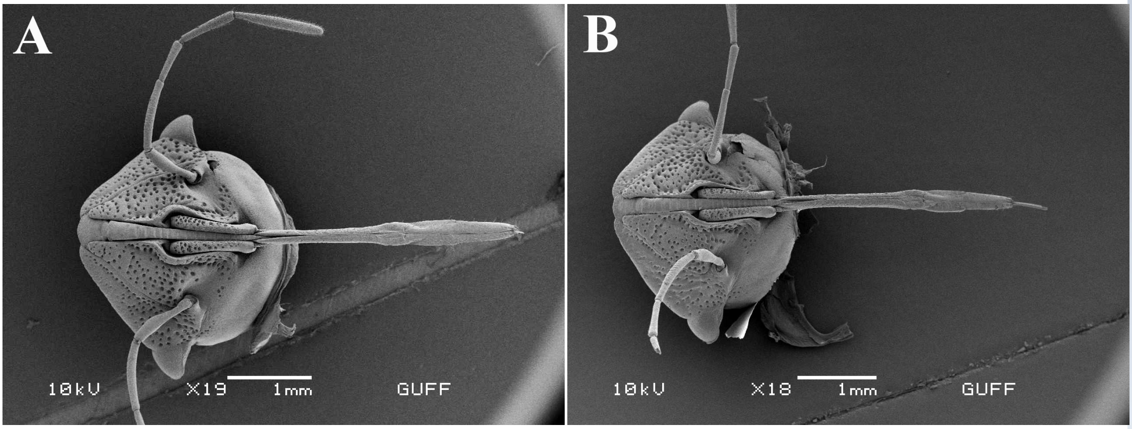

Figure 1. SEM micrographs of the head in *Eurygaster testudinaria.* A. Female individual; B. Male individual.

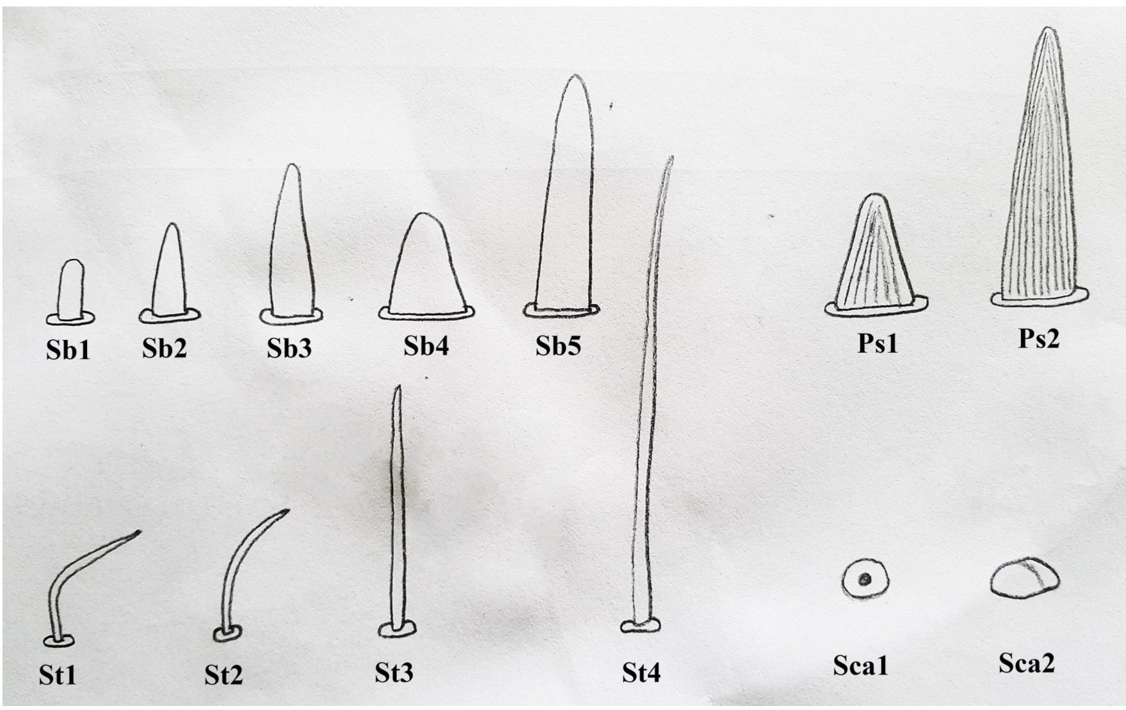

Figure 2. Diagrams of different types of sensilla on mouthparts, antenna, and head of *E. testudinaria.* Sb1, sensilla basiconica I; Sb2, sensilla basiconica II; Sb3, sensilla basiconica III; Sb4, sensilla basiconica IV; Sb5, sensilla basiconica V; Ps1, peg-like sensilla I; Ps2, peg-like sensilla II; St1, sensilla trichodea I; St2, sensilla trichodea II; St3, sensilla trichodea III; and St4, sensilla trichodea IV; Sca1, sensilla campaniformia I; Sca2, sensilla campaniformia II.

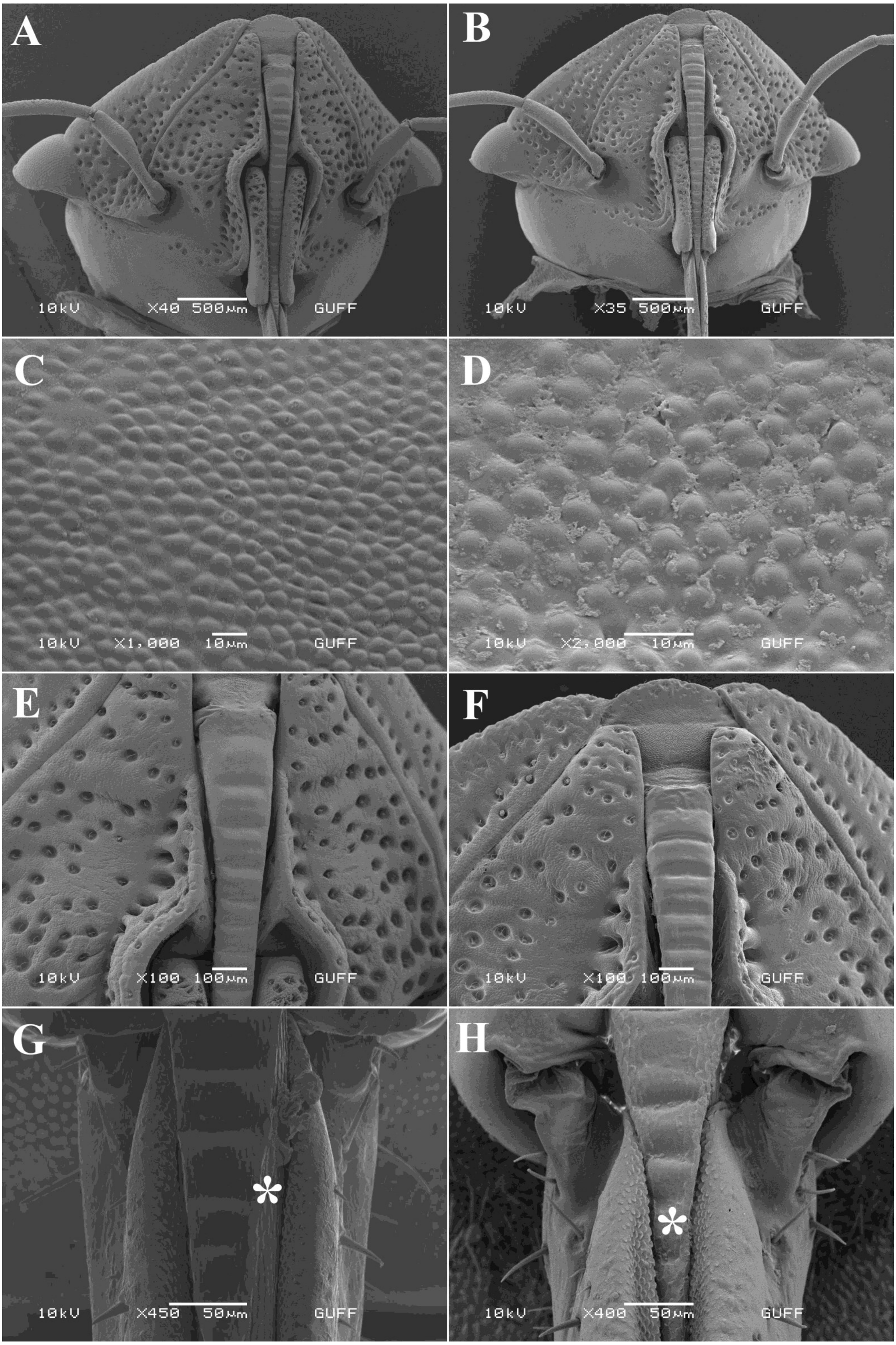

Figure 3. SEM micrographs of the head in *E. testudinaria;* A. Female individual; B. Male individual; C-D. Short dome-shaped protrusions on the surface of the proximal region; C. Female individual; D. Male individual; E-F. The surface of the other region of the labrum; E. Female individual; F. Male individual; G-H. Plate-shaped structures (*) at the end edges of the labrum; G. Female individual; H. Male individual.

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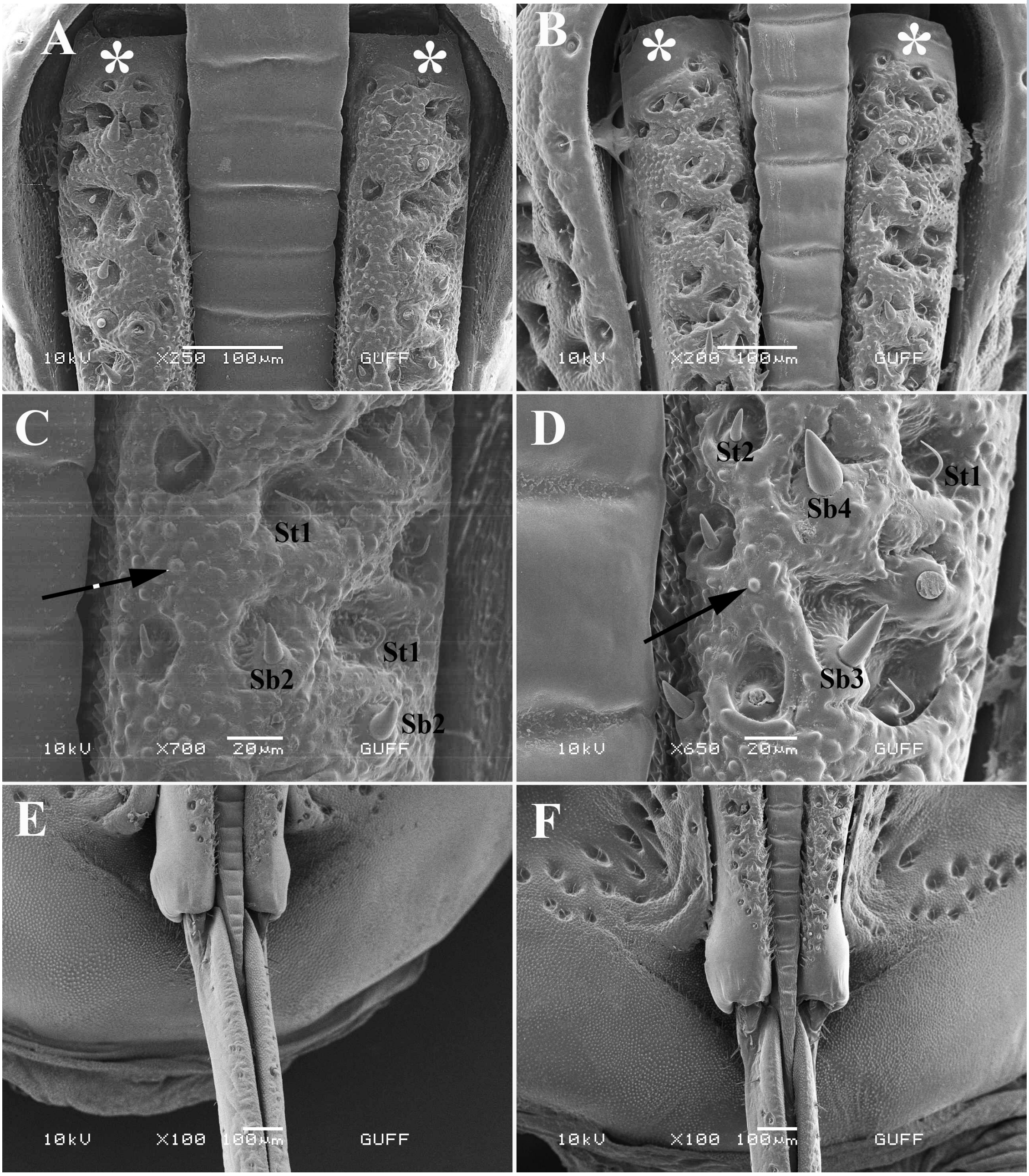

Figure 4. SEM micrographs of the first segment of labium in *E. testudinaria.* A. Female individual; B. Male individual; C. Different size sensilla in the middle part of the labium in female; D. Different size sensilla in the middle part of the labium in male; E. The last part of the first segment of the labium in female; F. The last part of the first segment of the labium in male. (*), the surface of the apex of the first segment; (→), small protrusions; St1, sensilla trichodea I; St2, sensilla trichodea II; Sb2, sensilla basiconica II; Sb3, sensilla basiconica III; Sb4, sensilla basiconica IV.

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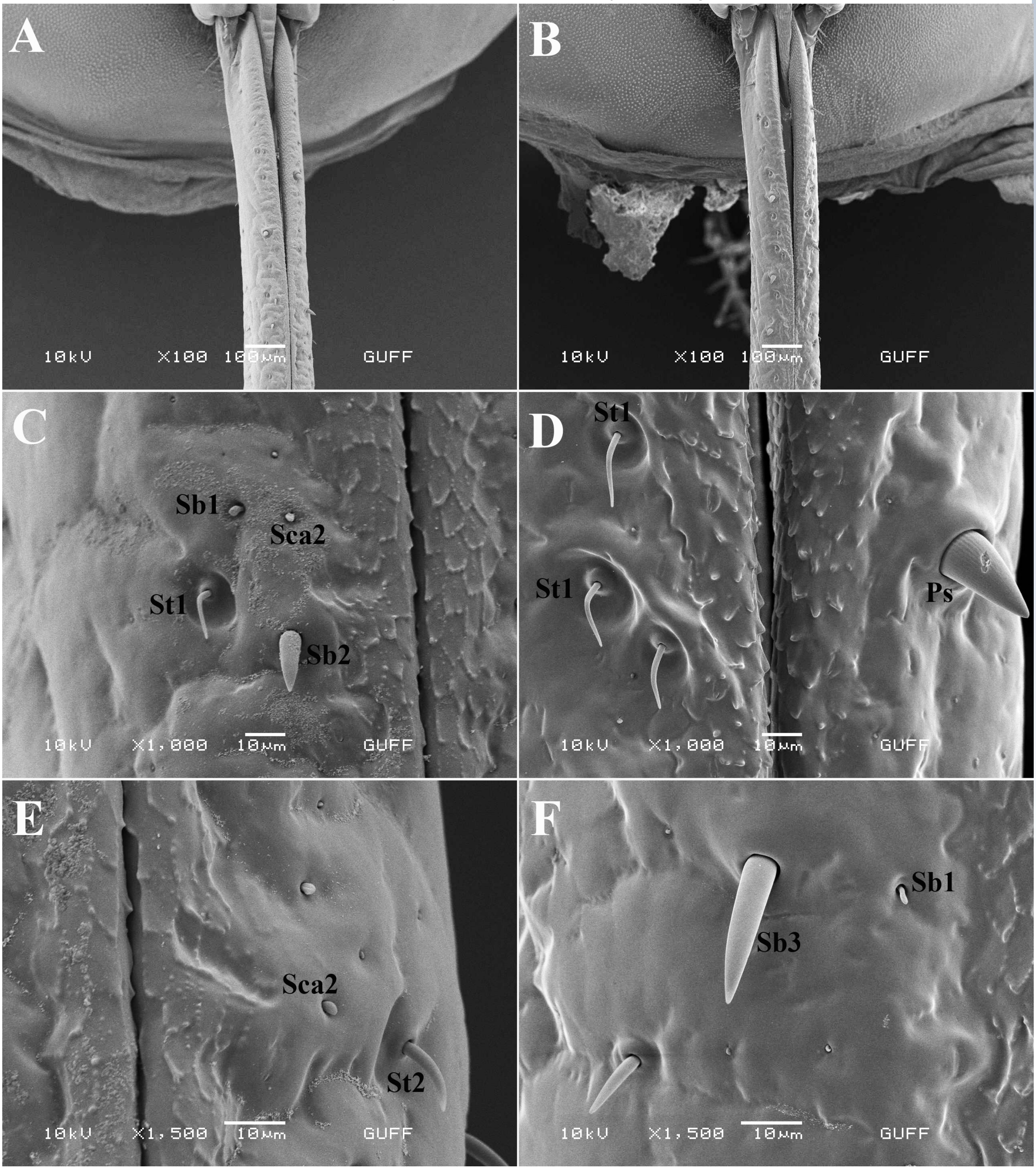

Figure 5. SEM micrographs of the second segment of labium in *E. testudinaria* A. Female individual; B. Male individual; C-F. SEM micrograph of four types of sensilla. C. and E. Female individual; D. and F. Male individual. Sb1, sensilla basiconica I; Sb2, sensilla basiconica II; Sb3, sensilla basiconica III; St1, sensilla trichodea I; St2, sensilla trichodea II; Sca2, sensilla campaniformia II; Ps1, peg-like sensilla I.

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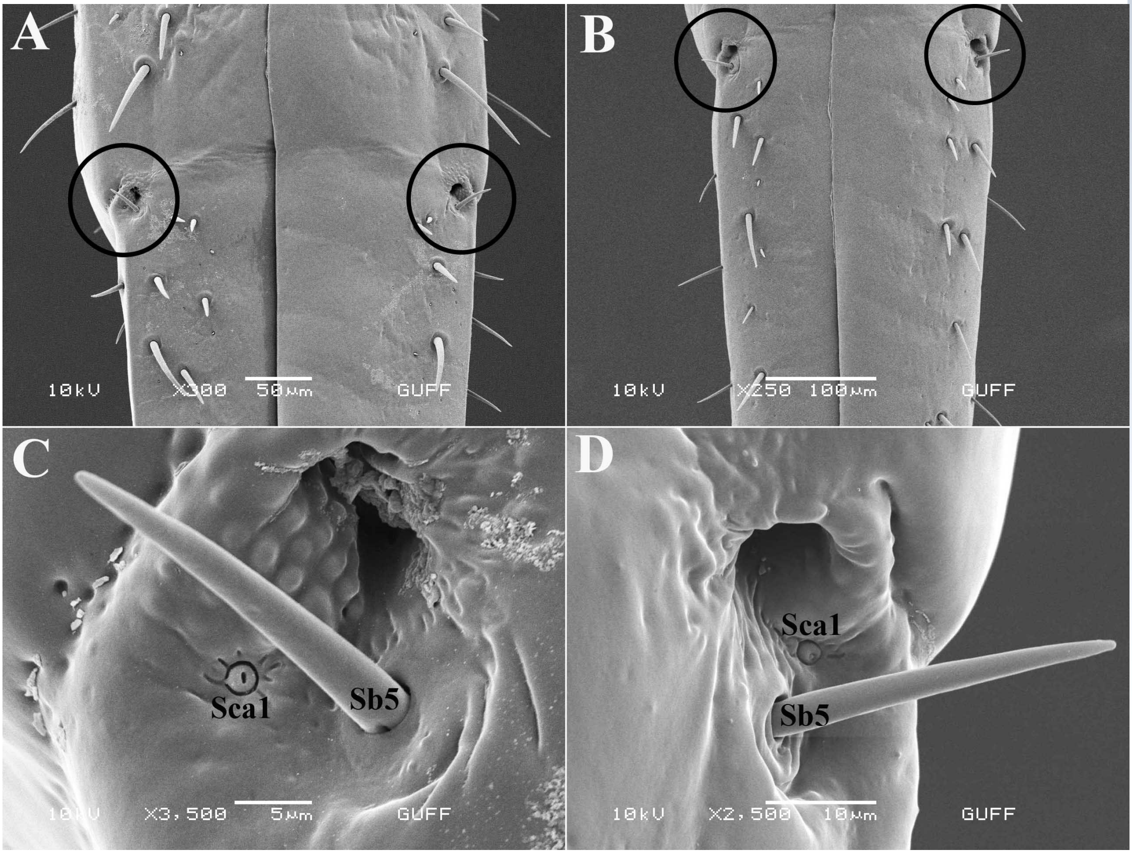

Figure 6. SEM micrographs of the junction of the second and third and the last segments in *E. testudinaria.* A. Female individual; B. Male individual; C-D. Sensilla campaniformia I (Sca1) and sensilla basiconica V (Sb5) type sensilla. C. Female individual; D. Male individual. (O), small canal structure.

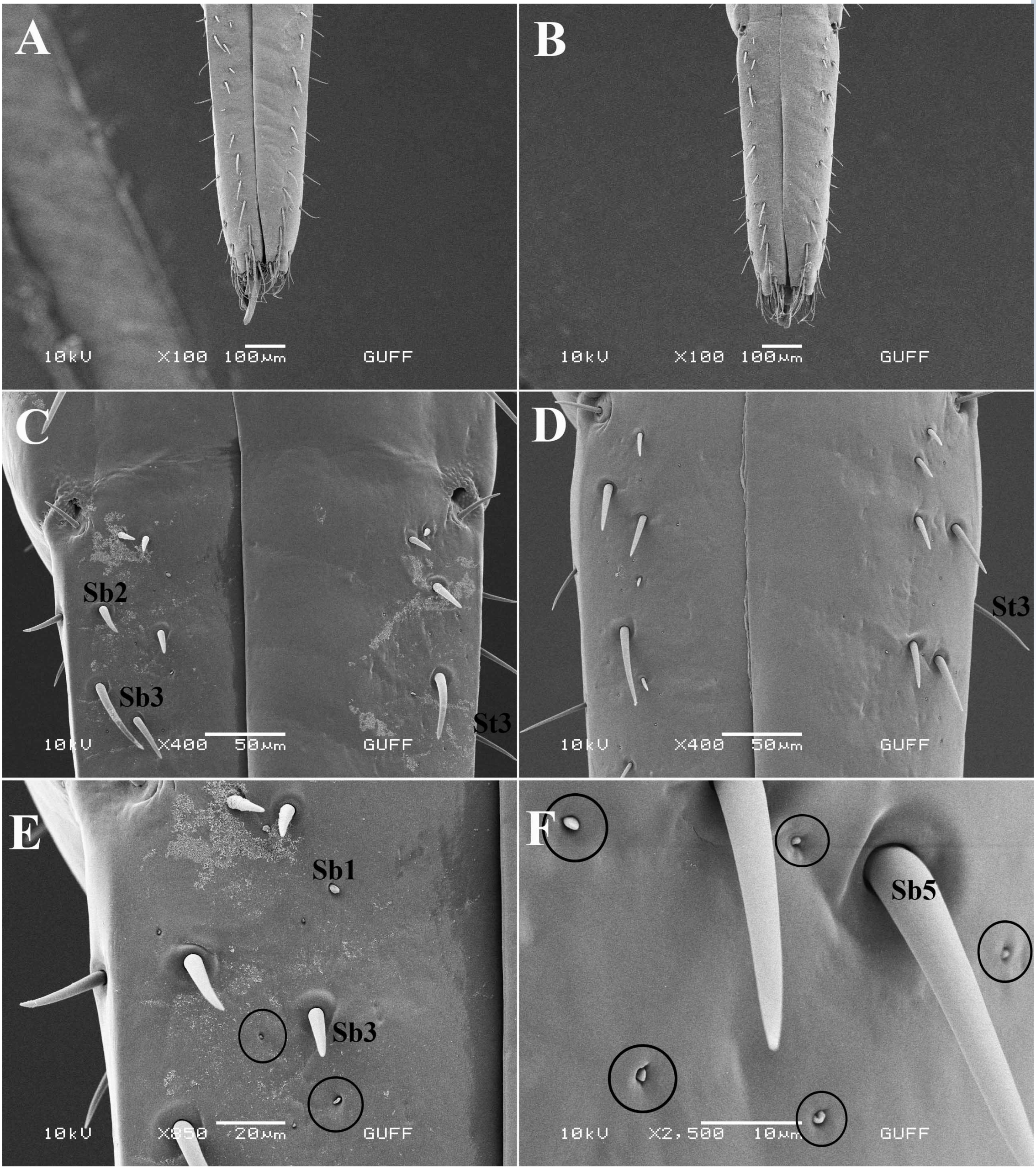

Figure 7. SEM micrographs of the third and the last segment in *E. testudinaria.* A. Female individual; B. Male individual; C-F. Sensilla basiconica (Sb), sensilla trichodea (St) and sensilla campaniformia (Sca) type sensila. C. and E. Female individual; D. and F. Male individual. Sb1, sensilla basiconica I; Sb2, sensilla basiconica II; Sb3, sensilla basiconica III; Sb5, sensilla basiconica V; St3, sensilla trichodea III; (O), Sca2, sensilla campaniformia II.

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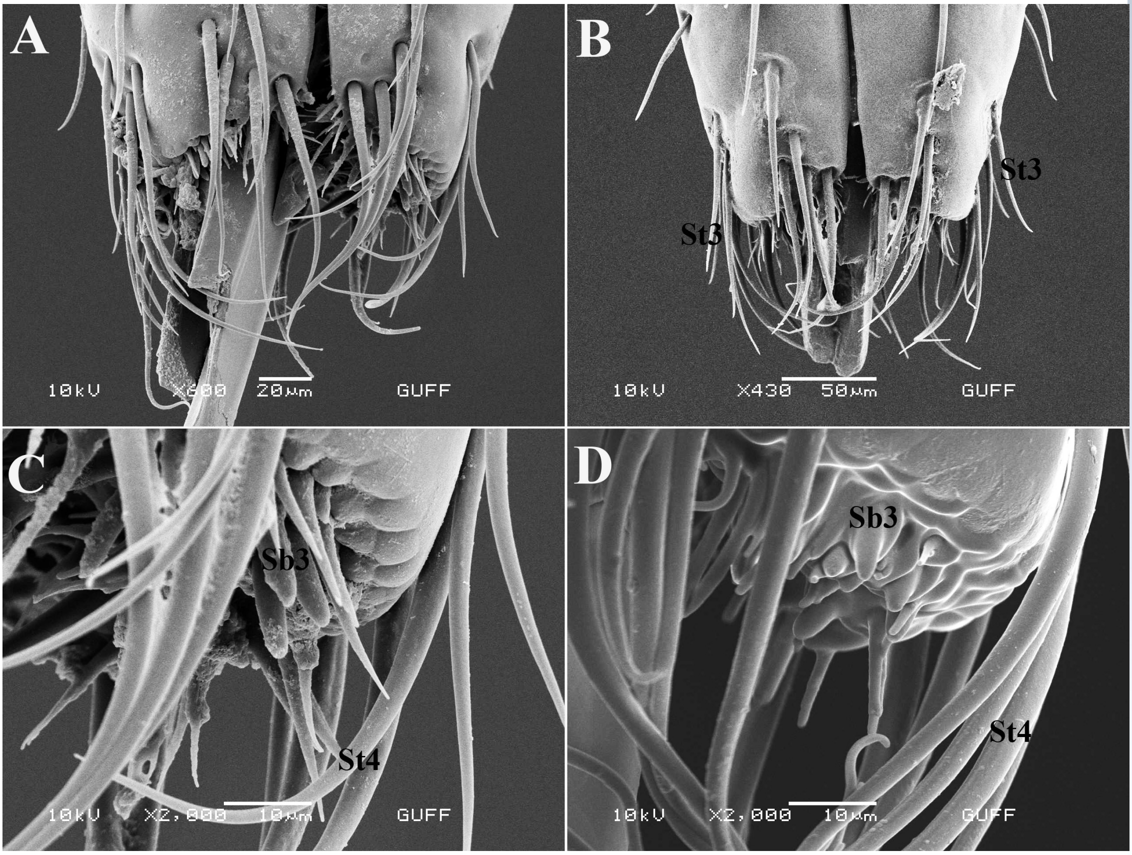

Figure 8. SEM micrographs of the last part of the third segment in *E. testudinaria*. A. Female individual; B. Male individual; C-D. Sensilla trichodea III (St3), sensilla trichodea IV (St4), and sensilla basiconica III (Sb3) type sensilla. C. Female individual; D. Male individual

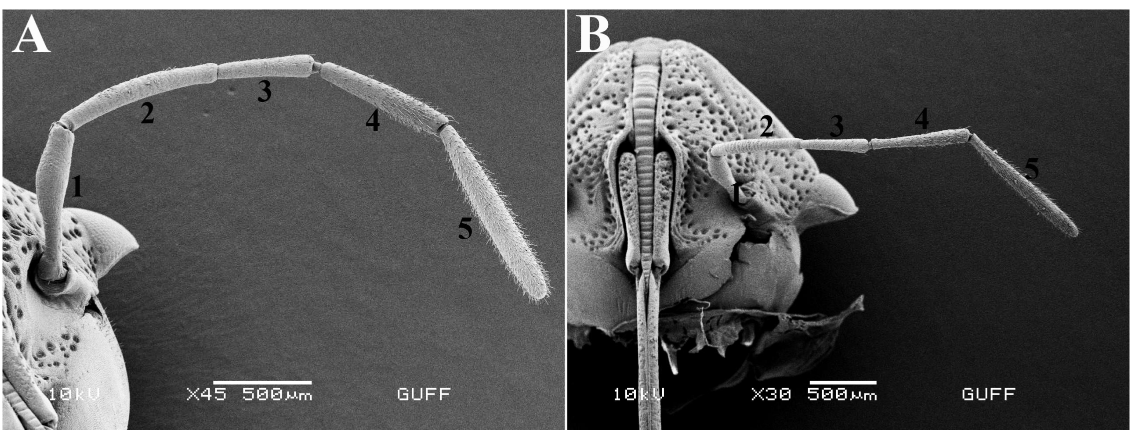

Figure 9. SEM micrographs of the antenna in *E. testudinaria*. A. Female individual; B. Male individual.

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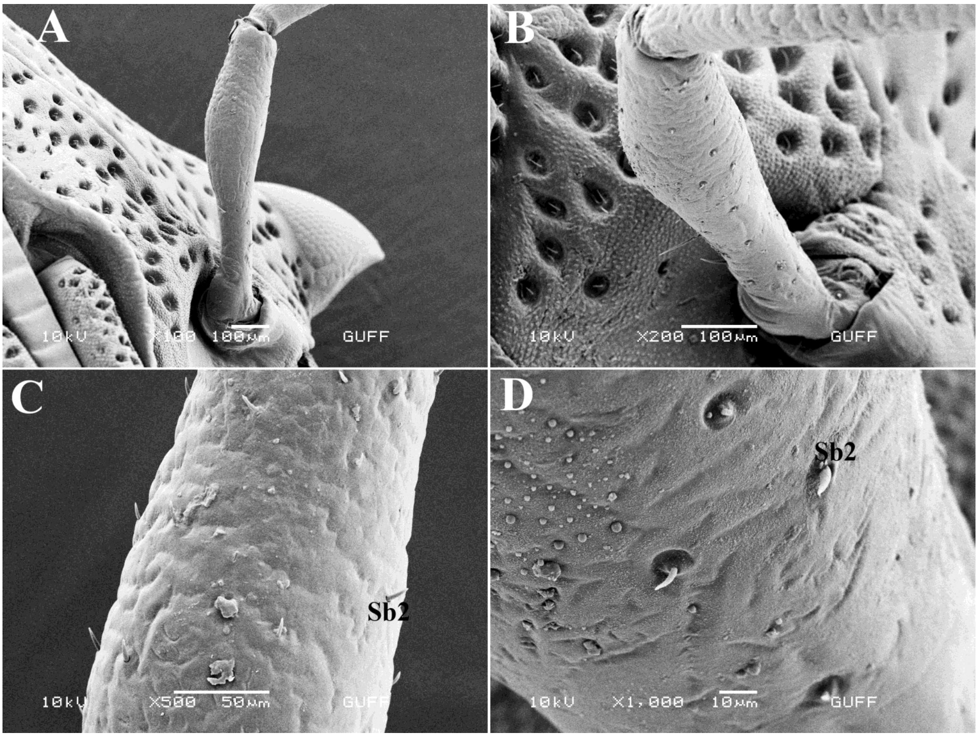

Figure 10. SEM micrographs of the first segment of the antenna. A. and C. Female individual; B. and D. Male individual. Sb2, sensilla basiconica II.

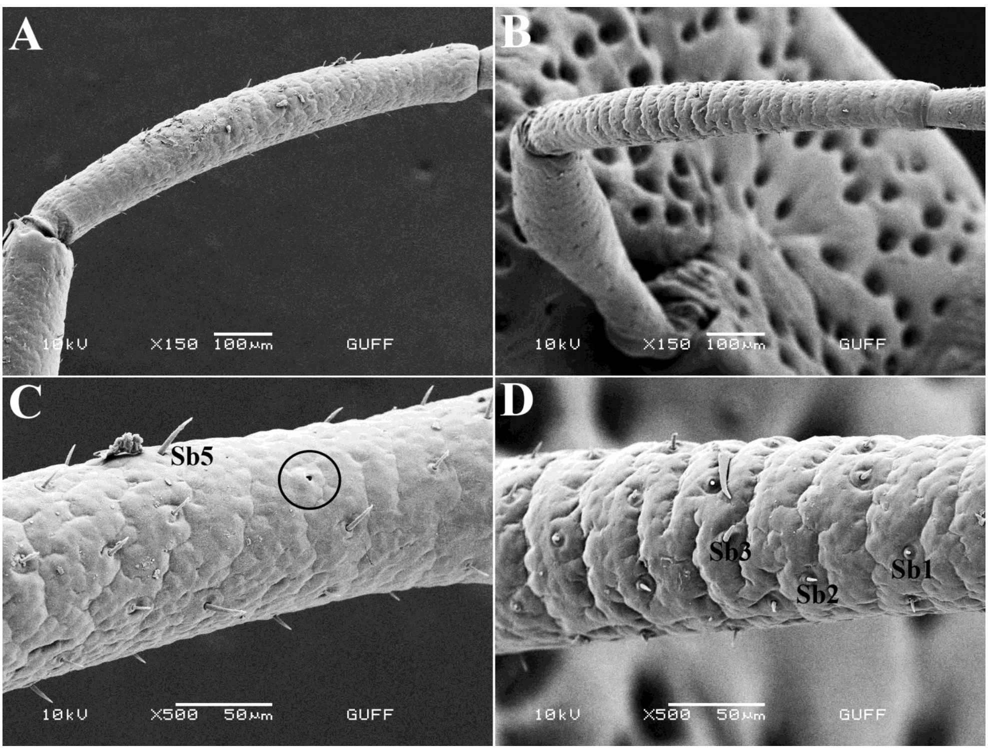

Figure 11. SEM micrographs of the second segment of the antenna. A. and C. Female individual; B. and D. Male individual. Sb1, sensilla basiconica I; Sb2, sensilla basiconica II; Sb3, sensilla basiconica III; Sb5, sensilla basiconica V; (O), Sca2, sensilla campaniformia II.

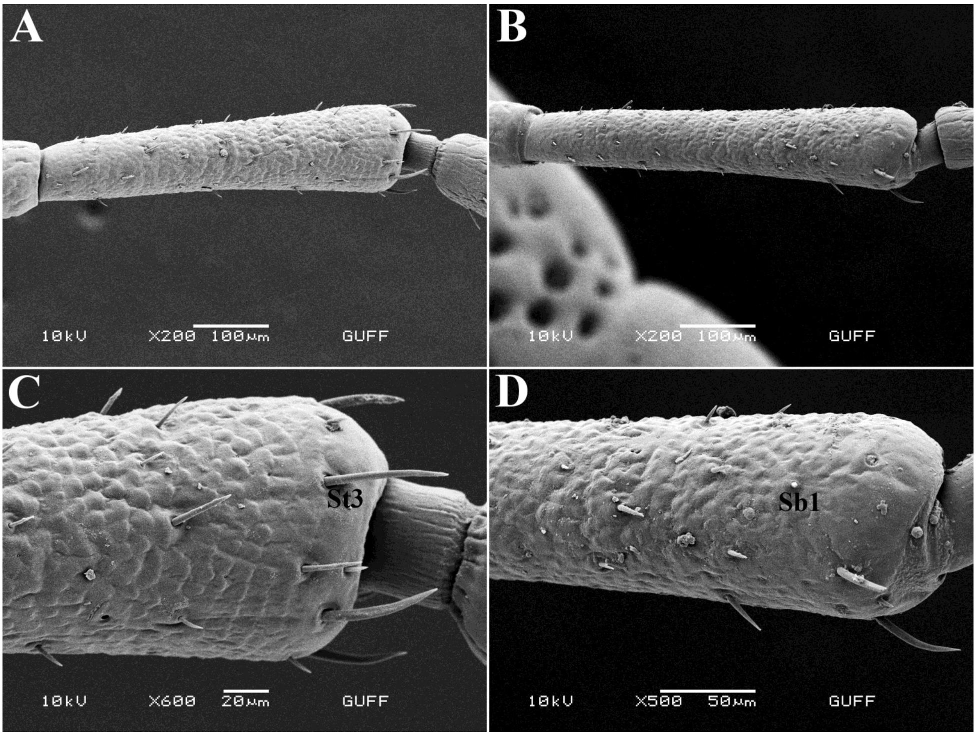

Figure 12. SEM micrographs of the third segment of the antenna. A. and C. Female individual; B. and D. Male individual. Sb1, sensilla basiconica I; St3, sensilla trichodea III.

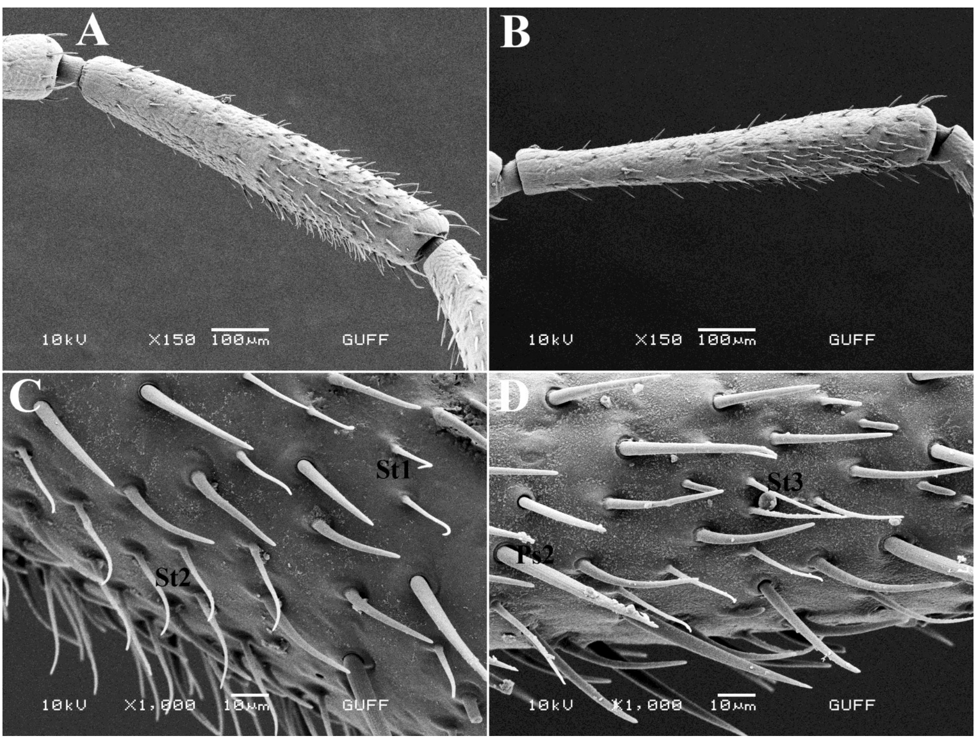

Figure 13. SEM micrographs of the fourth segment of the antenna. A. and C. Female individual; B. and D. Male individual. St1, sensilla trichodea I; St2, sensilla trichodea II; St3, sensilla trichodea III; Ps2, peg-like sensilla II.

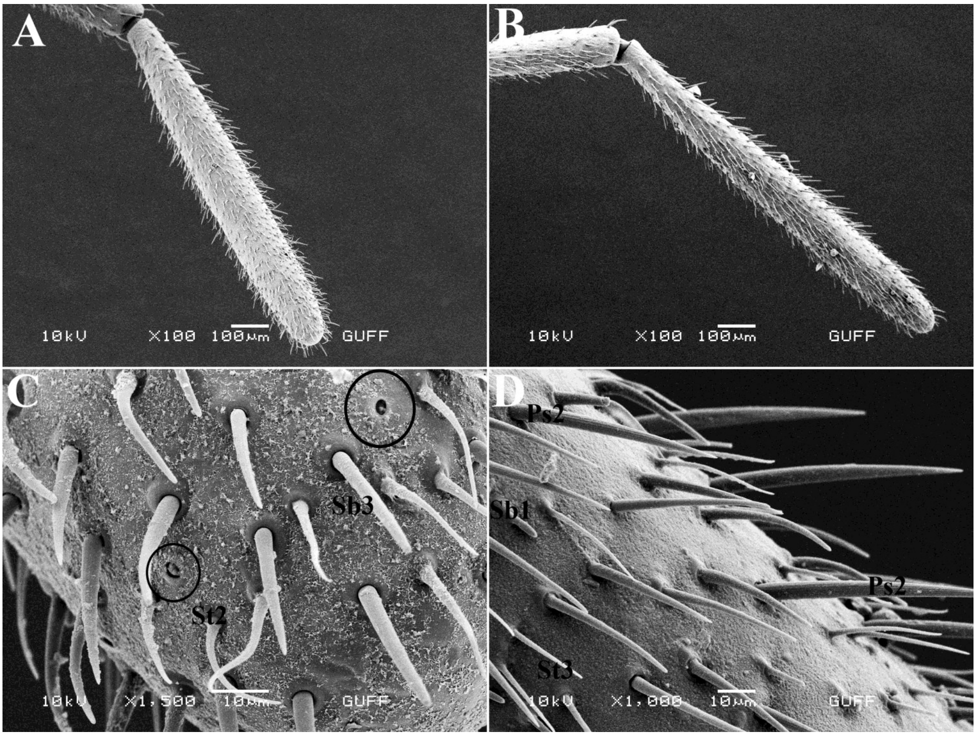

Figure 14. SEM micrographs of the fifth segment of the antenna. A. and C. Female individual; B. and D. Male individual. St2, sensilla trichodea II; St3, sensilla trichodea III; Sb1, sensilla basiconica I; Sb3, sensilla basiconica III; (O), Sca2, sensilla campaniformia II.

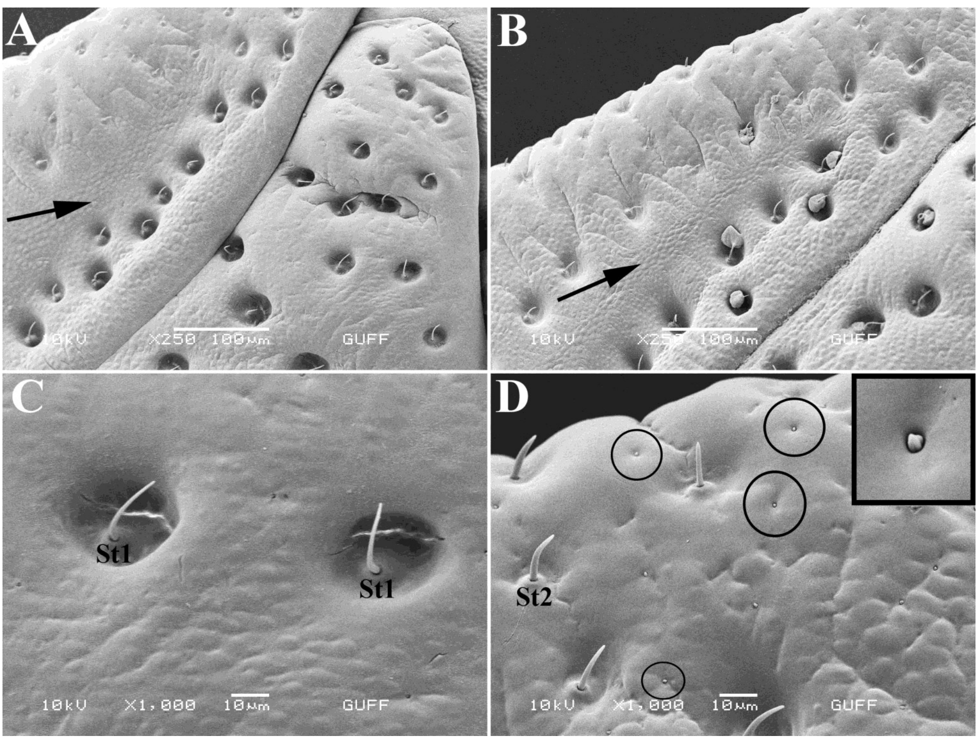

Figure 15. SEM micrographs of the surface of the head. A. and C. Female individual; B. and D. Male individual. (→), short dome-shaped protrusions on the surface; St1, sensilla trichodea I; St2, sensilla trichodea II; (O), Sca2, sensilla campaniformia II. The high magnification view of Sca2, sensilla campaniformia II (O) type sensilla is shown in the corner of Figure 5D. Its magnification is 5,000.

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