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Scanning electron microscopy of eggs of *Haemagogus leucocelaenus* (Diptera: Culicidae)

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ABSTRACT

Objective

To observe morphological details of the eggs of *Haemagogus* (*Conopostegus*) *leucocelaenus*, seen for the first time by scanning electron microscopy (SEM), with morphometric analysis of the main structures.

Methods

Eggs of *Hg. leucocelaenus* were obtained from females captured in the Biological Reserve of Tinguá, State of Rio de Janeiro. Some of the eggs were kept for hatching and others underwent processing for scanning electron microscopy studies. Three eggs were submitted to morphometric analysis. The material was fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, both in 0.1M, pH

7.2 sodium cacodylate buffer, then dehydrated in ethanol and dried using the critical point method. This was then set up on metallic supports, covered with gold and observed using the Jeol 5310 scanning electron microscope. Measurements were made with the aid of the Semafore analysis software coupled to the electron microscope.

Results

The eggs presented elliptical outlines of approximately 574 μm in length and 169 μm in width, with an egg index (*l/w ratio*) of 3.39 μm. The exochorion was extremely regular and had ornamentation that was usually hexagonal but sometimes pentagonal. Tubercles were observed on the chorionic cells, symmetrically arranged in relation to the longitudinal axis. Inside the cells, there were smaller, individualized tubercles, some arranged peripherally and others grouped to a greater or lesser extent in the center. The surface of the chorionic reticulum did not present rugosity. The micropylar apparatus was formed by a prominent and continuous collar of 8.32 μm in thickness, with a slightly irregular surface. The micropylar disk was very evident, and was continuous with the collar. The micropyle was seen at the center of this disk, measuring 1.6 μm and with a micropylar apparatus of 27.3 μm in diameter.

Conclusions

The ornamentation of the exochorion presents differences in relation to the tubercles of chorionic cells and the external chorionic reticulum between the eggs of *Hg. Leucocelaenus*, in comparison with the eggs of *Hg. janthinomys* and *Hg. equinus*, and also in relation to those of *Aedes aegypti*, *Ae. albopictus* and *Ae bahamensis*. In various aspects, the eggs of *Hg. leucocelaenus* have more resemblance to those of *Hg. Equinus* than those of *Hg*. *janthinomys*, with greater differences presented in relation to the eggs of *Hg. spegazzinii* and *Hg. lucifer.*

Keywords

Diptera, ultrastructure. Culicidae, ultrastructure. Eggs. Microscopy, electron, scanning. *Haemagogus*.

INTRODUCTION

The Haemagogus genus presents large specific diversity and consists of 32 species (Arnell,[1] 1973). Many of them are extremely important from an epidemiological point of view because of their involvement in the transmission of the forest yellow fever virus and other arboviruses, thereby acting in the maintenance of the natural cycle of these zoonoses. The forest yellow fever transmitters at present known in Brazil are exclusively mosquitoes and the vertebrate hosts, which are mainly primates, including man (Degallier et al,[5] 1992).

Haemagogus (Conopostegus) leucocelaenus is an essentially forest species whose preferred habitat is the crown of trees. It is active during the daytime (Chadee et al,[3] 1995; Forattini & Gomes,[6] 1988). This mosquito is common in Brazil and its epidemiological importance is in relation to its role in the transmission of arboviruses, among which yellow fever (Kumm & Cerqueira,[12] 1961). *Haemagogus*

(Conopostegus) leucocelaenus was recently implicated as a primary vector for forest yellow fever in southeastern Brazil.* The arboviruses Wyeomyia, Ilhéus, Maguari, Tucunduba and Una have also been isolated from this species (Karabatsos,[11] 1985; Hervé et al,[8] 1986). In the laboratory, it has been shown to be a more efficient vector for the yellow fever virus than *Aedes aegypti* (Waddell,[15] 1949). *Hg. leucocelaenus* has recently received special attention due to its increasing medical importance (Forattini & Gomes,[6] 1988). Its geographical distribution extends from Trinidad to southern Brazil and northern Argentina. In Brazil, it occurs mainly in the states of the southern, southeastern and western-central regions (Consoli & Lourenço-de-Oliveira,[4] 1988).

***Personal communication from Professor Pedro Vasconcellos, Arbovirus Laboratory of Instituto Evandro Chagas.**

The first study on mosquito eggs using scanning electron microscopy was made by Matsuo & Kunou[13] (1972). Studies have only been made on the eggs of four species of *Haemagogus*: *Hg. spegazzinii* Brèthes, *Hg. lucifer* (Howard, Dyar & Knab) (Mattingly,[14] 1973), *Hg. equinus* and *Hg. janthinomys* (Linley & Chadee,[10] 1991). The present study had the objective of observing the eggs of *Hg. leucocelaenus* using scanning electron microscopy (SEM) and performing morphometric analysis of the main structures.

METHODS

Eggs of *Hg. leucocelaenus* were obtained from females captured in the Biological Reserve of Tinguá, in the municipality of Nova Iguaçu, State of Rio de Janeiro, at the latitude of S 22º28 - 22º39' and longitude of W 43º13' - 43º34'. These were captured when already naturally engorged, using a manual mouth aspirator and taken to the laboratory on the same day. Only females in perfect condition were utilized. They were individually isolated in flat-bottomed glass tubes measuring 25 mm in diameter and 50 mm in height that, at the bottom, contained a piece of dampened cotton wool covered with filter paper. This had the function of serving as a substrate for egg-laying (Bates & Roca-Garcia,[2] 1945). Around 10 females were utilized, and 20 eggs were obtained. Three of these were submitted to morphometric analysis.

Immediately after being laid, the eggs were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, both in a 0.1M, pH 7.2 sodium cacodylate buffer. After washing in the same buffer, the eggs were dehydrated in a series of increasing ethanol concentrations and submitted to the critical point drying method, using superdry CO_2 in Balzer's apparatus. Following this, the material was set up on metallic supports, covered with gold and observed using the Jeol 5310 scanning electron microscope.

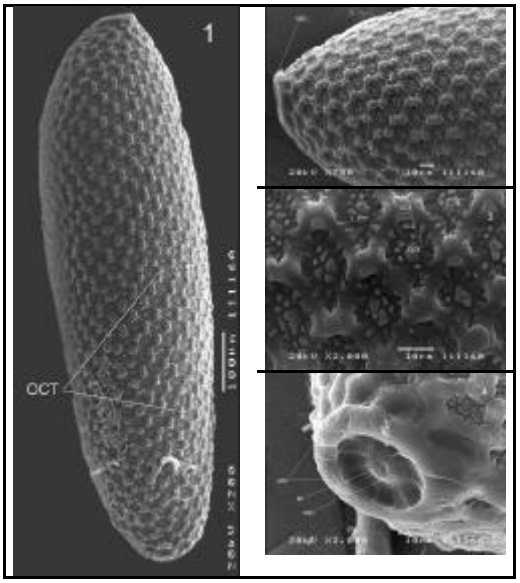
The measurements were made directly from the images obtained, with the aid of the Semafore analysis software coupled to the microscope. The following parameters were utilized: total length, total width, thickness of the micropylar collar and size of the micropyle. Maximums, minimums, averages and standard deviations are cited in the Table.

The terminology utilized for the description of the eggs follows Harbach & Knight[7] (1980).

RESULTS

The eggs had a deep black color and were laid separately, with firm adherence to the substrate. They presented an elliptical outline of approximately 574 µm in length and 169 µm in width at their central region (Figure, part 1). At the extremities, the anterior region measured 50 µm at the level of the

micropyle and 61.9 μm in the posterior region. The egg index, or in other words the ratio between length and width, was calculated by using the width of the central region, thus obtaining 3.39 μm.



Figures - ***Part 1*** - *Haemagogus leucocelaenus*: whole egg. Scale 100 μm. OCT – External chorionic tubercle. ***Part 2*** - Anterior region of the egg, micropyle and exochorion. Scale 20 μm. MiC – Collar of the micropyle. ***Part 3*** - Structures of the chorionic cells. Scale 20 μm. OCR – External chorionic reticulum. OCC – External chorionic cell. OCT – External chorionic tubercle. ***Part 4*** - Detail of the micropylar apparatus. Scale 10 μm. MiC – Collar of the micropyle. Mi – Micropyle. MiD – Disk of the micropyle.

The external coating of the eggs presented an extremely regular exochorion. The majority of the exochorion cells had ornamentation with a hexagonal appearance, but sometimes this was pentagonal (Figure, part 2).

On the margins of these chorionic cells, tubercles measuring 1.49 to 6.32 μm in diameter were observed, symmetrically arranged in relation to the longitudinal axis. Inside them, there were smaller, individualized tubercles, some arranged peripherally and others grouped to a greater or lesser extent in the center (Figure, part 3).

The tubercle density was 18 to 25 (21.1±2.08; n=10) per cell in the anterior ventral region. The tubercles had a smooth appearance, without any type of nodule on their surface. The surface of the chorionic reticulum did not present rugosity. The micropylar apparatus was observed in the anterior region of the egg. It was formed by a prominent and continuous collar of around 8.32 μm in thickness, with a slightly irregular surface (Figure, part 4).

In its center, a very evident micropylar disk was observed, measuring around 7.29 μm in diameter. It was continuous with the collar, in the form of membranous creases arranged uniformly in a helical manner. A very evident orifice, the micropyle, was viewed at the center of this disk, measuring 1.6 μm. The total external diameter of the micropylar apparatus was 27.3 μm (Figure, part 4).

DISCUSSION

The ornamentation of the exochorion is an excellent parameter for making comparisons between species and reveals significant differences, especially in relation to the presence of tubercles in the chorionic cells. The eggs of *Hg. janthinomys* and *Hg. equinus* examined by Linley & Chadee[10] (1991) were differentiated by the shapes of the cells and distribution of these tubercles. In *Hg. janthinomys*, these authors observed chorionic cells with a hexagonal appearance and sometimes slightly ovaloid. In *Hg. Equinus*, these cells usually had a hexagonal appearance, but sometimes were pentagonal, which was also observed in the present study, in *Hg. leucocelaenus* (Figure, part 2). *Hg. equinus* presents small chorionic tubercles scattered uniformly in the center and periphery of the cell, while in *Hg. janthinomys* they are arranged at the center of the cell. No similarity was found in the distribution of these tubercles in *Hg. leucocelaenus*. In *Hg. Equinus*, many of the tubercles present fused appearance, while others are clearly scattered and differ from *Hg. leucocelaenus* by presenting small nodules on their surfaces. The external chorionic reticulum observed in *Hg. leucocelaenus* has a smooth appearance, thus differing from what was observed by Linley & Chadee[10] (1991) in *Hg. janthinomys* and *Hg. equinus*. Linley[9] (1989) observed that the external chorionic reticulum in *Aedes aegypti*, *Ae. albopictus* and *Ae. bahamensis* also presented rugosity, with a fine reticular mesh. The same author reported that, in these species, there was only one central tubercle inside the chorionic cells, thus diverging from observations made regarding *Haemagogus*.

In *Hg. Spegazzinii*, Mattingly[14] (1973) did not view the micropyle in their observations under the optical microscope. Differing greatly from the eggs of *Hg. spegazzinii* and *Hg. lucifer*, which do not present a circular apical collar, the eggs of *Hg. leucocelaenus* resemble more those of *Hg. equinus* than those of *Hg. janthinomys.* The micropyle is also smaller than in those two species. The micropylar disk, located at the center of the micropylar apparatus, differs from what was observed in eggs of *Hg. janthinomys* (Linley & Chadee,[10] 1991), since in the latter there was no continuity of the disk with the collar.

Linley & Chadee[10] (1991) reported that, in *Hg. janthinomys* and *Hg. Equinus*, the dorsal surface of the egg was found to have adhered to the substrate by means of filamentous tubercles. These authors pointed out that these structures would have the possible function of making the egg more secure, thereby impeding physical removal by predators, as well as allowing them to float in rainwater. Such filaments were not observed in *Hg. leucocelaenus*.

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