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Ultrastructural studies of sensilla in one fly of forensic importance

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Abstract. Taxonomic identification is essential in the field of forensic entomology. Insects are important in the decomposition of cadavers. The flies are generally attracted to cadavers and one of the most important contributions is to estimate the postmortem interval. The scanning electron microscopy (SEM), which allows rapid and accurate identification of different species of forensics flies, has been highlighted as it allows better visualization of the external morphology of immature and some adults. The purpose of this study was to examine in details the morphological aspects of sensilla on the antennae of female and male of *Xanthacrona bipustulata* Wulp using SEM, since many of them could not be observed just by the use of light microscopy.

Keywords: Acalyptratae; Arthropod; Brazil; Morphology; Scanning Electron Microscopy.

The flies of the family Ulidiidae measure around 2 to 14 mm, usually with a dark and yellow coloration. There are 716 species differentiated in 113 genera (Kameneva *et al.* 2010; Tepedino *et al.* 2017). Most species of this family are saprophagous, but some species have developed the phytophagous habit. The larval and adult stages can feed on a variety of vegetables. Some adults present in this family are attracted by organic matter in the process of decomposition as leaves, fruits, trunks, carcasses and feces (Souza & Linhares 1997; Marchiori & Silva 2001). The presence of Ulidiidae species in animal carcasses is rare, the exudates from decaying carcasses could serve as substrate for the development of the larvae, which are usually found in decaying plant material (Moretti *et al.* 2008).

Ultrastructure studies using Scanning Electron Microscopy (SEM) have been extensively conducted to clarify some structures on Hexapoda: Lopes *et al.* (2002) found two types of basiconic sensilla on antennae of *Phoracantha semipunctata* (Fabricius) (Coleoptera); Sukontason *et al.* (2004) studied the antennal sensilla of the families Calliphoridae, Sarcophagidae and Muscidae (Diptera); Nascimento *et al.* (2013) who described, the antennae sensilla of *Melipona scutellaris* Latreille (Hymenoptera); Goyal *et al.* (2011) described the egg, larval and pupal stages of three species of the Ulidiidae family: *Chaetopsis massyla* (Walker), *Euxesta eluta* Loew and *Euxesta stigmatias* Loew; Zhang *et al.* (2013a) described the sensilla on antennae and maxillary palps of the adults of *Lispenei mongola* Tian et Ma and observed three types of sensilla (trichoidea, basiconic and chaotic) distributed in these sensorial organs; Zhang *et al.* (2013b) observed three types of sensilla (microtrichia, basiconic and trichoidea) on the flagellum of the adults of *Fannia scalaris* (Fabricius) and *Fannia canicularis* (Linnaeus); Carriço *et al.* (2015) describe sensilla on antennae of the adults of *Ophyra albuquerquei* Lopes and *Ophyra aenescens* (Wiedemann) and observed four types of sensilla distributed on theses organs (microtrichia, basiconic, chaetic and trichoidea); Pezzi *et al.* (2016) observed six types of sensilla (microtrichia, basiconic, clavate, chaotic, styloconic and trichoidea) on antennae and maxillary palps of the adults of *Sarcophaga tibialis* Macquart; Carriço *et al.* (2017) described the antennae, maxillary palps and Terminalia of males of *Peckia* (*Peckia*) *chrysostoma* (Wiedemann) and observed three types of sensilla (microtrichia, basiconic and trichoidea) distributed in theses sensorial and reproductive organs; Caetano *et al.* (2018) analyzed the aspects of the sensilla on antennae and maxillary palps of *Mesembrinella bellardiana* Aldrich; *Mesembrinella bicolor* (Fabricius) and *Mesembrinella semihyalina* Mello and observed four types of sensilla (microtrichia, basiconic, clavete and trichoidea) distributed in theses sensorial organs.

An ultrastructure analysis of the morphological aspects of sensilla on the antennae of *X. bipustulata* have not been performed yet. The purpose of this study was to examine in details these sensorial organs on the aforementioned species to help increase the anatomical database.

MATERIAL AND METHODS

This research was carried out through the cooperation of "Fundação Oswaldo Cruz" (FIOCRUZ) and "Instituto Samambaia de Ciências Ambientais e Ecoturismo" (ISCAE), process number 07/2018.

The flie *X. bipustulata* was obtained from one carrion of *Oryctolagus* sp and the collections were performed daily, during january of 2018, in an Atlantic rainforest fragment at "Instituto Samambaia de Ciências Ambientais e Ecoturismo" (ISCA) - Petrópolis, Rio de Janeiro, Brazil (22°46'90" S and

43º14'82" W). Number authorization for scientific research ICMBio/SisBio ("Instituto Chico Mendes de Conservação da Biodiversidade/Sistema de Autorização e Informação em Biodiversidade") was 54279.

Specimens were properly identified following Brown *et al.* (2009). The terminologies of adult's flies used in this study followed Mello (2003) and Kosmann *et al.* (2013), the classification of sensilla followed by Setzu *et al.* (2011); Zhang *et al.* (2013a, b); Carriço *et al.* (2015) and Caetano *et al.* (2018).

The heads were dissected from one male and five females under dissecting microscope. Then, these structures were processed for SEM examination by transferring to 2.5% of glutaraldehyde mixture in phosphate buffered saline (PBS) for 24 h. Afterwards, specimens were rinsed twice with PBS during 10-minute- intervals and post fixed with 1% of osmium tetroxide at room temperature for 3 days. This post fixation step was carried out under a well-ventilated fume hood. The flies were then rinsed twice with PBS and dehydrated in an increasing graded series of ethanol (30, 50, 70, 80 and 90%) for 12 h during each step. Thereafter, flies were placed in absolute alcohol, followed by a treatment in absolute acetone. On the next step, the specimens were subjected to critical point drying and they were later placed on metallic supports, coated with a thin gold layer (20-30 nm) and examined under JEOL 6390LV scanning electron microscope (SEM) (Akishima, Tokyo, Japan).

RESULTS

SEM observations of the head of male and female of *X. bipustulata* revealed a pair of antennae, frontally situated, between the large compound's eyes (Figure 1). Antenna morphology consisting in three segments: the first called scape (Sc), the second pedicel (Pe) and a third flagellum observed in female composed of a seta called arista (Ar) located laterally and an enlarged basal flagellomere (Fn) (Figure 1). All antennal segments in this species have sensilla.

Male: The scape is densely covered by microtrichia, and only one type of sensilla was observed: eight-sensilla chaetica (Ch I) of similar length and organized in a single row (Figure 2A). The surface of the pedicel is covered by microtrichia similar to those found in the scape and presents two types of chaetica sensilla (Ch I and ChII). The first type (Ch I) is represented by 14 bristles, similar to those found in the scape. The second type (Ch II) is represented by a single bristle located in the distal region (Figure 3A).

Female: The scape is densely covered by microtrichia, and only one type of sensilla was observed: eight-sensilla chaetica (Ch I) of similar length and arranged in a single row (Figure 2B). The surface of the pedicel is covered by microtrichia similar to those found in the scape and presents two types of chaetica sensilla (Ch I and ChII). The first type (Ch I) is represented by 15 bristles, similar to those found in the scape. The second type (Ch II) is represented by a single bristle located in the distal region (Figure 3 B). The flagellum is the largest segment of the antenna and numerous sensilla are found in this segment. Arising closed to the base of the first flagellomere on its dorsolateral surface is the arista, which is composed of two segments (I-II) (Figure 4A). The Fn is densely covered by microtrichia similar to those found in the scape and pedicel. Among the microtrichia has a type of sensilla: trichoidea (tr) (Figure 4B).

DISCUSSION

The ultrastructure of the antennae of Ulidiidae species using SEM are limited in the literature, theses sensorial structure have been performed, such as: Sukontason *et al.* (2006) described the ultrastructure of the adhesive device in S*arcophaga* (*Liosarcophaga*) *Dux* Thomson showing the smooth surface and their implication as mechanical carriers of pathogens; Goyal *et al.* (2011) described the egg, larval and pupal stages of three species of the Ulidiidae family: *C. massyla* , *E. eluta*, and *E. stigmatias* and Pezzi *et al.* (2016) investigated the antenna and maxillary palp of S. *tibialis* and described the sensilla in these sensorial structures.

The external morphology of the antennae segments of *X. bipustulata* is generally similar to that of other Ulidiidae and calyptrate flies (Sukontason *et al.* 2006; Carriço *et al.* 2015; Pezzi *et al.* 2016; Caetano *et al.* 2018). In this study, the microtrichia morphology of the antennae is similar to that of other calyptrate and are present on all antennal surface with a variable distribution (Sukontason *et al.* 2004; Sukontason *et al.* 2007; Setzu *et al.* 2011; Zhang *et al.* 2013a; Zhang *et al.* 2013b; Carriço *et al.* 2015; Pezzi *et al.* 2016; Caetano *et al.* 2018).

The chaetic sensilla were observed in the scape (chI) and pedicel segments (ChI-ChII). This distribution pattern was also found in *O. albuquerquei*, *O. aenescens* (Carriço *et al.* 2015) and *Ophyra chalcogaster* (Wiedemann) (Sukontason *et al.* 2007), but differs of *S. tibialis*, *M. bellardiana*; *M. bicolor* and *M. semihyalina* that have tree type of chaetic sensilla (Pezzi *et al.* 2016; Caetano *et al.* 2018).

The flagellum is the largest segment of the antenna and its

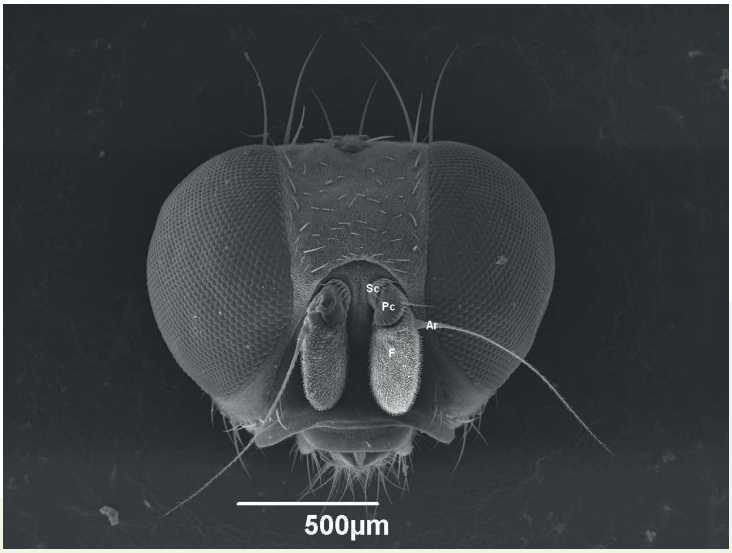

Figure 1. Scanning electron micrographs showing the heads of female, in frontal view, of *Xanthacrona bipustulata*: female (X 50). Sc = scape; Pe = pedicel; Ar = arista; Fn = first flagellomere.

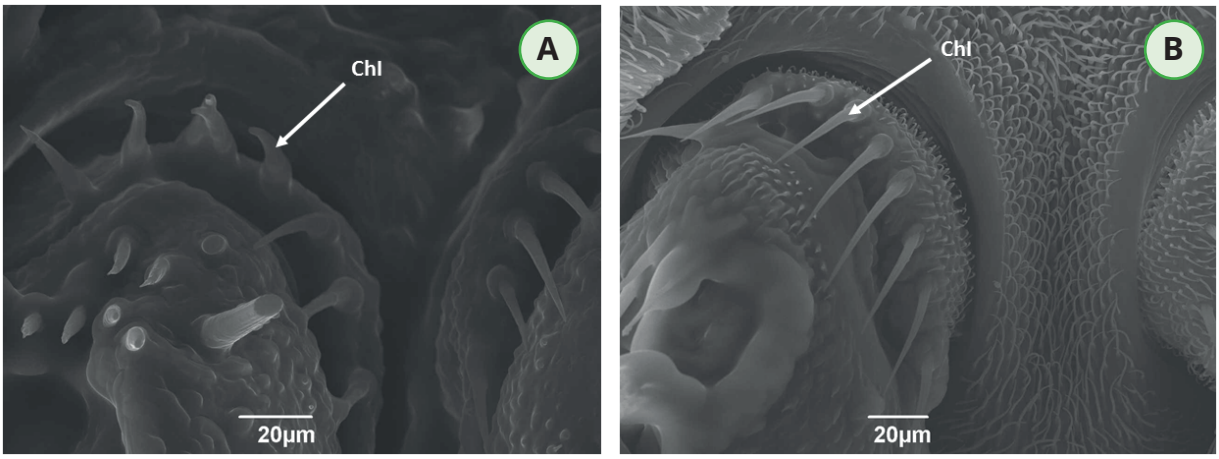

Figure 2. Scanning electron micrographs showing the cuticular surface of scape in both genders of *Xanthacrona bipustulata*: (A) male (X 800), (B) female (X 650). Sc = scape; ChI = chaetic sensilla.

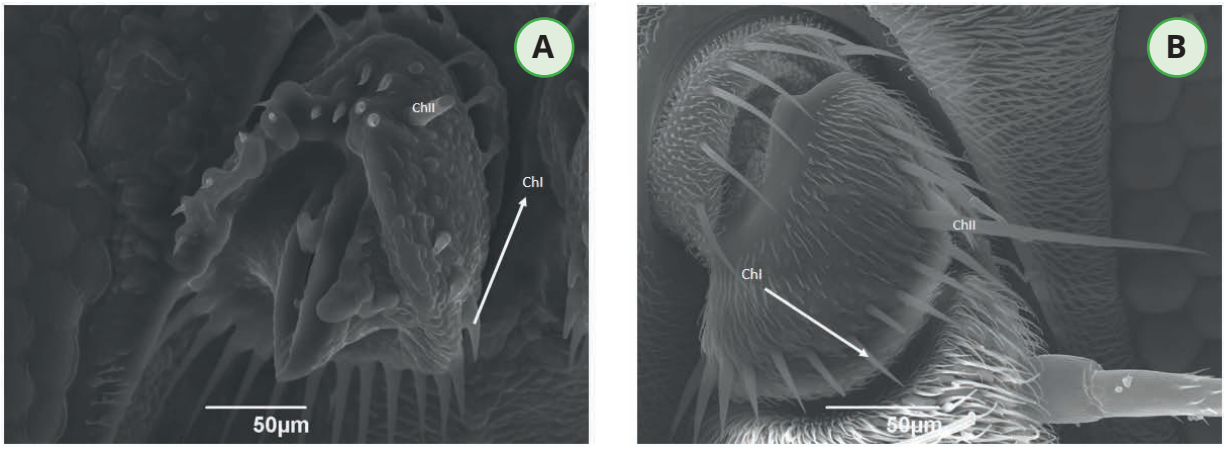

Figure 2. Scanning electron micrographs showing the cuticular surface of scape in both genders of *Xanthacrona bipustulata*: (A) male (X 800), (B) female (X 650). Sc = scape; ChI = chaetic sensilla.

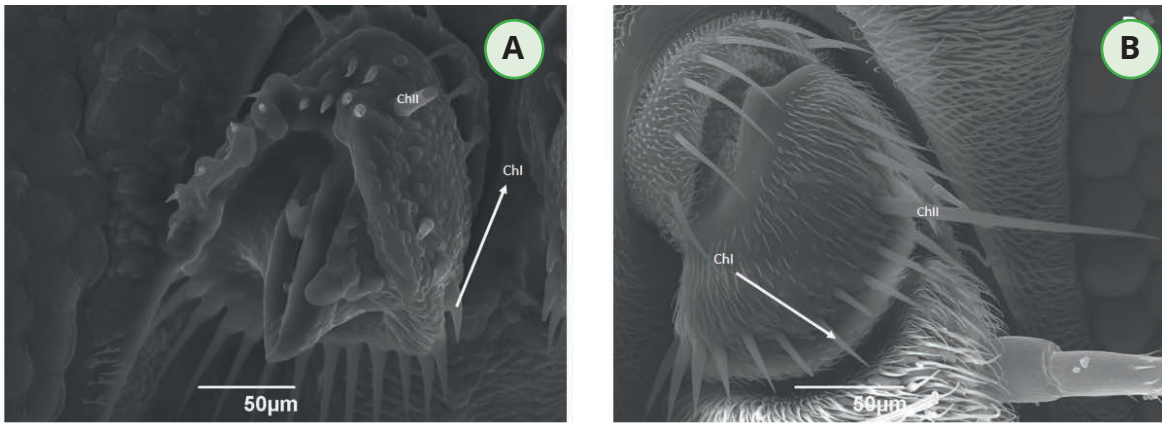

Figure 3. Scanning electron micrographs showing the cuticular surface of pedicel in both genders of *Xanthacrona bipustulata*: (A) male (X 450), (B) female (X 500). Pe = pedicel; ChI-ChII = chaetic sensilla I and II.

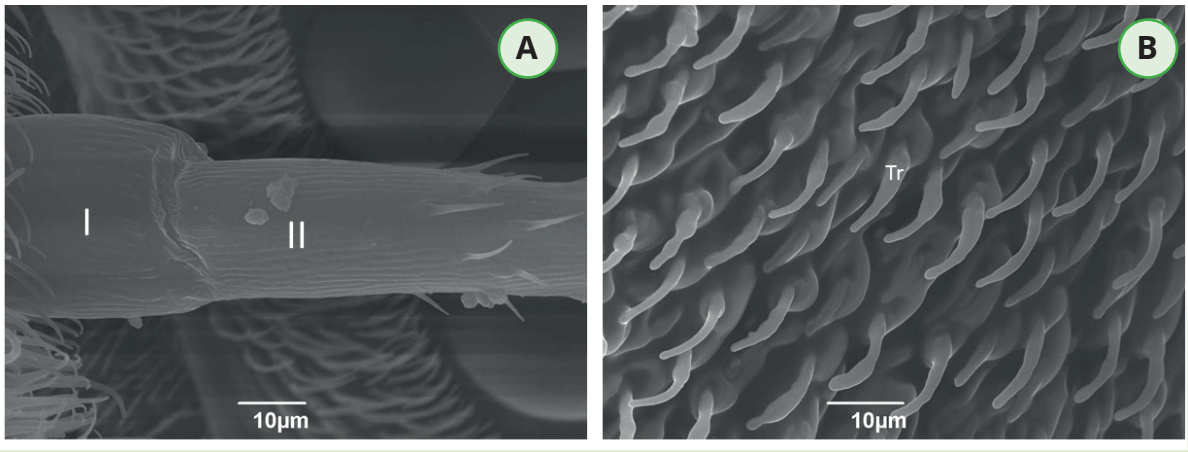

Figure 4. Scanning electron micrographs showing the cuticular surface of flagellum of female *Xanthacrona bipustulata:* (A) arista of female divided in two (I-II) segments (X 450). (B) Flagellomere (X 1700). Fn = first flagellomere; Tr = trichoidea.

number of sensory sensilla can vary from species (Sukontason *et al.* 2004; Carriço *et al.* 2015). One types of sensilla on female was observed: trichoidea (tr); those distribution differ of other dipteran species, such as *O. chalcogaster* (Sukontason *et al.*, 2007), *Musca domestica* Linnaeus (Sukontason *et al.* 2004), *F. scalaris* and *F. canicularis* (Zhang *et al.* 2013b), *L. mongola* (Zhang *et al.* 2013a) *P. terraenovae* (Setzu *et al.* 2011), *O. albuquerquei* and *O. aenescens* (Carriço *et al.* 2015) and *M. bellardiana*; *M. bicolor* and *M. semihyalina* (Caetano *et al.* 2018).

In conclusion, taxonomical studies are important to the biological sciences in general, because erroneous species identifications can mislead expert reports, additionally, this investigation provided new findings of some diagnostic structures of flies using SEM, since many of them are not possibly observed just by the use of light microscopy

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