ARTÍCULO CIENTÍFICO

ULTRASTRUCTURAL MORPHOLOGY OF THE ANTENNA AND BUCCAL APPARATUS OF FOUR FLIES OF FORENSIC IMPORTANCE

César Carriço[1,2]✉
Tayra Pereira-Sato[3,4]
Rebecca Leal-Caetano[1,5]
Rosane Acri[1]
Déborah Paredes-Soares da Silva[5]
Júlio Vianna-Barbosa[1]
Zeneida Teixeira-Pinto[1]

[1]Instituto Oswaldo Cruz (FIOCRUZ). Laboratório de Educação em Ambiente e Saúde
julio.jub@gmail.com
zeneida@ioc.fiocruz.br
[2]Instituto Brasileiro de Medicina de Reabilitação (IBMR). Laureate International Universities
rosaneacri@gmail.com

[3]Instituto Oswaldo Cruz (Fiocruz). Laboratório de Referencia Nacional em Vetores das Riquet-sioses.
satotayra@yahoo.com.br

[4]Univesidade Federal de Juiz de Fora (UFJF), Programa de Pós-Graduação em Comporta-miento e Biología Animal

[5]Universidade Estácio de Sá (UNESA)
rebeccalealcaetano@gmail.com

✉ carrico82@gmail.com

[1,3]Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, CEP 21040-900, Brasil.
[2]Av. das Américas, 2603 - Barra da Tijuca, Rio de Janeiro - RJ, CEP 22631-002, Brasil.
[4]Campus Universitário, Rua José Lourenço Kelmer, s/n - São Pedro, Juiz de Fora - MG, 36036-900, Brasil.
[5]Rua do Riachuelo, número 27, Centro 20230-010 Río de Janeiro, Estado de Río de Janeiro, Brasil.

Folia Entomológica Mexicana (nueva serie), 6(2): 47-58, 2020.

Recibido: 27 de junio 2019
Aceptado: 29 de abril 2020
Publicado en línea: 31 de agosto 2020

ISSN: 2448-4768

ULTRASTRUCTURAL MORPHOLOGY OF THE ANTENNA AND BUCCAL APPARATUS OF FOUR FLIES OF FORENSIC IMPORTANCE

Morfología ultraestructural de las antenas y aparatos bucales de cuatro moscas de importancia forense.

César Carriço[1,2] ,Tayra Pereira-Sato[3,4], Rebecca Leal-Caetano[1,5], Rosane Acri[1], Déborah Paredes-Soares da Silva[5], Júlio Vianna-Barbosa[1] y Zeneida Teixeira-Pinto[1.]

[1]Instituto Oswaldo Cruz / Fundação Oswaldo Cruz. Laboratório de Educação em Ambiente e Saúde.
[2]Instituto Brasileiro de Medicina de Reabilitação (IBMR). Laureate International Universities.
[3]Instituto Oswaldo Cruz (Fiocruz). Laboratório de Referência Nacional em Vetores das Riquetsioses.
[4]Univesidade Federal de Juiz de Fora (UFJF), Programa de Pós-Graduação em Comportamiento e Biología Animal.
[5]Universidade Estácio de Sá (UNESA)

*Autor de correspondencia: carrico82@gmail.com

ABSTRACT. Insects and other arthropods are found in decomposing vertebrate corps and carcasses. In forensic entomology, the right identification is essential. The purpose of this study was to examine in detail the morphological aspects of sensilla on the antennae and maxillary palps of Cochliomyia macellaria (Fabricius, 1775), Chloroprocta idioidea (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae), Graphomya maculata (Scopoli, 1973) and Sarcopromusca pruna (Shannon and Del Point, 1926) (Diptera: Muscidae). Adults of aforementioned species were collected on carcass of Oryctolagus sp Lilljebord 1873 in Instituto Samambaia de Ciências Ambientais e Ecoturismo (ISCAE), Petropolis - Rio de Janeiro State, Brazil. In this investigation, four types of sensilla (microtrichia, trichoide, basiconic and chaetic) were distributed throughout the different antennae segments. Buccal apparatus (proboscis and labellum) were also observed. This investigation provided new diagnostic structures on the distribution of the sensilla on the antennae and buccal apparatus of flies of forensic importance using SEM (Scanning Electron Microscopy), since many of them could not be observed just by the use of light microscopy.

Keywords: Atlantic Rainforest, Brazil, Diptera, SEM.

RESUMEN. Insectos y otros artrópodos se encuentran en cuerpos de vertebrados en descomposición y cadáveres. En la entomología forense, la identificación es esencial. El propósito de este estudio es el examinar a detalle características morfológicas de la sensilla en la antena y los palpos maxilares de Cochliomyia macellaria (Fabricius, 1775), Chloroprocta idioidea (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae), Graphomya maculata (Scopoli, 1973) y Sarcopromusca pruna (Shannon and Del Point, 1926) (Diptera: Muscidae). Adultos de las previamente mencionadas especies fueron colectados de los cadáveres de Oryctolagus sp Lilljebord 1873 en el Instituto Samambaia de Ciências Ambientais e Ecoturismo (ISCAE), Petrópolis - Rio de Janeiro, Brasil. En esta investigación, cuatro tipos de sensilla (microtrichia, trichoide, basiconic y chaetic) fueron distribuidos a través de todos los diferentes segmentos de la antena. Los aparatos bucales (probóscide y labelo) también fueron observados. Esta investigación aporta nuevas estructuras de diagnóstico en la distribución de la sensilla en la antena y el aparato bucal de las moscas de importancia forense usando MEB (Microscopio electrónico de barrido), ya que muchos de ellos no pudieron ser observados bajo la simple luz microscópica.

Palabras Clave: Bosque Atlántico, Brasil, Diptera, MEB

Introduction

Many insect species and other arthropods occur on or around a cadaver, using this ephemeral habitat to feed, live and breed (Von Zuben, 2001; Amendt *et al*., 2011). Flies are attracted by the odor of carcasses and their immature are responsible for the decomposition of carcasses (Marchenko, 2001; Martinez *et al*., 2007).

Insects that demonstrate a great potential for forensic area belong to the Diptera order (Archer, 2003). The composition of the representatives of the Diptera order in a body is influenced by the body decomposition stage and by environmental conditions (Oliveira-Costa, 2011).

The Calliphoridae family has great value in estimating the postmortem interval, because many

species begin the colonization of corpses few hours after exposure (Liu and Greenberg, 1989; Byrd and Butler, 1996; Lee, 1996). In many countries where the practice of forensic entomology is applied, many species belonging to this family are recorded as the most important in the carcasses of degradation process (Nuorteva *et al*., 1967; Greenberg, 1991; Iannacone, 2003; Sukontason *et al*., 2003; Arnaldos *et al*., 2004). Although some Muscidae species feed and develop on carcasses, there are only a few species which have forensic interest in Brazil (Carvalho and Mello-Patiu, 2008).

Generally, in adult flies, accurate species identification is complicated due to anatomical and morphological similarities among different species belonging to the same group (Pancorbo *et al*., 2006). To solve this problem, the information contained in the DNA molecule began to be used, facilitating the taxonomic identification at any stage of development (Wells and Stevens, 2008), and scanning electron microscopy was another tool used, having its use increased as it allows a better view of the external morphology of immature (Liu and Greenberg, 1989; Peterson and Newman, 1991; Greenberg and Singh, 1995; Sukontason *et al*., 2006) as well as of adult flies (Carriço *et al*., 2015).

Many researchers have studied the ultrastructure in adult flies to help increase the anatomical database on flies: Zhang *et al*. (2013a); Zhang *et al*. (2013b); Carriço *et al*. (2015); Pezzi *et al*. (2016); Carriço *et al*. (2017); Caetano *et al*. (2018).

An ultrastructure analysis of the morphological aspects of sensilla on the antennae and buccal apparatus (proboscis and labellum) of *Cochliomyia macellaria* (Fabricius, 1775), *Chloroprocta idioidea* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae), *Graphomya maculata* (Scopoli, 1973) and *Sarcopromusca pruna* (Shannon and Del Point, 1926) (Diptera: Muscidae) have not been performed yet. Studies like this help to improve forensic entomology. For example, if parts of the flies (as head) are found in a crime scene, these parts can be analyzed using SEM for accurate taxonomic identification. Choosing the head for the present study is due to the fact that in this part of the body, the greater number and greater diversity of sensilla among the different species are concentrated (Zhang *et al*., 2013a; Zhang *et al*., 2013b; Pezzi *et al*., 2016;). The purpose of this study was to examine in details these sensorial organs on the aforementioned species to help increase the anatomical database for flies.

Materials and Methods

Specimens of *C. macellaria, C. idioidea, G. maculata* and *S. pruna* were obtained from carcasses of *Oryctolagus* sp, and the collections were daily performed, during two weeks in august of 2015, in Instituto Samambaia de Ciências Ambientais e Ecoturismo (ISCAE), Petrópolis - Rio de Janeiro State, Brazil (22º46'90"S and 43º14'82"W). Scientific Research Authorization Number ICMBio / SisBio (Instituto Chico Mendes de Conservação da Biodiversidade / Sistema de Autorização e Informação em Biodiversidade) is 54279.

The identification of flies following the key of Mello (2003), Carvalho and Couri (2002) and Carvalho *et al*. (2002). Adults morphology used in this study followed Triplehorn and Johnson (2011) and McAlpine (1981), the classification of sensilla followed Setzu *et al*. (2011) and Zhang *et al*. (2013 a, b).

The heads and buccal apparatus were dissected from five males and two females of *C. macellaria*, two males of *C. idioidea*, five males of *G. maculata* and six males and two females of *S. pruna* 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, the specimens were rinsed twice with PBS during 10 minute- intervals and postfixed with 1% of osmium tetroxide at room temperature for 3 days. This postfixation 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 (20-30nm) and examined under JEOL 6390LV scanning electron microscope (SEM) (Akishima, Tokyo, Japan).

Results and Discussion

The use of Scanning Electron Microscopy (SEM) has been used to increase the general knowledge of sensor structures in medical and forensic import flies (Carriço *et al*., 2015; Carriço *et al*., 2017). Sensory organs are distributed all over the body surface of the flies (Sukontason *et al*., 2008; Carriço *et al*., 2015).

All insects as well as these calliphorid and muscid are equipped with a pair of antennae located on the frontal region of the head, between the large compound eyes (Figures 1, 2 and 3). The antennal morphology consists in three segments: Scape (Sc), Pedicel (Pc) and distal Flagellum with a basal funiculus (F) and a feathered arista (Ar) (Figures 1,2 and 3).

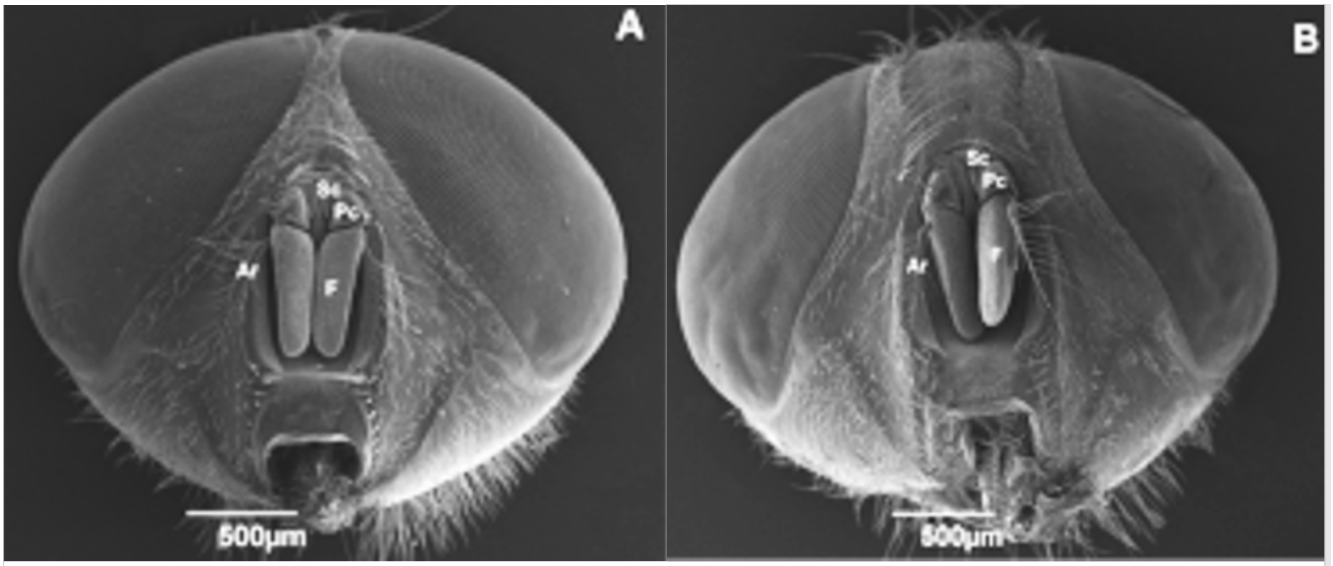

Figure 1. Scanning electron micrographs of features on the head of male and female of *Cochliomyia macellaria* (Diptera: Calliphoridae). A- head of male X 37 B- head of female X 37. Scape (Sc), Pedicel (Pc) and Flagellum with Arista (Ar) and Funiculus (F).

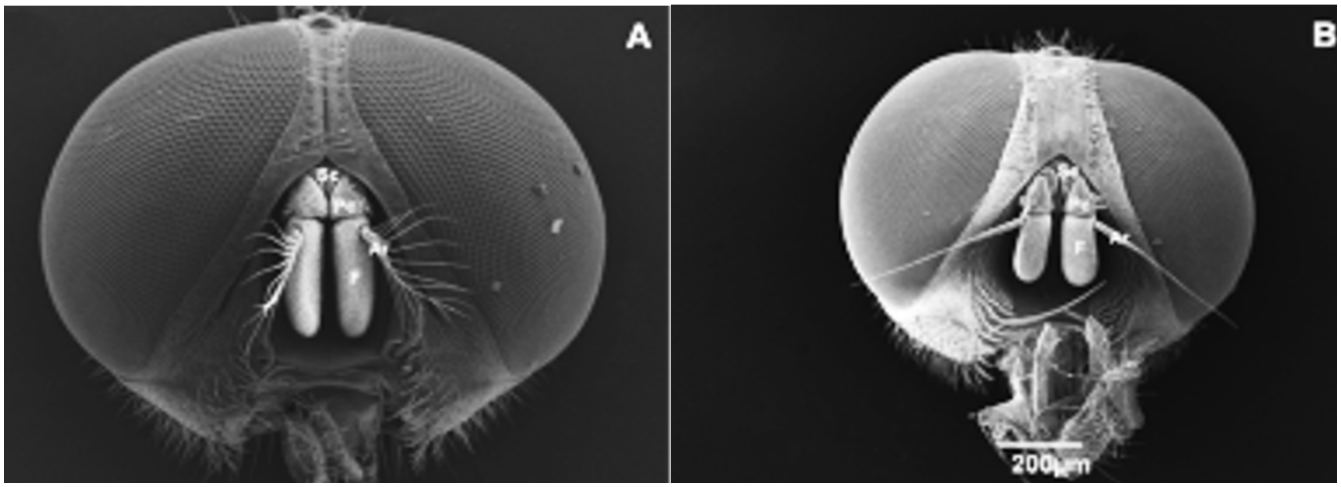

Figure 2. Scanning electron micrographs of features on the head of male and female of *Sarcopromusca pruna* (Diptera: Muscidae). A- head of male X 35 B- head of female X 37. Scape (Sc), Pedicel (Pc) and Flagellum with Arista (Ar) and Funiculus (F).

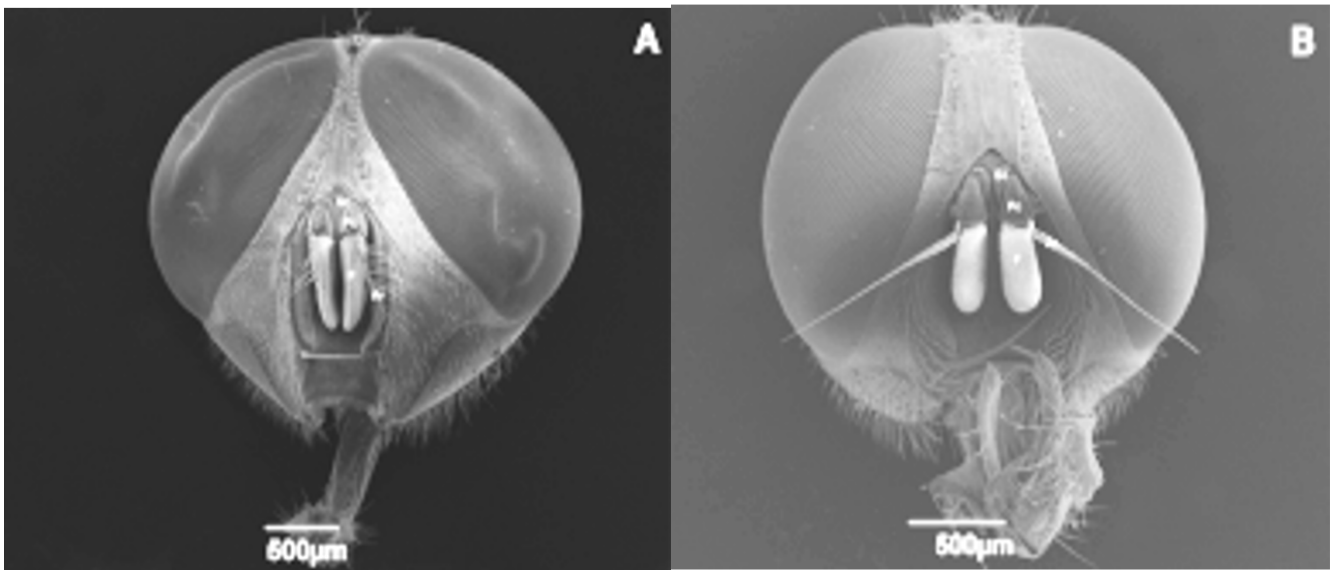

Figure 3. Scanning electron micrographs of features on the head of *Chloroprocta idioidea* (Diptera: Calliphoridae) and *Graphomya maculata* (Diptera: Muscidae). A- head of male of C. idioidea X27 B - head of female of G. maculate X37. Scape (Sc), Pedicel (Pc) and Flagellum with Arista (Ar) and Funiculus (F).

Sensilla in the antennae and others parts of the insects have a sensitive structure role (Sukontason *et al*., 2004). These structures are responsible for detecting several stimuli as the opposite sex, food sources, orientation in shape, and other aspects (Zhang *et al.*, 2013 a,b). Sensilla was identified on the three antennal segments of *C. macellaria, C. idioidea, G. maculata* and *S. pruna* had similar characters of antennae described in many other flies species (Setzu *et al.,* 2011; Sukontason *et al*., 2004; Sukontason *et al*., 2007; Zhang *et al.*, 2013a,b; Carriço *et al.*, 2015; Carriço *et al*., 2017; Caetano *et al*. 2018).

The Sc in *C. macellaria* has two types of sensilla in both genders: four chaetic sensilla (Ch) of similar length in a single row and densely covered by microtrichia (Mc) (Figure 4 A, B), using the grid technique described by Kelling-Johannes (2001). The second antenna segment is called Pc and it is covered by microtrichia, trichoidea (Tr) and three morphological types of chaetic sensilla are observed (ChI, ChII and ChIII) in both genders (Figure 5 A, B).

The Sc of male *S. pruna* has two chaetic sensilla (Ch) and the female nine chaetic sensilla (ChI) of similar length. In both genders, the Sc is covered with microtrichia (Mc) (Figure 6 A, B). The Pc of male of S. pruna has two types of chaetic sensilla (ChI and ChII) randomly inserted (Figure 6 A); the female has three types of chaetic sensilla (ChI, ChII and ChIII) (Figure 6 A, B).

In the species *C. idioidea*, the Sc of male has three chaetic sensilla (Ch) of varying in length in a single row and densely covered by microtrichia (Mc) (Figure 7 A), using the grid technique described by Kelling-Johannes (2001). On the Pc, only ChI and ChII are noticed, but less numerous and not varying in length among them (Figure 7 A).In the species *G. maculata* the Sc of female has five chaetic sensilla (Ch) all of them varying in length and covered by microtrichia (Mc) (Figure 7 B). The Pc has three morphological types of chaetic sensilla (ChI, ChII and ChII), and varying in length (Figure 7B).

The flagellum is the largest segment of the antenna and its number of sensory sensilla can vary from species to species (Sukontason *et al*., 2004, Carriço *et al*., 2015). In this study, the flagellum of all species presented a great number of sensilla (Figures 8-9). The segments of Ar of all species are around, covered by short microtrichia (Mc), using the grid technique described by Kelling-Johannes (2001) and divided in three segments (Figures 8-9). Anterior surface of F of all species, three morphological types of sensilla are distributed on the entire surface: trichoidea sensilla (Tr), basiconic sensilla (Ba) and microtrichia (Figures 8-9).

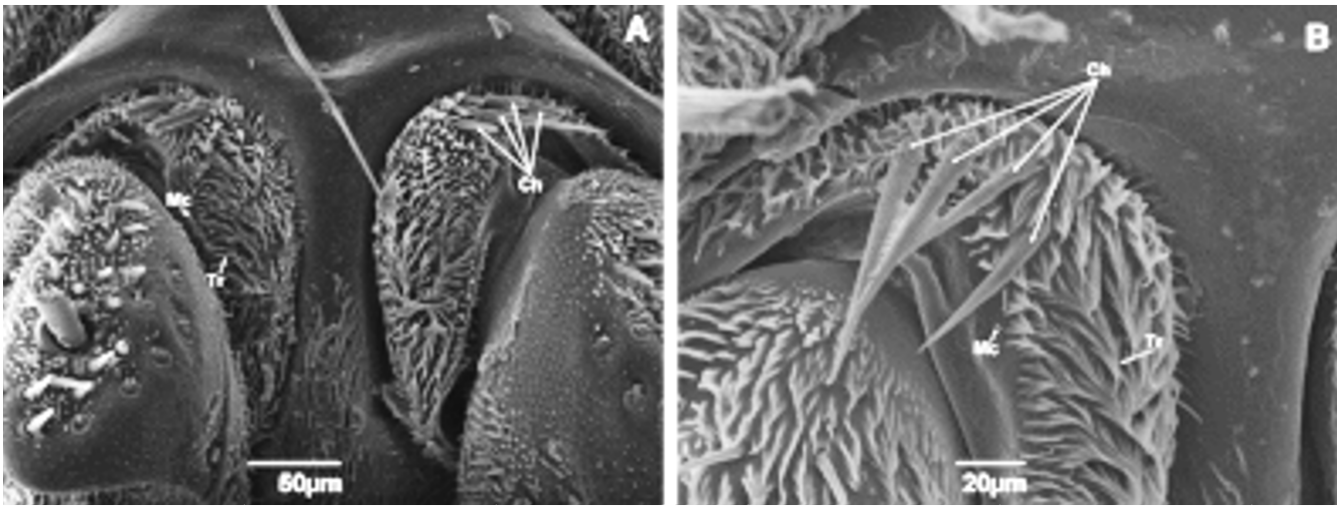

Figure 4. Scanning electron micrographs of features on the scape of male and female of *Cochliomyia macellaria* (Diptera: Calliphoridae). A- scape of male X350 B- scape of female X650. Chaetic sensilla (Ch I); Microtrochia (Mc) and Trichoidea sensilla (Tr).

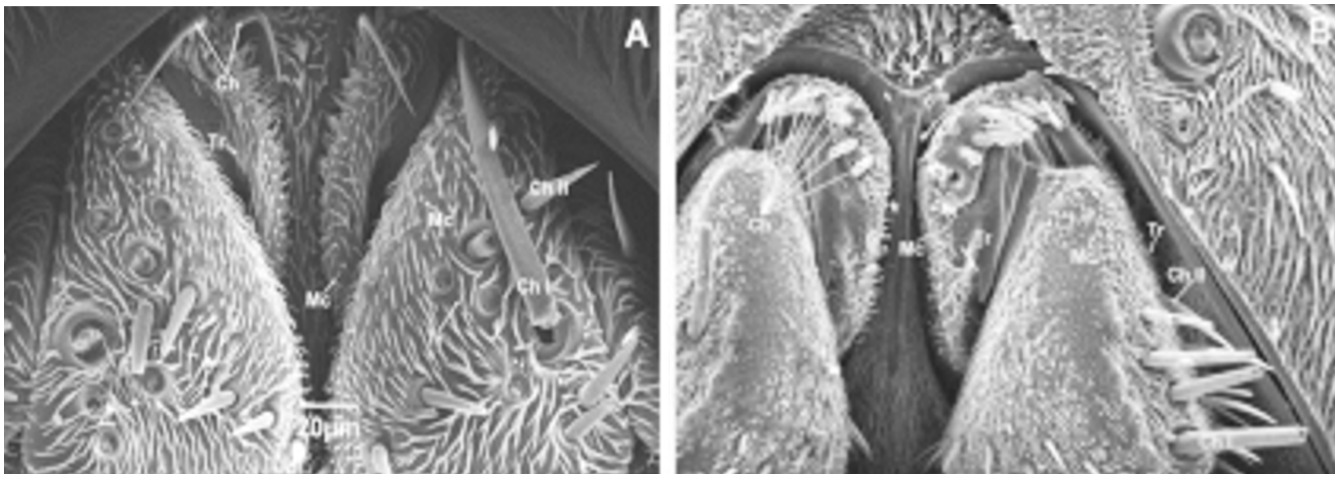

Figure 5. Scanning electron micrographs of features on the pedicel of *Cochliomyia macellaria* (Diptera: Calliphoridae). A- pedicel of male X230 B- pedicel of female X330. Chaetic sensilla (ChI, ChII and ChII); Microtrichia (Mc) and Trichoidea sensilla (Tr).

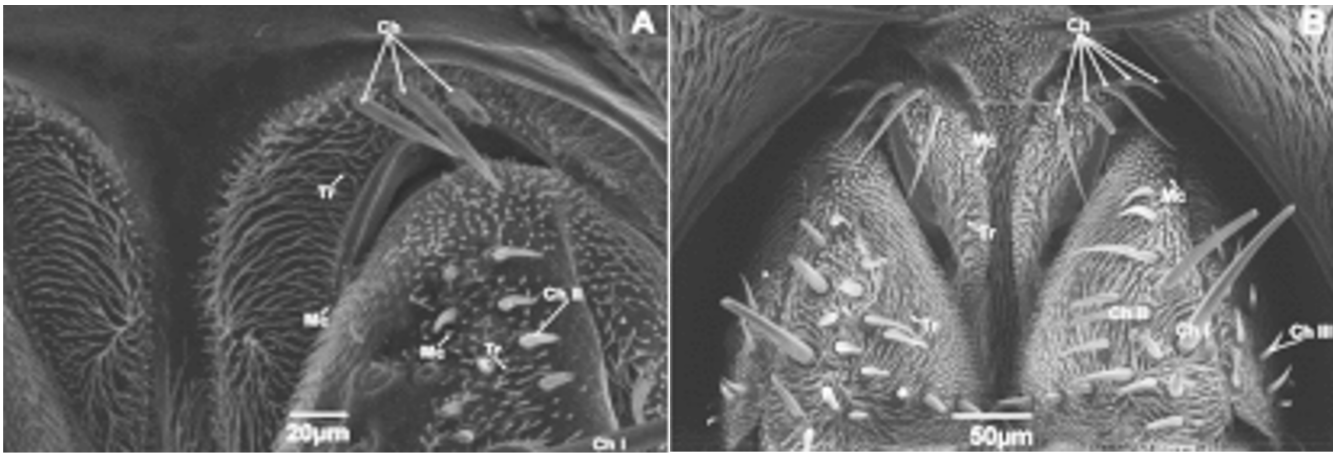

Figure 6. Scanning electron micrographs of features on the scape and pedicel of male and female of *Sarcopromusca pruna* (Diptera: Muscidae). A- scape and pedicel of male X550 B- scape and pedicel of female X300. Chaetic sensilla (ChI, ChII and ChIII); Microtrichia (Mc) and Trichoidea sensilla (Tr).

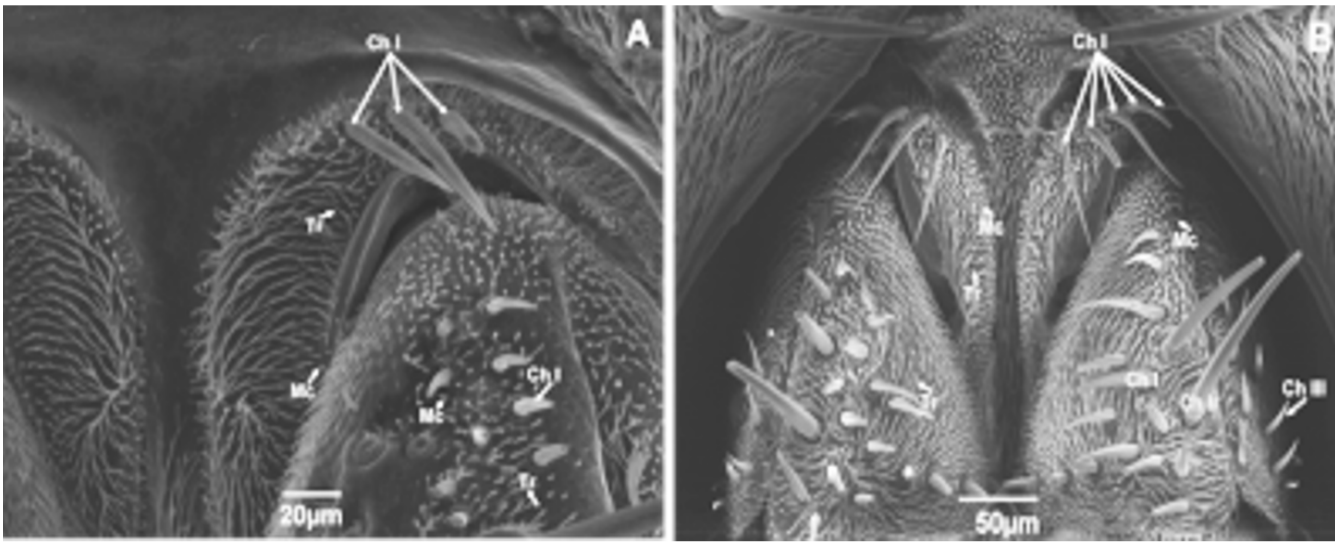

Figure 7. Scanning electron micrographs of features on the scape and pedicel of A- *Chloroprocta idioidea* (Diptera: Calliphoridae) male X550 and B- *Graphomya maculata* (Diptera: Muscidae) female X300. Chaetic sensilla (ChI, ChII and ChIII); Microtrichia (Mc) and Trichoidea sensilla (Tr).

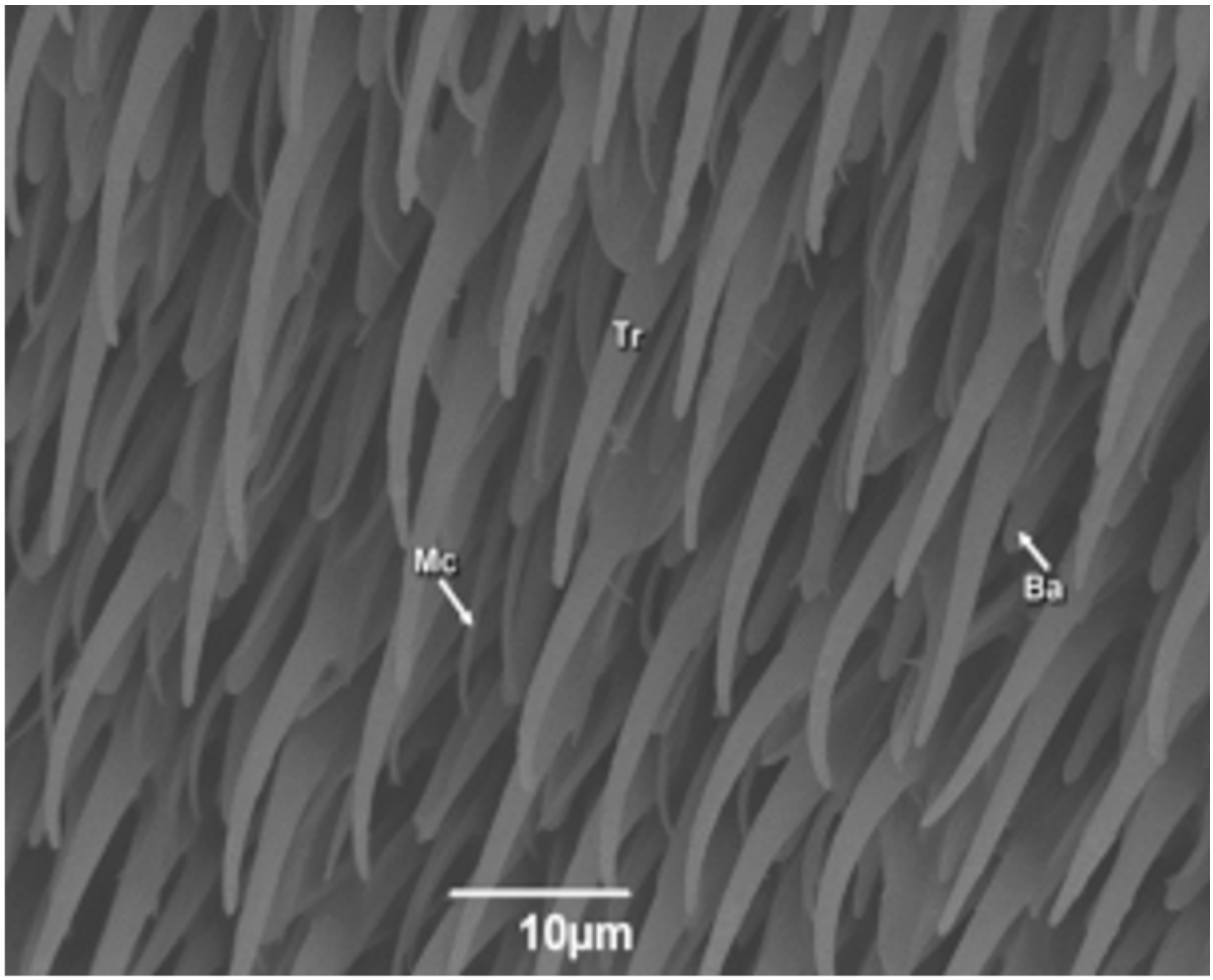

Figure 8. Scanning electron micrographs of features on the Flagellum of *Cochliomyia macellaria* (Diptera: Calliphoridae). Basal funiculus of female X1900. Basiconic sensilla (Ba); Microtrichia (Mc) and Trichoidea sensilla (Tr).

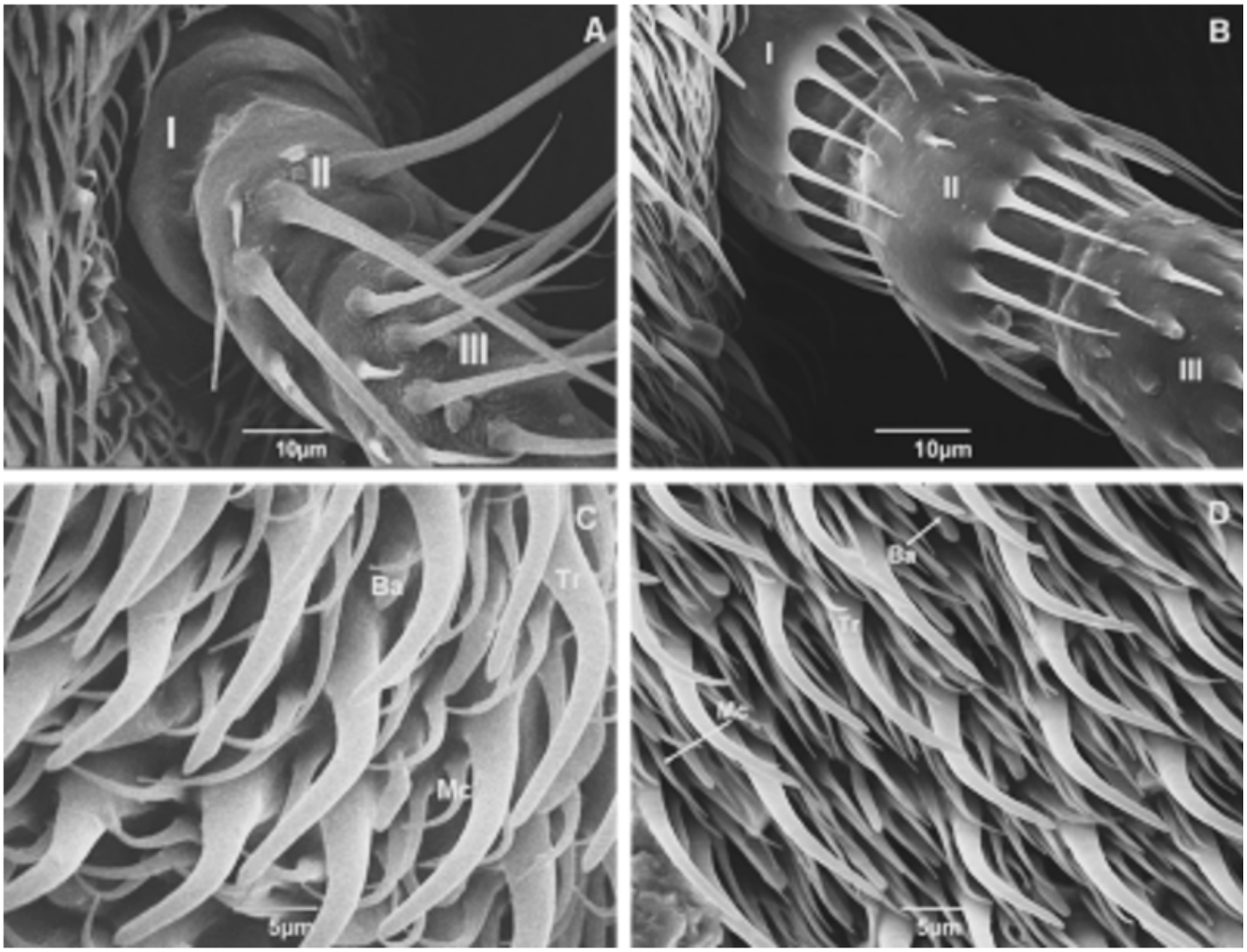

Figure 9. Scanning electron micrographs of features on the Flagellum of *Sarcopromusca pruna* (Diptera: Muscidae). A- arista of male X 2000 B- arista of female X 2000 C- basal funiculus of male X 4000 D- basal funiculus of female X 3000. Basiconic sensilla (Ba); Microtrichia (Mc) and Trichoidea sensilla (Tr).

In the flies *C. macellaria* and *S. pruna.* sensilla microtrichia, trichoidea and basiconic were observed in this segment and resembled those described in other dipteran species, such as *Ophyra chalcogaster* (Wiedemann, 1824) (Sukontason *et al*., 2007), *M. domestica* (Sukontason *et al*., 2004), *Fannia scalaris* (Fabricius, 1794) and *F. canicularis* (Linnaeus, 1761) (Zhang *et al.* 2013b), *Lispenei mongola* Tian et Ma (Zhang *et al*., 2013a) *Protophormia terraenovae* Robineau-Desvoidy, 1830 (Setzu *et al*., 2011), *Ophyra albuquerquei* Lopes, 1985 and *Ophyra aenescens* (Wiedemann, 1830) (Carriço *et al*., 2015).

In this investigation, four types of sensilla (microtrichia, trichoide, basiconic and chaetic) were distributed throughout the different antennae segments, but only microtrichia and trichoidea sensilla were observed in all of them. These observations met those of Carriço *et al*. (2015) in their studies with *O. albuquerquei* and *O. aenescens*.

The presence of chaetic sensilla was observed only on the scape and pedicel of all species. In relation to the Sc, chaetic sensilla varied among all the studied species. In male and female of *C. macellaria,* four chaetic sensilla were observed. In *S. pruna* male only two chaetic sensilla were observed, and nine chaotic sensilla in the female. In the Sc of *C. idioidea* male, three chaetic sensilla were observed, and in the female specimen of *G. maculata* five chaetic sensilla. In Pc, however, it was only possible to observe the different types of chaetic sensilla, the amount varying between the same sex, the opposite sex and between the different species studied. This distribution pattern was also found in *O. chalcogaster*

(Sukontason *et al*., 2007), *Mesembrinella bellardiana* Aldrich, 1922; Mesembrinella bicolor (Fabricius, 1805) and *Mesembrinella semihyalina* Mello, 1967 (Caetano *et al.*, 2018)

Unlike the antennal characters, regarding the presence of sensilla, the proboscis and labellum of all the species observed do not show sexual dimorphism (Figures 10-12). These segments present different morphological types of sensilla distributed on the entire surface like trichoidea sensilla (Tr) and chaetic (Ch) (Figures 10-12). Microtrichia (Mc) covers the surface of the palps, but not as densely as on the antennae (Figures 10-12). It was similar to other dipterans such as *O. chalcogaster, Fannia hirticeps* (Stein, 1982), *L. mongola* and *Sarcophaga tibialis* Macquart, 1851 (Sunkontason *et al*., 2007; Wang *et al*., 2012; Zhang *et al*., 2013a; Pezzi *et al*., 2016).

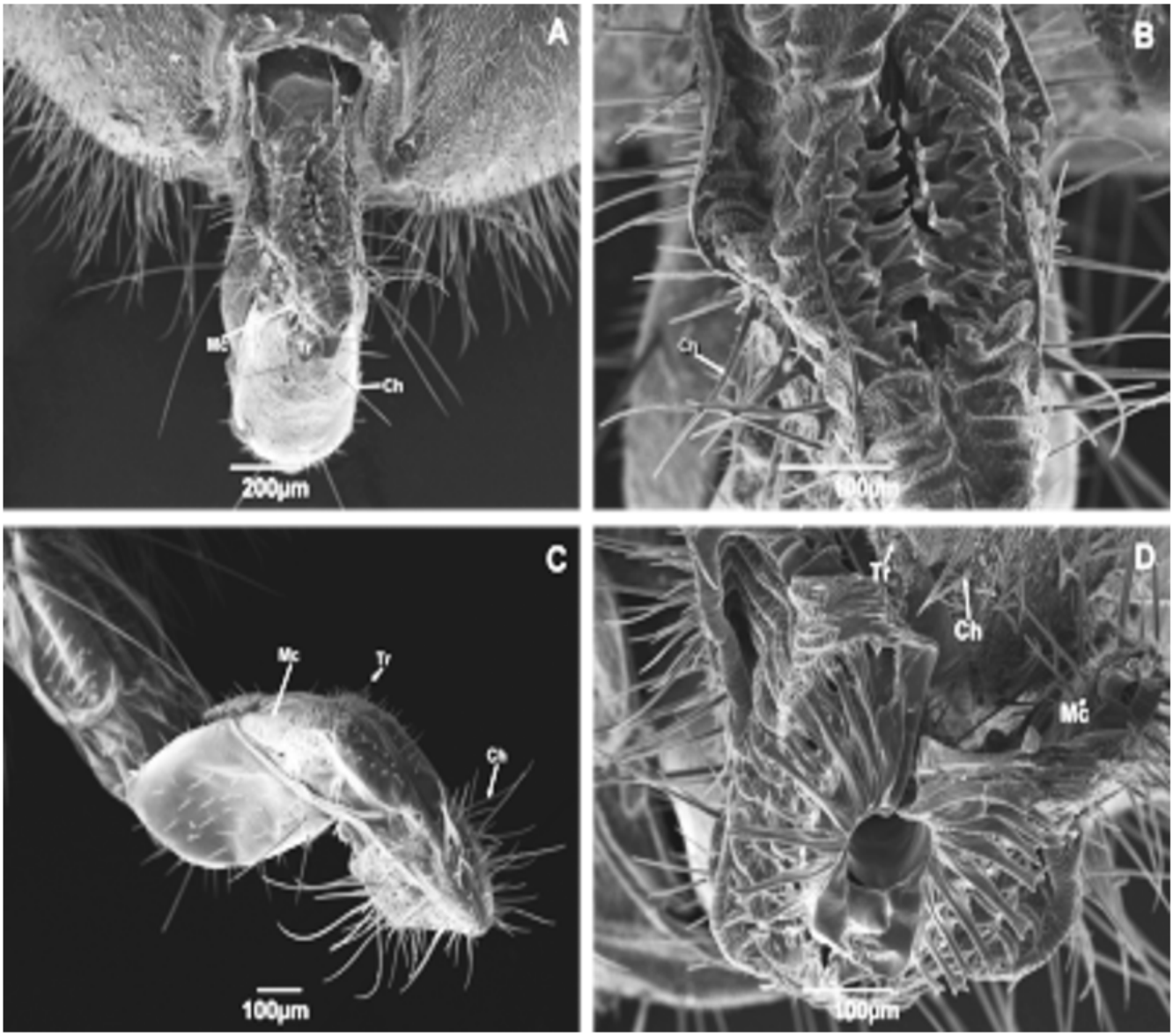

Figure 10. Scanning electron micrographs of the palps of *Cochliomyia macellaria* (Diptera: Calliphoridae). A - palp of male X80 B- palp of male X250 C – palp of female X110 D- palp of female X200. Chaetic sensilla (Ch); Microtrichia (Mc) and Trichoidea sensilla (Tr).

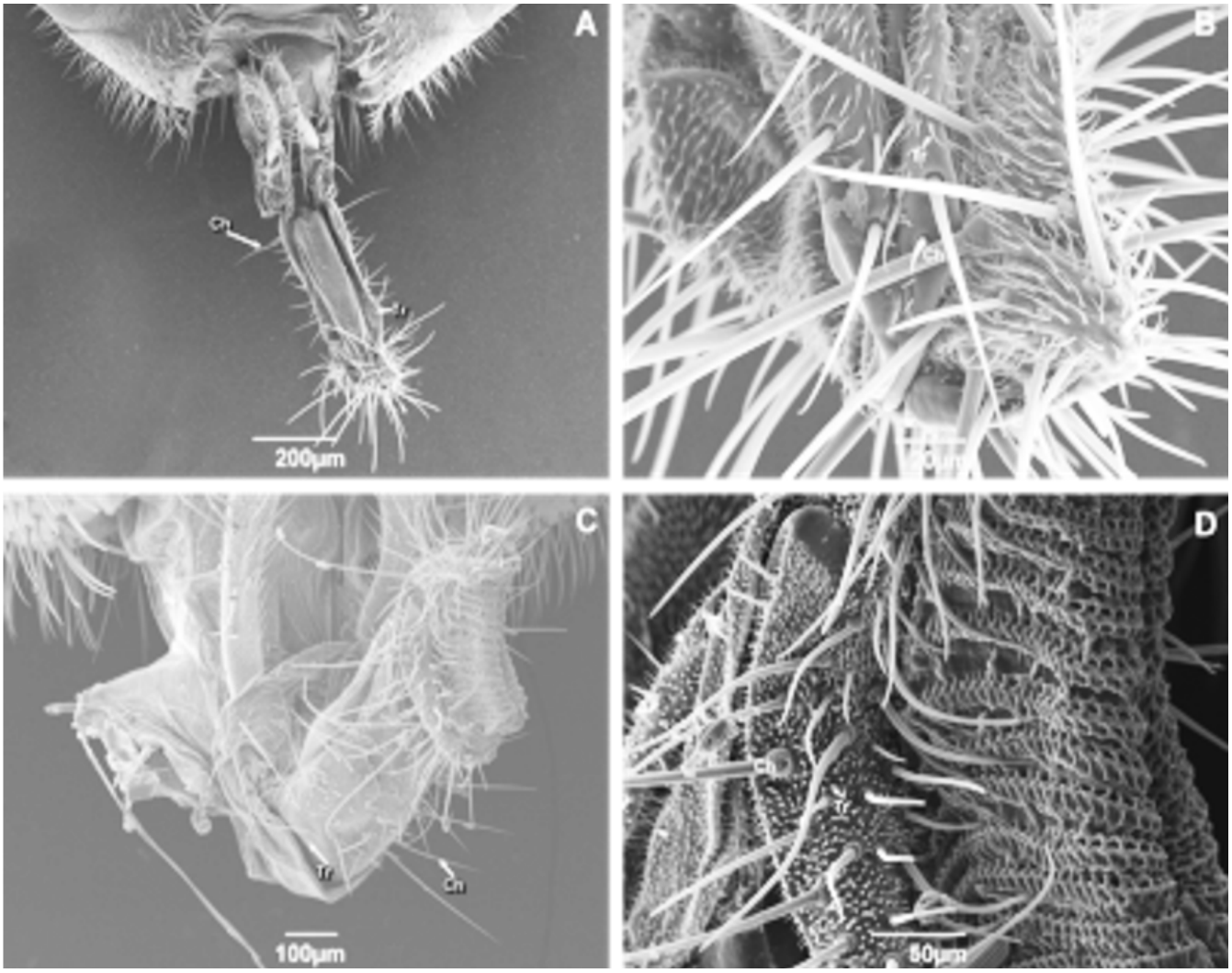

Figure 11. Scanning electron micrographs of the palps of *Sarcopromusca pruna* (Diptera: Calliphoridae). A - palp of male X70 B- palp of male X750 C – palp of female X110D- palp of female X450. Chaetic sensilla (Ch) and Trichoidea sensilla (Tr).

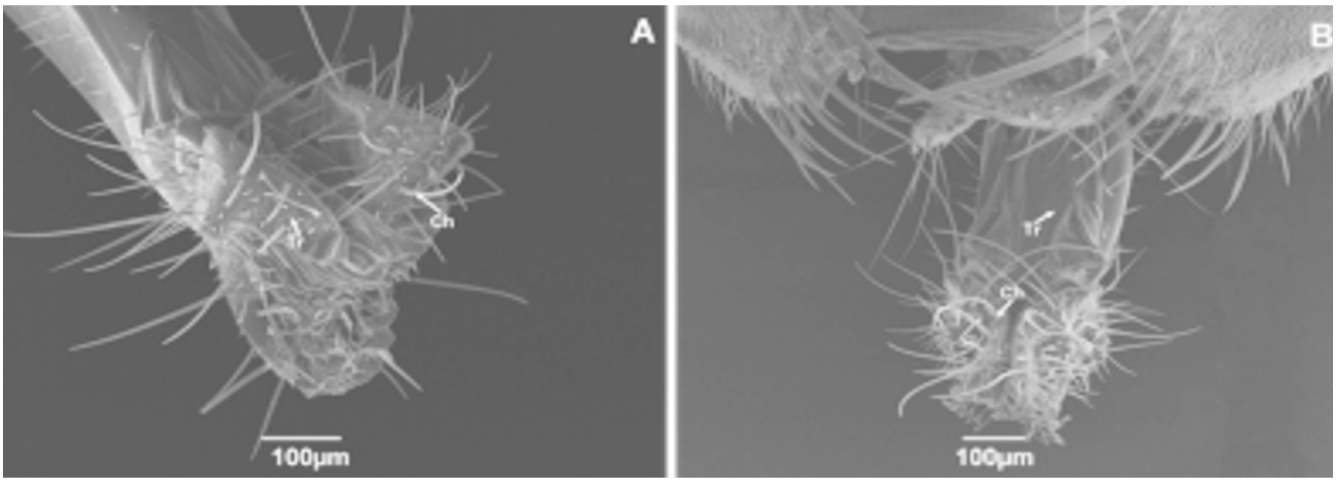

Figure 12. Scanning electron micrographs of the palps *Chloroprocta idioidea* (Diptera: Calliphoridae) and *Graphomya maculate* (Diptera: Muscidae). A- palp of male of *C. idioidea* X150 B - palp of female of *G. maculate* X120. Chaetic sensilla (Ch) and Trichoidea sensilla (Tr).

Dipteran trichoide sensilla can be responsible for detecting various types of substances. Chaetic plays a role of mechanosensitive sensilla whereas basiconic sensilla does not have a defined function (Shanbhag *et al*., 1999; Wang *et al*., 2012). Buccal apparatus of male/female of C.macellaria, male of *C. idioidea*, female of *G. maculata* and male/female of *S. pruna* were long when stretched and the sensilla observed on buccal apparatus are common in other dipteran as *M. bellardiana; M. bicolor* and *M. semihyalina* (Caetano *et al*., 2018).

Conclusions

This investigation provided a description of some ultrastructure of sensorial organs using SEM, since many of them could not be observed just by the use of light microscopy. Moreover, these micrographs help increase the anatomical database on flies for forensic importance.

Acknowledgements

We thank Paulo Vander Ferreira Santana (teacher from Prefeitura Municipal do Rio de Janeiro) for language editing. All authors acknowledge the Electron Microscopy Platform Rudolf Barth of Instituto Oswaldo Cruz (IOC/FIOCRUZ) for the use of the scanning electron microscope.

References

Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R. and Hall, M. J. R. 2011. Forensic entomology: applications and limitations. *Forensic Science, Medicine, Pathology,* 7:379–392. https://doi.org/10.1007/s12024-010-9209-2

Arnaldos, M. I., Romera, E., Presa, J. J. and Luna A. 2004. Studies on seasonal arthropod succession on carrion in the southeastern Iberian Peninsula. *International Journal of Legal Medicine,* 118:197-205. https://doi.org/10.1007/s00414-004-0446-3

Archer, M. S. 2003. Annual variation in arrival and departure times of carrion insects at carcasses: implications for succession studies in forensic entomology. *Austin Journal of Zoology,* 51:569-576. https://doi.org/10.1071/ZO03053

Byrd, J. H. and Butler, J. F. 1996. Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development. *Journal of Medical Entomology;* 33:901-905. http://dx.doi.org/10.1093/jmedent/33.6.901

Caetano, R. L., Carriço, C., Freitas, D. M. A. and Pinto, Z. T. 2018. Ultrastructure of sensilla on antennae and maxillary palps in three Mesembrinellidae species. *Revista Chilena de Historia Natural,* 91:7. http://dx.doi.org/10.1186/s40693-018-0077-6

Carriço, C., Mendonça, P. M., Cortinhas, L. B., Mallet, J. R. S., Queiroz, M. M. C. 2015. Ultrastructural studies of some character of Diptera (Muscidae) of forensically importance. *Acta Tropica,* 142:96-102. https://doi.org/10.1016/j.actatropica.2014.11.005

Carriço, C., Caetano, R. L., Barbán- Alvarez, L. D. R. and Pinto, Z. T. 2017. Morphology of Flesh *Fly Peckia (Peckia) chrysostoma* (Diptera: Sarcophagidae) Revealed by Scanning Electron Microscopy. *Austin Journal of Forensic Science and. Criminology,* 4(2):1063. Available in: https://austinpublishinggroup.com/forensics-cience-criminology/fulltext/ajfsc-v4-id1063.php

Carvalho, C. J. B. and Couri, M. S. 2002 *Part I. Basal Groups.* Pp 17-132. In: Carvalho C. J. B. (Ed) *Muscidae (Diptera) of the Neotropical Region: Taxonomy*. Editora UFPR, Curitiba. ISBN-13 : 978-8573350913

Carvalho, C. J. B., Moura, M. O. and Ribeiro, P.B. 2002. Chave para adultos de dípteros (Muscidae, Fanniidae, Anthomyiidae) associados ao ambiente humano no Brasil. *Revista Brasileira de Entomologia;* 46 (2):107-114. Disponible en: https://www.scielo.br/pdf/rbent/v46n2/a01v46n2.pdf

Carvalho, C. J. B. and Mello-Patiu, C. A. 2008. Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia;* 52 (3):390 - 406. https://doi.org/10.1590/S0085-56262008000300012

Greenberg, B. 1991. Flies as forensic indicators. *Journal of Medical Entomology*; 28(5):565-577. https://doi.org/10.1093/jmedent/28.5.565

Greenberg, B. and Singh, D. 1995. Species identification of calliphorid (Diptera) eggs. *Journal of Medical Entomology;* 32:21-26. https://doi.org/10.1093/jmedent/32.1.21

Iannacone, J. 2003. Artropodofauna de importancia forense en un cadáver de cerdo en el Callao, Perú. *Revista Brasileira Zoologia,* 20(1):85-90. https://doi.org/10.1590/S0101-81752003000100010

Lee, H. L. 1996. Recovery of forensically important insect larvae from human cadavers in Malaysia. *Malaysian Journal of Pathology;* 18:125-127. PMID: 10879234

Liu, D. and Greenberg, G. 1989. Immature stages of flies of forensic importance. *Annals of the Entomological Society of America;* 83 (1):80-93.

Marchenko, M. L. 2001. Medicolegal relevance of cadaver entomofauna for the determination of the time of death. *Forensic Science International*; 120:89-109. https://doi.org/10.1016/s0379-0738(01)00416-9

Martinez, E., Duque, P. and Wolff, M. 2007. Succession pattern of carrion-feeding insects in Paramo, Colombia. *Forensic Science International,* 166:182-189. https://doi.org/10.1016/j.forsciint.2006.05.027

McAlpine, J. F. 1981. Morphology and terminology – adults. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM. *Manual of Neartic Diptera -1.* Biosystematic Research Institute monograph. 27:9-63. Agriculture Canada, Otawa.

Mello, R. P. 2003. Chave para identificação das formas adultas das espécies da família Calliphoridae (Diptera, Brachycera, Cyclorrhapha) encontradas no Brasil. *Entomología y Vectores,* 10(2): 255-268.

Nuorteva, P., Isokoski, M. and Laiho K. 1967. Studies on the possibilities of using blowflies (Diptera) as medicolegal indicators. In: Finland. 1. Report of four indoor cases from the city of Helsinki. *Acta Entomologica Fennica;* 33(4):217-225. Available in: https://www.cabdirect.org/cabdirect/abstract/19691000170

Oliveira-Costa, J. 2011. *Entomologia Forense, quando os insetos são vestígios.* Tratado de Perícias Criminalísticas. 3ª edição. Campinas. (ed) Millenium; 520 p.

Pancorbo, M. M., Ramos, R., Saloña, M. Y Sánchez, P. 2006. Entomología molecular forense. *Ciência forense*, 8:107-130. Disponible en: https://ifc.dpz.es/recursos/publicaciones/26/57/7.Entomologmolecular.pdf

Peterson, R. D., Newman and Junior, S. M. 1991. Chorionic structure of the egg of the screwworm *Cochliomyia hominivorax* (Diptera: Calliphoridae). *Journal of Medical Entomology;* 28:152-160. https://doi.org/10.1093/jmedent/28.1.152

Pezzi, M., Whitmore, D., Chicca, M., Semeraro, B., Brighi, F. and Leis M. 2016. Ultrastructural morphology of the antenna and maxillary palp of *Sarcophaga tibialis* (Diptera: Sarcophagidae). *Journal of Medical Entomology,* 0(0): 1-8. http://dx.doi.org/10.1093/jme/tjw061

Shanbhag, S. R., Muller, B. and Steinbrecht, R. A. 1999. Atlas of olfactory organs of *Drosophila melanogaster* 1. Types, external organization, innervation and distribution of olfactory sensilla. *International Journal of Insect Morphology and Embryology,* 28:377–397. https://doi.org/10.1016/S0020-7322(99)00039-2

Setzu, M. D., Poddighe, S. and Angioy, A. M. 2011. Sensilla on the antennal funiculus of the blow fly, *Protophormia terraenovae* (Diptera: Calliphoridae). *Micron;* 42:471-477. https://doi.org/10.1016/j.micron.2011.01.005

Sukontason, K., Sukontason, K. L., Piangjai, S., Chaiwong, T., Boonchu, N., Kurahashi, H. and Vogtsberger, R. C. 2003. Larval ultrastructure of *Parasarcophaga dux* (Thomson) (Diptera: Sarcophagidae). *Micron;* 34:359-364. https://doi.org/10.1016/j.micron.2003.07.001

Sukontason, K., Sukontason, K. L., Piangjaia, S., Boonchua, N., Chaiwonga, T., Ngern-kluna, R., Sripakdeea, D., Vogtsbergerb, R. C. and Olsonc, J. K. 2004. Antennal sensilla of some forensically important flies in families Calliphoridae, Sacorphagidae and Muscidae. *Micron*, 35:671-679. https://doi.org/10.1016/j.micron.2004.05.005

Sukontason, K. L., Bunchu, N., Methanitikorn, R., Chaiwong, T., Kuntalue, B. and Sukontason, K. 2006. Ultrastructure of adhesive devise in fly in families Calliphoridae, Muscidae and Sarcophagidae, and their implication as mechanical carries of pathogens. *Parasitological Research*; 98:477-481. https://doi.org/10.1007/s00436-005-0100-0

Sukontason, K., Methanitikorn, R., Chaiwong, T., Kurahashi, H., Vogtsberger, R.C. and Sukontason, K. L. 2007. Sensilla of the antenna and palp of *Hydrotaea chalcogaster* (Diptera: Muscidae). *Micron*; 38:218-223. https://doi.org/10.1016/j.micron.2006.07.018

Sukontason, K., Methanitikorn, R., Kurahashi, H., Vogtsberger, R. C. and Sukontason, K. L. 2008. External morphology of *Chrysomya pinguis* (Walker) (Diptera:Calliphoridae) revealed by scanning electron microscopy. *Micron;* 39:190–197. https://doi.org/10.1016/j.micron.2007.01.004

Triplehorn, C. A. and Johnson, N.F. 2011. *Estudo dos insetos: tradução da 7ª edição de Borror and Delong's introduction to the study of insects.* São Paulo, Cengage Learning, 809p.

Von Zuben, C. J. 2011. Zoologia aplicada: Recentes avanços em estudos de entomologia forense. *Entomología y vectores;* 8:173-183.

Wang, Q. K., Zhang, M., Li, K. and Zhang, D. 2012. Olfactory sensilla on antennae and maxillary palps of *Fannia hirticeps* (Stein, 1982) (Diptera: Fanniidae). *Micron Research and Technology;* 75:1313-1320. https://doi.org/10.1002/jemt.22066

Wells, J. D. and Stevens, J. R. 2008. Application of DNA-based methods in forensic entomology. *Annual Review of Entomology,*53:103-120. https://doi.org/10.1146/annurev.ento.52.110405.091423

Wunderer, H. and Smola, U. 1982. Fine structure of ommatidia at the dorsal eye margin of *Calliphora erythrocephala* Meigen (Diptera: Calliphoridae): an eye region specialized for the detection of polarized light. *International Journal of Insect Morphology and Embryology,* 11:25-38. https://doi.org/10.1016/0020-7322(82)90035-6

Zhang, D., Wang, Q. K., Liu, X. H. and Li, K. 2013a. Sensilla on antenna and maxillary palp of predaceous fly, *Lispeneimongola tianet* Ma (Diptera: Muscidae). *Micron;* 49:33-39. https://doi.org/10.1016/j.micron.2013.02.012

Zhang, D., Wang, Q. K., Yang, Y. Z., Chen, Y. O. and Li, K. 2013b. Sensory organs of the antenna of two *Fannia species* (Diptera: Fanniidae). *Parasitological Research;* 112:2177-2185. https://doi.org/10.1007/s00436-013-3377-4