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Ultrastructural studies of some character of Diptera (*Muscidae*) of forensically importance

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ABSTRACT

Insects are important in the decomposition of cadavers. In the field of forensic entomology, the taxonomic identification is essential to proceed to any procedure. The use of these insects in medico criminal investigation is the object of forensic entomological studies; the flies are generally attracted to cadavers and one of the most important contributions is to estimate the postmortem interval. The scanning electron microscopy, which allows rapid and accurate identification of character could be used to help identify different species of forensics flies, has been highlighted as it allows better visualization of the external morphology of immature and some adults. The aim of this study was to describe the katepisternals in females of *Morellia humeralis* and *Biopyrellia bipuncta*; the ommatidia of the compounds eyes of the male of *B. bipuncta*; the antennae of females of *Ophyra aenescens* and *Ophyra albuquerquei* and the ocellar triangle of the last two species and *O. chalcogaster* examined by scanning electron microscopy to help increase the anatomical database on flies for forensic importance. The katepisternals of *M. humeralis* and *B. bipuncta* were densely covered by different groups of sensilla. The surface of the ocellar region of *O. aenescens* was not covered by sensilla, but in *O. albuquerquei and O. chalcogaster* were densely covered by different types of sensilla. The coeloconic sensilla were only found in the flagellum of *O. albuquerquei*.

1. Introduction

Forensic entomology is a tool used by justice system which makes use of insects as criminal evidence, not only in estimating the *post mortem* interval, but also in cases in which displacement of the body, neglect of children, elderly or disabled; use of chemicals and the route of narcotics have been noticed (Benecke, 2001; Benecke and Lessig, 2001; Benecke et al., 2004; Introna et al., 1998; Okiwelu et al., 2008).

Groups of insects which show great potential for forensic information belong to the Diptera order, especially *Calliphoridae*, *Muscidae* and *Sarcophagidae* families (Archer, 2003). These families are mostly associated with decaying meat or corpses (Carvalho and Mello-Patiu, 2009). The *Muscidae* is a large cosmopolitan family, and some species are considered associated to human habitats in Brazil (Carvalho et al., 2002).

Some species of *Biopyrellia* Townsend, 1932 and *Morellia* Robineau-Desvoidy, 1830 adults showed a strong attraction to human faeces and are considered potential vectors of pathogens to humans (Uribe-M et al., 2010; Carvalho et al., 2005).

Ophyra Robineau-Desvody, 1830 adults can be floricultural or be attracted by substances in process of fermentation, decomposition and blood or wounds (Pamplona and Couri, 1989). The *Ophyra* species is a fly of medical and forensic importance in many parts of the world (Sukontason et al., 2007). The larvae were observed in human cadavers, carcasses of animals and others (Pamplona and Couri, 1989; Barbosa et al., 2009).

The ultrastructure in adults flies have been the subject of many researchers, such as Stoffolano et al. (1988) who described, by using SEM, the abdominal plaques of both sexes of *Tabanus nigrovittatus* Macquart, 1847, *Apiocera barri* Cazier, 1982, *Hydrophorus viridiflos* (Walker, 1852) and *Dolichopus consanguineous* (Wheeler, 1899), revealing the presence of small pits, and the authors suggested that these cuticular plaques have a sensory function; Angioy et al. (1999)

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confirmed in their ultrastructure study the different functional roles of the abdominal heart and the aorta of *Protophormia terraenovae* Robineau-Desvoidy, 1830; Sukontason et al. (2004) studied the antennal sensilla of some forensically important flies of the families *Calliphoridae*, *Sarcophagidae* and *Muscidae*, and they observed that the sensilla types were morphologically similar and suggested that each type of sensilla had a function.

Sukontason et al. (2006) analyzed the ultrastructure of the adhesive device in flies of the following families: *Calliphoridae*, *Muscidae* and *Sarcophagidae*, and the results provided anatomical information about the successful attachment of theses flies to smooth surfaces as well as the role of these ultrastructure as a mechanical carrier of microorganisms; Sukontason et al. (2007) provided an extensive description of the sensilla on the antenna and palp of a fly specie of medical and forensic importance - *Ophyra chalcogaster* (Wiedemann, 1824), by using SEM to observe many structures which could not be clearly studied by making use of light microscopy: the chaetic sensilla, trichoidea sensilla and microtrichia, for instance. Zhang et al. (2013a) studied aspects of sensilla on the antenna and maxillary palps of *Lispe neimongola* Tian et Ma, 2000 and observed that the antennal scape had only chaetic sensilla, the pedicel had two subtypes and the funiculus had three types of sensilla (trichoidea, basiconic and chaetic sensilla).

The structures selected in this study were those used in dichotomous keys which offered diagnostic characters, observable through light microscopy. The scanning electron microscopy was used in order to identify ultrastructure that might be responsible for the state of the characters.

The purpose of our paper is to describe the ultrastructure of five flies belonging to the *Muscidae* family: first the katepisternal and humeral callus in adult females of *Morellia humeralis* (Stein, 1918) and the katepisternal *Biopyrellia bipuncta* (Wiedemann, 1830); second the male ommatidia the compounds eyes of *B. bipuncta*; third the antennae of females of *Ophyra aenescens* (Wiedemann, 1830) and *Ophyra albuquerquei* Lopes, 1985 and fourth the ocellar triangle of the last two species and *O. chalcogaster* examined by scanning electron microscopy to help increase the anatomical database on flies for forensic importance.

2. Material and methods

The flies *M. humeralis, B. bipuncta, O. aenescens*, *O. albuquerquei* and *O. chalcogaster* were obtained from the Forensic Entomology Project regularly performed by our group. Two domestic pigs carcasses were used as a bait (*Sus scrofa* L.) and the collections were performed in January 2012, which were put in the West side of Rio de Janeiro, Parque Estadual da Pedra Branca—PEPB (Pedra Branca State Park), (23°52′ and 23°04′S and 43°23′ and 43°32′W), Brazil. The authorization for scientific research INEA (Instituto Estadual do Ambiente—a State Environment Institute) is 043/2011. The traps model and the field protocols were the same as those proposed by Salviano (1996). The adults were daily collected, between 09 am and 2 pm and transported to Fundação Oswaldo Cruz—Fiocruz–Oswaldo Cruz Foundation). In the laboratory, the collector tube was placed in a freezer at −17 °C for 40 min. After freezing, the desired insects were transferred to test tubes. Ten specimens were placed on metallic supports, coated with thin layer gold (20–30 nm) and examined under JEOL 6390LV scanning electron microscope (SEM) (Akishima, Tokyo, Japan). It was not necessary any kind of fixing or drying, because of the natural chitin coverage of the specimens. The terminologies of adults flies used in this study followed McAlpine (1981), Carvalho and Couri (2002) and the classification of sensilla followed that of Setzu et al. (2011) and Zhang et al. (2013a,b).

3. Results

3.1. Morellia humeralis

The specimens used were adults female. The humeral callus with four setae of similar shape, but which different size; the surface is covered by small setae (Fig. 1A). The analysis of katepisternals shows in the lateral view, a total of two larger setae in opposite sides, both have similarities in shape; one shorter setae with the same shape was noticed, located above the other two and the surface of the katepisternals is densely covered by two types of different groups of sensilla (Fig. 1B and C).

3.2. Biopyrellia bipuncta

The katepisternals in adults females, shows in the lateral view, a larger setae, and the surface of the katepisternals shave one type of scattered sensilla (Fig. 2A and B). The male of this specie shows the anterointernal ommatidia very enlarged and the lower third one with reduced ommatidia (Fig. 2C and D).

3.3. Ophyra aenescens

SEM observations of the ocellar triangle in females revealed that this is large and reaches the frontal structure with the end of the rounded tip (Fig. 3A). The surface of the ocellar region is not covered by sensilla and it has two long bristles, five medium ones and two small ones (Fig. 3B and C).

3.4. Ophyra albuquerquei

The ocellar triangle in the females of *O. albuquerquei* is slender does not reach the frontal one, tuned tip (Fig. 4A). The surface of the ocellar region is covered by sensilla and has two long bristles, nine medium ones and one small one (Fig. 4B and C).

3.5. General shape of the antennae of O. aenescens and O. albuquerquei

The ultrastructure of the head of both flies is equipped with a pair of antennae frontally situated, between the large compound eyes (Figs. 3A and 5A). Antennal morphology of two species consists of three segments: short proximal scape (Sc), pedicel (Pe) and a distal flagellum, the latter is composed of a seta called arista (Ar) located laterally and an enlarged basal funiculus (F) (Fig. 5B and C).

3.5.1. Scape

The Sc of *O. albuquerquei* have two types of sensilla, fourteen small chaetic sensilla (ChI) of similar length which were noticed arranged in a single row and trichoidea sensilla (Tr), were more numerous, densely and variable in length (Fig. 6A). However, the Sc of *O. aenescens* have approximately six chaetic sensilla (ChI), like the first one and arranged in a single row and trichoidea sensilla (Tr) variable in length (Fig. 6B). The cuticular surface of the Sc of both species is covered by small spinules of microtrichia (Mc).

3.5.2. Pedicel

The second antenna segment is called pedicel, in both species, and the surface is covered by short Mc. Besides, the Tr is similar to those found on the scape and it is also more numerous, densely and variable in length in *O. albuquerquei* than *O. aenescens* (Fig. 6B and A). Two morphological types of chaetic sensilla (small ChI and bigger ChII) are distributed on the pedicel, variable in length and shape in *O. aenescens*, and the ChII is similar to those found in the Sc (Fig. 6B). However, in *O. albuquerquei*, only ChI was noticed on the Pc, but more numerous and varying in length (Fig. 6A).