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Ultrastructure of immature stages of *Lucilia cuprina* (Diptera: Calliphoridae) using scanning electron microscopy

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ABSTRACT

The blowfly *Lucilia cuprina* is distributed worldwide and is a mechanical vector of pathogens. It can cause myiasis in humans and is strongly related to forensic entomology, as it is frequently found on human and animal corpses. However, most of the *L. cuprina* found on corpses are the immature stages of this fly. Correct identification is very important for forensic entomology but at present only the identification keys of adult *L. cuprina* are available. Thus, the aim of this paper was to describe and analyze the morphological characteristics of all larval instars and the puparia of *L. cuprina* using scanning electron microscopy (SEM).

1. Introduction

Lucilia cuprina (Wiedemann, 1830) (Diptera: Calliphoridae) is a blowfly that is found worldwide and it displays typical synanthropic behavior, being intimately associated with humans and human habitations (Norris, 1965). This fly acts as a mechanical vector of pathogens, since it is associated with anthropic environments, and is frequently found on carcasses (Ferreira and Lacerda, 1993; Linhares, 1981). It is an insect of great medical and veterinary importance as it can cause myiasis in humans and in animals, principally in sheep. This fly is commonly known as the Australian sheep blowfly and can have an economic impact on the sheep industry (Stevens and Wall, 1996; Zumpt, 1965).

Lucilia cuprina is considered an important forensic specie, since it has been frequently found associated with human and animal corpses according to many authors (Byrd and Castner, 2001; Early and Goff, 1986; Goff, 2000; Greenberg and Kunich, 2002; Smith, 1986). This fly is the dominant specie during the active decomposition stage, and is responsible for the removal of most of the tissue (Early and Goff, 1986). The blowflies (Diptera: Calliphoridae) are the first insects to arrive at a dead body and they readily oviposit on it (Smith, 1986). Therefore, knowledge of the length and activity of the larval stages of these flies can help determine the postmortem interval (Erzinclioglu, 1989).

Most of these insects found on carcasses are the immature forms, and in order to help entomologists estimate the postmortem interval the correct identification of the immature forms is very important. The immature forms collected from corpses are commonly preserved in ethanol for later identification. However, there is a need to rear these larvae until adults in order to identify and analyze all the immature stages.

The calliphorid larvae are very similar and some authors have described the first and third instar of *L. cuprina* using light microscopy focusing on the cephalopharyngeal skeleton, which is

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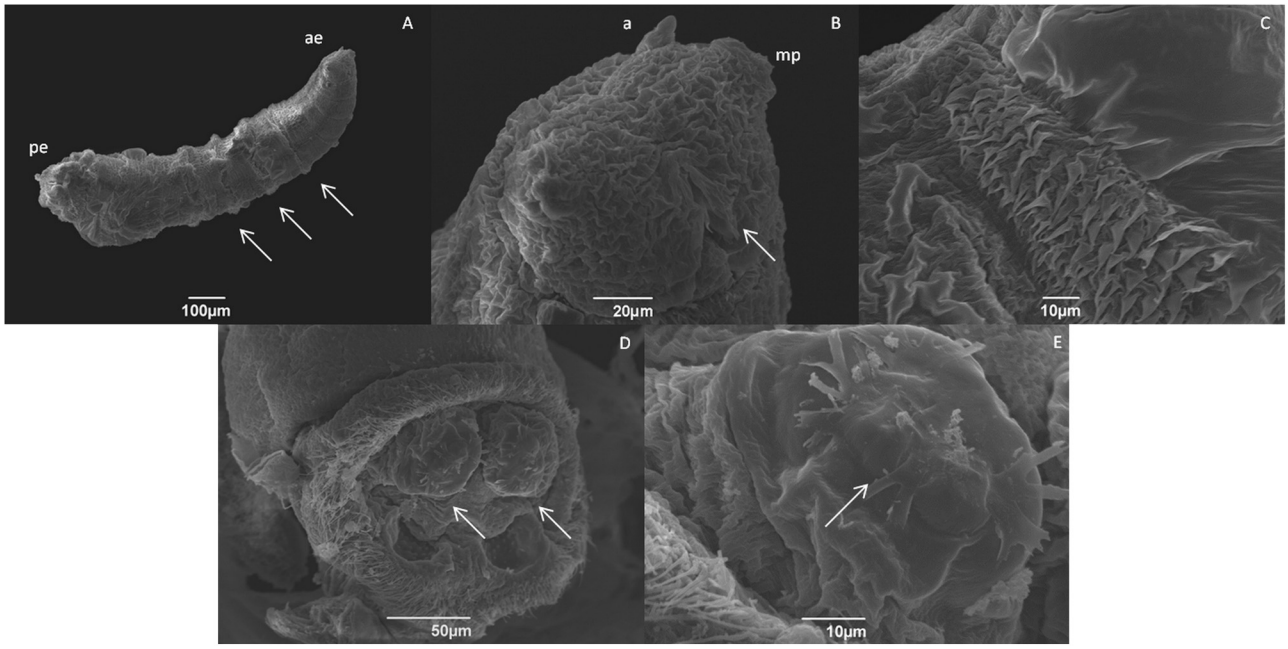

Fig. 1. Scanning electron micrographs of a first instar larva of *Lucilia cuprina* (Diptera: Calliphoridae). (A) Larval body with groups of spines located between the segments (arrows); the anterior (ae) and posterior ends (pe) of the larval body (×110). (B) Cephalic region with antennae (a), maxillary palps (mp) and bucal hooks (arrow) (x900). (C) Detail of spines between the first segment and thoracic region (×1100). (D) Anal segment with anal tubercles and posterior spiracles (arrows) (×500). (E) Posterior spiracles (arrow) (×1900).

the main structure visible using this technique (Sukontason et al., 2010; Szpila et al., 2013). However, light microscopy does not provide details of other characteristics that could have a diagnostic value (Liu and Greenberg, 1989; Mendonça et al., 2010).

Thus, the aim of this study was to describe and analyze the morphological characteristics of all larval instars and puparia of *L. cuprina* using scanning electron microscopy (SEM) in order to elucidate new diagnostic features of this specie.

2. Material and methods

The immature samples of *L. cuprina* used in this study were collected in Macapá, Amapá State, Brazil, using pig carcasses as bait, according to Barbosa et al. (2009). The colonies were reared and maintained at the Setor de Entomologia Médica e Forense, which is part of the Laboratório de Transmissores de Leishmanioses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, in the state of Rio de Janeiro, as previously described (Queiroz and Milward-de-Azevedo, 1991). Insects were kept in cages at room temperature and supplied ad libitum with water and sugar. Bovine meat was used as a source of protein to stimulate oviposition and as food for the immature larvae.

The third generation of the laboratory colony was used in this study. Ten specimens of each immature stage were analyzed. The terminology used in describing the morphology in this paper followed Margaritis (1985) and Mc Alpine et al. (1981).

The three larval instar forms were washed several times in distilled water. Larvae were killed by placing them in hot water for 5 min and then they were fixed in 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer, pH 7.2, for 1 h and postfixed in 1% osmium tetroxide in the same buffer for 1 h at room temperature in the dark. Then these immature forms were washed with sodium cacodylate buffer three times for 10 min each. Afterward, they were dehydrated in an ascending series of acetone up to 100% and submitted to the critical point drying method, using superdry CO_2 in a Balzers apparatus (Hayat, 1970). Specimens were placed on metallic supports, coated with a thin layer of gold (20–30 nm) and examined under a Jeol JSM 6390LV scanning electron microscope (Akishima, Tokyo, Japan). The SEM images were transferred directly to a computer.

Puparia were not submitted to any kind of chemical fixation. Puparia were put under refrigeration for 5 min and then they were placed onto double-stick tape on metallic supports, coated with gold and examined under the same SEM.

3. Results

3.1. First larval instar

The larval body of *L. cuprina* is composed of 12 segments (one cephalic; three thoracic and eight abdominal) and has the vermiform shape typically found in muscoids (Fig. 1A). The anterior end, comprising the cephalic region, is pointed and the posterior end is blunt. The larval body is 3.76 ± 0.27 mm long and 0.199 ± 0.037 mm wide.

The cephalic region is slightly bilobed and bears the sensorial structures: a pair of antennae and a pair of maxillary palps. Because of its localization, antennae are also known as dorsal organs and maxillary palps are known as terminal organs. The maxillary palps are formed by a complex of sensitive papillae. This cephalic region also includes the buccal hooks and oral cristae, but these structures are not well developed in the first instar (Fig. 1B).

There is a band of spines between the cephalic region and the first thoracic segment. Two types of spines were observed: one type is flattened with tapered ends and the other is narrower with tapered tips, all of them are projected backward (Fig. 1C). The body tegument is smooth with the intersegmental line similar to those of the first thoracic segment. In this larval instar, the anterior spiracle is not visible.

The posterior end or anal segment is covered with filiform spines and only the anal tubercles can be seen easily; the other tubercles are too small to be seen. The posterior spiracle is located at the top of an elevation and the single spiracular or peritrema opening is sustained by spiracular muscles (Fig. 1D and E).