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ARTICLE

Ultrastructural analysis of the antennae of *Hemilucilia segmentaria* (Diptera: Calliphoridae), a blowfly of forensic importance

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Abstract. *Hemilucilia segmentaria* (Fabricius, 1805) is a Neotropical blowfly species of forensic importance, with necrophagous and asynanthropic habits. In this study, the antennal ultrastructure of *H. segmentaria* was examined using scanning electron microscopy. The three antennal segments are covered by microtrichia. Sensilla chaetica were detected only on the scape and pedicel. Setiferous plaques and a pedicellar button were observed on the pedicel. Four types of sensilla were found on the postpedicel, including s. trichoidea, s. basiconica (subtype I, II and III), s. coeloconica subtype I, and sensory pits with s. coeloconica subtype II. This is the first time that the fine structure of the antennae of *H. segmentaria* was studied. Our results constitute a solid base for research on comparative and functional morphology in *H. segmentaria* and other blowflies.

Keywords. Fine structure; Necrophagous fly; Sensilla; Scanning electron microscopy.

INTRODUCTION

Hemilucilia (Calliphoridae: Chrysomyinae: Chrysomyini) is an endemic Central and South American genus composed by six species (Dear, 1985). One of its species, the blowfly *Hemilucilia segmentaria* (Fabricius, 1985) is an asynanthropic and necrophagous species (Amat & Medina, 2021), distributed throughout the Neotropical region (Dear, 1985). This species has been used in real medicolegal cases to estimate the postmortem interval (Kosmann *et al.,* 2011; Souza *et al.,* 2014) and the adults can act as biological vectors of eggs of *Dermatobia hominis* (Linnaeus, 1781), a fly that causes furuncular myiasis (Marinho *et al.,* 2003).

Flies, like *H. segmentaria,* are endowed with specialized sensory organs that aid them in their survival and their use of resources in the environment. In this sense, the antennae have an important role since they carry multiple sensilla, which function as chemoreceptors, mechanoreceptors, thermoreceptors and hygroreceptors that facilitate the search for food, oviposition sites, hosts, mating partners, and refuge (Chapman, 1998; Elgar *et al.,* 2018). For instance, when searching for oviposition sites, blowflies can detect minute traces of odor in air currents that come from wounds or decomposing remains of either humans or other animals (Chaudhury *et al.,* 2016; Liu *et al.,* 2016).

Fine morphology of antennae from several dipterans have been characterized (*e.g.,* Sukontason *et al.,* 2004; Zhang *et al.,* 2016; Roh *et al.,* 2020), including some blowflies of forensic, medical, and veterinary importance (Fernandes *et al.,* 2004; Zhang *et al.,* 2013; Zhang *et al.,* 2014). However, no information is available on *H. segmentaria* or on the other species of the genus. A study of this type is essential to better understand the olfactory perception in these species and provide tools for comparative morphology studies at the ultrastructural level, of ecology and niche evolution. Thus, the present work aimed to characterize for the first time the antennal ultrastructure of *H. segmentaria* using scanning electron microscopy.

MATERIAL AND METHODS

Adult specimens of *H. segmentaria* were collected using a fish-baited Van Sommeren-Rydon trap in a secondary forest fragment located at the campus of University of Sao Paulo, Brazil (24°26′09″S, 47°16′11″W). The antennae of two

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adult specimens (one male and one female) were examined. Both specimens were pinned and deposited at the Museu de Zoologia da Universidade de Sao Paulo (MZSP). The taxonomic determination is based on the keys of Dear (1985) and Carvalho & Ribeiro (2000).

For ultrastructural observation, the flies were kept in a humid chamber for 24 hours for the dissection of the antennae. An ultrasonic wash in detergent and degreaser solution was performed to clean the structures in an Ultra Cleaner 800A (15 min at 55°C). The antennae were, then, dehydrated in an alcoholic series (70%, 85%, 90%, 95% and 100%, 2 hours each), dried at room temperature, mounted on stubs with double-sided adhesive tape, and finally, coated with gold in a Balzers Sputter Coater SCD 050. Scanning electron microscopy (SEM) images were taken in a Carl Zeiss Sigma VP Scanning Electron Microscope in the Centro de Aquisição de Imagens e Microscopia do Instituto de Biociências of University of Sao Paulo (CAImi-IBUSP). The figures were edited in Adobe Photoshop CC 2019 and organized on plates using Adobe Illustrator CC 2019. The morphological terminology of the antennae, the pedicel and the classification of the sensilla, follows the proposal of Cumming & Wood (2017), McAlpine (2011) and Schneider (1964), respectively. The size of sensilla was measured from the SEM images using GIMP Portable 2.6.3 to complement its description, although these data should be analyzed with a larger sample. The morphometric data obtained were presented as mean and standard deviation, and n corresponds to the number of measured sensilla. The distribution and density of sensilla was not measured due to the low number of specimens analyzed.

RESULTS

The antennae of both female and male of *H. segmentaria* consists of three segments: a scape attached to the head, followed by a pedicel (Pd) and later a postpedicel with a plumose arista (Fig. 1A). The scape is short, slightly flattened in lateral view, with distal region covered with microtrichia, and with a row of sensilla chaetica (= bristles or setae) on dorsal margin (Fig. 1B). This is the only type of sensilla detected in the scape.

The pedicel, connected proximally to the concave distal end of the scape, is triangular in lateral view, covered with microtrichia and with a pedicellar cleft (Fig. 1A-B). There are several sensilla chaetica on the dorsal region, among which a prominent bristle stands out (Fig. 1B). Smaller sensilla chaetica form a row at the lateroventral margin (Fig. 1B). In the dorsal region, next to the most prominent bristle, there are four setiferous plaques consisting of a circular rim with a central bulbous seta and a tuft of fine hairs posterior to the raised rim of the setal socket (Fig. 1B). When separating the pedicel from the postpedicel, a pedicellar cone was observed (Fig. 1C). The distal articular surface is concave and covered with rows of microtrichia, each row consisting of three microtrichia (Fig. 1D). A third type of sensilla located dorsolaterally to the annular ridge and near the pedicellar cleft was detected, the pedicellar button, which consists of an poreless central dome surrounded by a smooth and convex ring-shaped structure (Fig. 1E).

The postpedicel, the most prominent segment, is densely covered with microtrichia, and carries the largest number and variety of sensilla: s. trichoidea, s. basiconica, s. coeloconica, sensory pits, and an arista (Fig. 1F and Fig. 2). Sensilla trichoidea are straight or slightly curved toward the apex, and hair-like in overall appearance (Fig. 1A). They have a porous wall (Fig. 2B), with a wide base tapering towards tip. They are the most numerous and longest sensilla (24.6 ± 3.1 µm, n = 22), extending above the microtrichia. Sensilla basiconica resembles straight cones with multiporous walls and are divided into three subtypes (Ba-I, Ba-II and Ba-III). Subtype Ba-I is robust, with a basal width of 2.2 ± 0.3 µm and a length of 9.6 ± 1.7 µm (n = 15) (Fig. 2C). Subtype Ba-II is thinner than Ba-I, with a basal width of 1.8 ± 0.2 µm and a length of 10.1 ± 1.1 µm (n = 14) (Fig. 2A). Subtype Ba-III is approximately uniform in width from base to apex, with a pointed apex, and a basal width of 1.9 ± 0.4 µm and a length of 13.62 ± 1.4 µm (n = 4) (Fig. 2D). Subtype I sensilla coeloconica are small, peg-shaped structures (length 3.4 ± 0.3 µm, n = 27), with finger-like cuticular projections on the distal two-thirds (Fig. 2E). Also, several sensory pits scattered on the proximal half of posteroventral surface were observed (Fig. 1A). Within these pits, about four subtype II sensilla coeloconica were found. They are small, peg-shaped structures (length 2.8 ± 0.1 µm, n = 2) but with finger-like cuticular projections emerging from base (Fig. 2F). Finally, the arista is located at the dorsolateral basal region. It is trisegmented, the two most basal segments are short and with a few short microtrichia, while the third is long and carries two rows of long microtrichia (Fig. 1A).

DISCUSSION

The present study is the first to describe the fine morphology of the antennae and their sensory structures in both sexes of the blowfly *H. segmentaria.* As expected, we found no differences in composition and morphology between the male and female examined. In general, the composition and ultrastructure of segments and sensilla described herein are like that found in other blowflies (Fernandes *et al.,* 2004; Zhang *et al.,* 2013; Zhang *et al.,* 2014). As described in *H. segmentaria,* s. chaetica arranged in a row on the scape and dispersed on the pedicel had been observed in several species, such as *Cochliomyia hominivorax* (Coquerel, 1858), *Triceratopyga calliphoroides* Rohdendorf, 1931, *Protophormia terraenovae* (Robineau-Desvoidy, 1830) and *Hemipyrellia ligurriens* (Wiedemann, 1830) (Fernandes *et al.,* 2004; Setzu *et al.,* 2011; Zhang *et al.,* 2014; Hore *et al.,* 2017). Sensilla chaetica (= bristles or setae) are recognized as socketed mechanoreceptors with nonporous walls, although in some species of insects they exhibit one pore at the apex (Ma *et al.,* 2016) and have a chemosensitive function (Ma *et al.,* 2018).

Setiferous plaques are also commonly found on the pedicel in blowflies (Greenberg & Ash, 1972; Sukontason *et al.,* 2004; Zhang *et al.,* 2013; Hore *et al.,* 2017). In general, the bulbous seta tapers distally and end single-tip, although in *C. hominivorax* some of them have a three-pointed apex (Fernandes *et al.,* 2004). According to Greenberg & Ash (1972), setiferous plaques on the pedicel do not have an olfactory function. In the same way, a poreless pedicellar button as that detected in *H. segmentaria* is also present in *T. calliphoroides* (Zhang *et al.,* 2014), but unlike the pedicellar button with three pores observed in *L. sericata* (Meigen, 1826) (Zhang *et al.,* 2013). The function of the pedicellar button remains unclear although morphologically it looks like a sensilla campaniform associated with proprioception (Chapman, 1998).

As expected, the postpedicel is the antennal segment with the highest abundance and diversity of sensilla in *H. segmentaria*. The three subtypes of sensilla basoni-

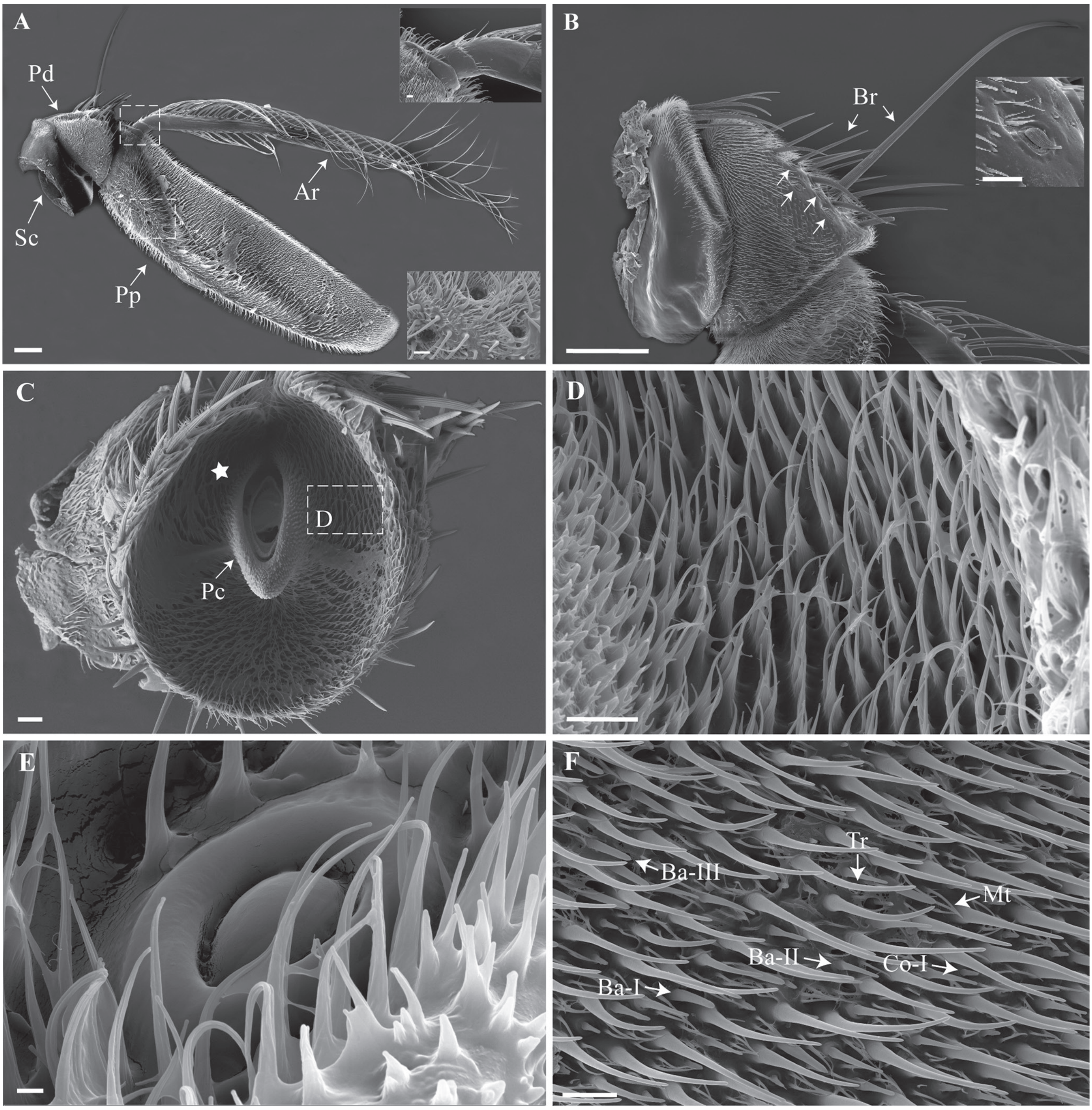

Figure 1. Antennal ultrastructure of *Hemilucilia segmentaria* (Fabricius). (A) General view of antenna. The upper box shows the basal region of arista. The lower box shows three sensory pits on the postpedicel. (B) Detail of scape and pedicel in lateral view. The four arrows on the pedicel show the location of setiferous plaques (detail in box). (C) Posterior view of the pedicel showing the pedicellar cone and distal articular surface. Box D corresponds to Fig. 1D. The star indicates the location of the pedicellar button. (D) Detail of microtrichia on the distal articular surface. (E) Pedicellar button. (F) Detail of surface of postpedicel showing different types of sensilla and microtrichia. Abbreviations: Ar = Arista; Ba-I, Ba-II and Ba-III = sensilla basiconica subtype I, I and III, respectively; Br = bristles or setae; Co = sensilla coeloconica; Mt = microtrichia; Pc = pedicellar cone; Pd = pedicel; Pp = postpedicel; Sc = scape; Tr = sensilla trichoidea. Scale bars (µm): A = 100 (upper and lower box = 10), B = 100 (box = 12.5); C = 20; D = 10; E = 1; F = 10.

ca and sensilla trichoidea are entirely formed by porous walls, as has been observed in other blowflies (Fernandes *et al.,* 2004; Setzu *et al.,* 2011; Zhang *et al.,* 2013; Zhang *et al.,* 2014). It is well known that odorous molecules diffuse through pores in the sensilla walls entering the sensillum lymph where they interact with odorant-binding proteins and are transferred through the aqueous medium towards the dendrites of olfactory sensory neurons (Fleischer *et al.,* 2018). Sensilla trichoidea and basiconica are the ones with the highest abundance and density not only in blowflies but also in other dipterans (Sukontason *et al.,* 2004; Zhang *et al.,* 2013; Zhang *et al.,* 2014). For its part, subtype I sensilla coeloconica resembles those of *H. ligurriens, P. terraenovae, C. hominivorax,* and *L. sericata* (Fernandes *et al.,* 2004; Setzu *et al.,* 2011; Zhang *et al.,* 2013; Hore *et al.,* 2017); while subtype II sensilla coeloconica into sensory pits resembles the basal-drum grooved coeloconic sensillum found in *C. hominivorax,* although in the latter this subtype of sensilla was not housed in pits (Fernandes *et al.,* 2004). Although the function of the s. coeloconica has not been studied in blowflies, it is possible that they are thermoreceptive, hygroreceptive or chemoreceptive as has been demonstrated in other insects (Yao *et al.,* 2005; Schneider *et al.,* 2018).

This study allowed us to characterize the antennal ultrastructure using scanning electron microscopy in the

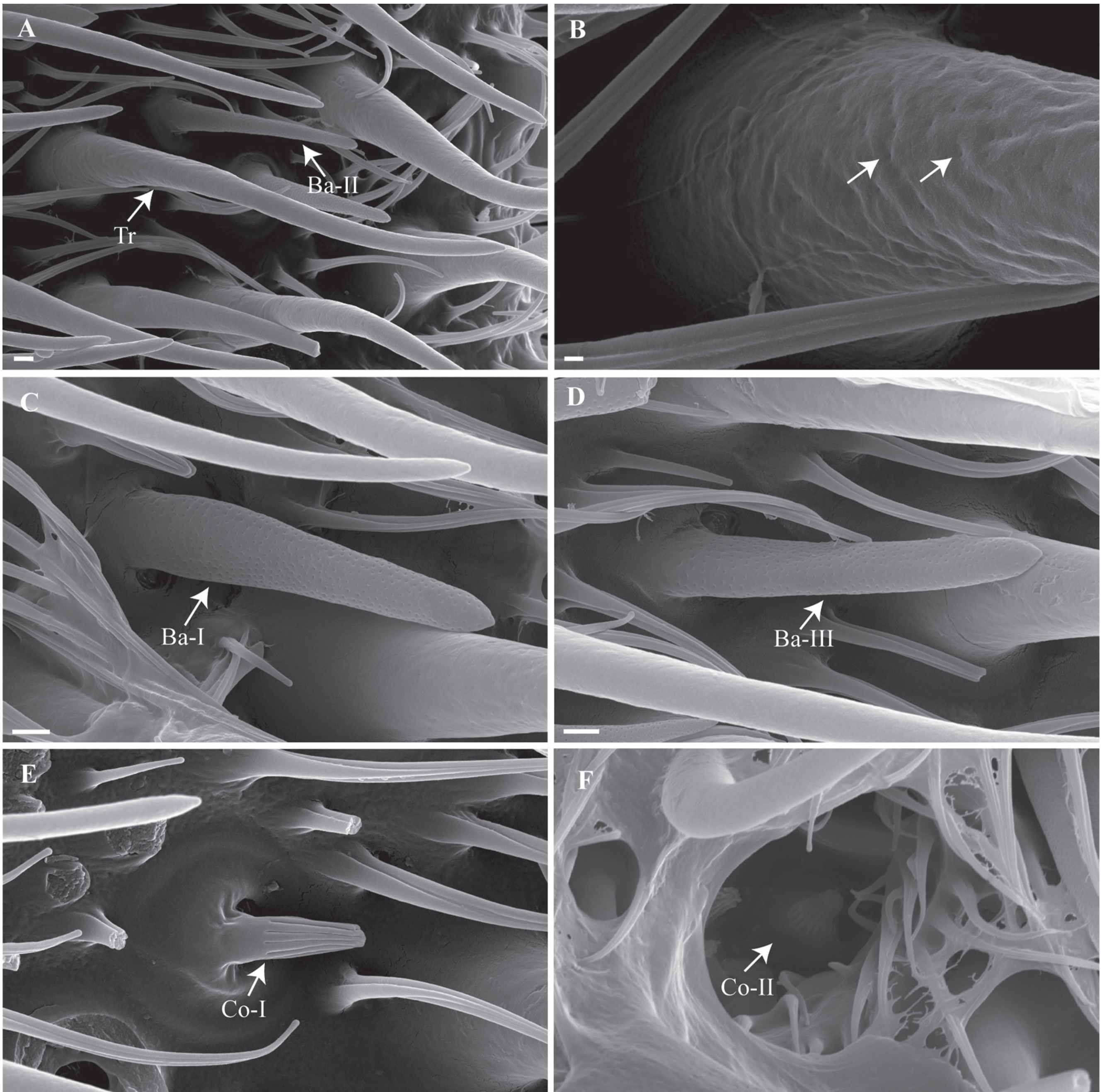

Figure 2. Detail of sensilla on the postpedicel of *Hemilucilia segmentaria* (Fabricius). (A) Sensilla trichoidea (Tr) and sensilla basiconica subtype II (Ba-II). (B) Basal region of sensilla trichoidea, some pores are indicated by arrows. (C) Sensilla basiconica subtype I (Ba-I). (D) Sensilla basiconica subtype III (Ba-III). (E) Sensilla coeloconica subtype I (Co-I). (F) Sensory pit with sensilla coeloconica subtype II (Co-II). Scale bars (µm): A, C, D, E = 1; B = 200; F = 2.

Neotropical blowfly *H. segmentaria*. Our results showed the antennal sensory equipment present in this necrophagous species. The putative function of each type of sensillum was analyzed through the comparison with previous studies on other species, although electrophysiological investigations, transmission electron microscopy and immunohistochemical studies are necessary to confirm its specific role. Also, this study provides the basis for future comparative morphological analysis in blowflies and other dipterans, which may be useful at the taxonomic and/or phylogenetic level.

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