CHAPTER 21

SCANNING ELECTRON MICROSCOPY OF THE EGG OF *HAEMAGOGUS TROPICALIS*

Acceptance date: 01/03/2024

Jeronimo Alencar
Department of Entomology, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Nicolas Dégallier
Institut de Recherche pour le Développement, Place Jussieu, Paris, France

Anthony Érico Guimarães
Department of Entomology, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Janira Martins Costa
Postgraduate Program in Zoology, Department of Entomology, National Museum, Federal University of Rio de Janeiro, Quinta da Boa Vista, São Cristóvão, Rio de Janeiro, RJ, Brazil

William de Almeida Marques
Department of Entomology, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Vanderlei C. Silva
Department of Entomology, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Jacenir Reis dos Santos-Mallet
Department of Entomology, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

ABSTRACT: *Haemagogus tropicalis* is strictly a forest-dwelling species from the fertile valley area of the Amazônia forest. It is a diurnal mosquito, and the oviposition sites for the species include tree holes. The eggs of *Hg. tropicalis* used in this study were from females captured on Combú Island, situated across from the city of Belém, Guajará Bay, state of Pará, at 1°25'S latitude and 48°25'W longitude. The eggs are elliptical and ~575 μm long with a width of ~144 μm. The ventral surface of the chorionic reticulum has regular chorionic cells with hexagonal and sometimes pentagonal ornamentation. Each chorionic cell has a thick external chorionic reticulum with regular borders. The interior of the chorionic cells have small, evenly distributed tubercles, and the dorsal external chorionic reticulum appears porous. The micropylar apparatus, located on the anterior area of the egg, was formed by a collar with a well-developed frame. Centrally, the micropylar disc had a

diameter of ~20 μm and the micropylar orifice is 2.1 mm in diameter. These data may enable construction of taxonomic keys for identifying eggs of *Haemagogus* species.
KEYWORDS: *Haemagogus tropicalis*, mosquitoes, eggs, morphology, ultrastructure, scanning electron microscopy

INTRODUCTION

Haemagogus tropicalis Cerqueira and Antunes, 1938, is a species that occurs in wild habitats and the flood-prone river margin areas ("varzea") in the Amazon forest. It is acrodendrophilic and diurnal, and tree holes serve as the larval habitats. The species is endemic to the complex of the islands of Marajo´ (Curralinho) and Combú, State of Pará in Brazil. There have been new records of the occurrence of this species in flood-prone river margin areas of the municipality of Abaetetuba, State of Pará. Alencar et al. (2003, 2005b) described and illustrated 2 neotropical species of *Haemagogus* on the basis of reticulum and outline patterns using scanning electron microscopy.

The morphological characteristics of the eggs are still poorly or incompletely known (Forattini 2002). This has stimulated the scientific commu- nity to discover and describe culicid eggs. Only ,16% of the eggs of Aedinii species have been described morphologically. However, this stage of development has received little attention for species identification (Reinert 2005). However, it is possible to make more detailed descriptions of the ornamentation of the exocorium of culicid eggs with scanning electron microscopy, and this may provide species diagnostic characters. The present study is a morphometric analysis and description of the eggs of *Hg. tropicalis* by means of scanning electron microscopy.

MATERIALS AND METHODS

The *Hg. tropicalis* eggs used in this study came from females captured on the island of Combú located in the bay of Guajará, opposite the city of Belém, in the state of Pará, 1º 25' S and 48º 25' W. The island of Combú has dry-land vegetation consisting predominantly of forest, although in certain areas there are natural pastures found between lakes and sandbanks or even on the margins of some rivers. These areas are periodically flooded by tidal overflow from the rivers, causing interactions between the aquatic and dry- land ecosystems (Sioli 1985). The climate according to the Köppen classification is Amazonian. The rainfall data show that the mean annual precipitation is ~2,500 μm, with a mean annual temperature of 32ºC.

Engorged females were captured using an oral suction tube (Castro trap) and transported to the laboratory on the same day. Only females in perfect condition were used, and these were isolated individually in flat-bottomed glass tubes 25 mm in diameter and 50 mm in height. At the bottom of the tube was a piece of cotton wool moistened with water and covered with filter paper, which served as a substrate for oviposition (Bates and Roca-

Garcia 1945). In total, 25 females were caught and 36 eggs were obtained. Eleven of the eggs were used for morphometric analysis. The species was confirmed using males and the dichotomous key of Arnell (1973).

Immediately after oviposition, eggs were removed from the filter paper using a fine brush.

They were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, both in 0.1 M sodium cacodylate buffer at pH 7.2. After washing in the same buffer, the eggs were dehydrated in a series of increasing ethanol concentrations and subjected to the critical-point drying method using superdry CO_2 in Balzer's apparatus. Next, they were mounted on gold-plated metal supports and observed under a Jeol 5310 scanning electron microscope (Akishima, Tokyo, Japan). The dorsal and ventral sides of eggs were photomicrographed at magnifications of 200–5,000X to view the exocorium and micropyle. Measurements were made directly on the images obtained with the aid of the Semafore analysis software (JEOL, Sollentuna, Sweden) coupled to the microscope. Total length, total width, micropyle collar thickness, and chorionic cell diameter and circumference were measured, but only the means are reported here. The terminology of Harbach and Knight (1980) is used to describe the eggs.

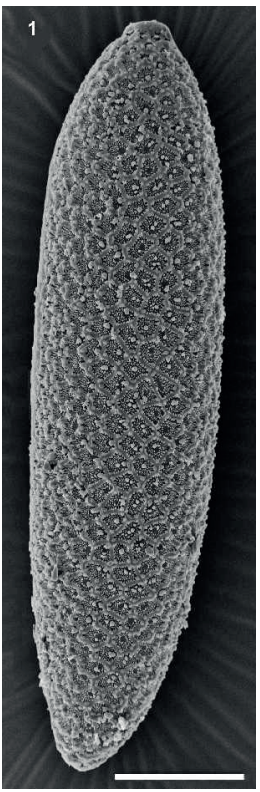

Fig. 1. Egg of *Hg. tropicalis*. Ventral (top) view, anterior end at top, showing micropylar collar. Scale, 100 mm.

RESULTS

The eggs are black and elliptical in outline (Fig. 1), with a length of ~575 mm and a width of 144 mm at the extremities. The anterior region presented micropyle height of ~42 mm, and the posterior region, ~38 μm. The egg index (l/w ratio) was 4.0. The anterior extremity tapered abruptly from the width of 140 mm. Tapering was more gradual at the posterior end from the width of 136 mm. The ventral surface (upper surface in the natural position) of the chorionic coating had regular chorionic cells with hexagonal and sometimes pentagonal ornamentation (Fig. 2). Each chorionic cell presented a thick raised external chorionic reticulum with regular edges (Fig. 3), with a longitudinal diameter of 15.3 ± 2.0 μm (n = 16) and a circumference of 180 μm.

Inside the chorionic cells (ventral) tubercles of various types with different shapes and diameters were viewed. Small individualized tubercles of rod-like appearance were found distributed along the whole extent of the cells. Some of them were bigger on the periphery, and the heights ranged from 0.6 to 277.0 μm, with a density of 60.5 per cell (Fig. 4). Others had a rounded shape and were grouped at the margins of most of the cells, following along the cell limits but distributed irregularly. In the central area, 1 or 2 more developed tubercles had diameters ranging from 1.95 to 4.10 μm. These were present in most of the cells and occurred in a very regular pattern (Fig. 5). In the dorsal region of the egg, no fused filaments were observed in the chorionic cells. In this region, the external chorionic reticulum had a porous appearance and its thickness ranged from 2.5 to 4.1 μm at the anterior extremity close to the micropyle and in the more medial area. The isolated tubercles varied widely in each cell (Fig. 6). In the central region of some chorionic cells, there were tubercles of greater diameter, which we call central tubercles. The micropyle, in the anterior region of the egg, had a collar with very evident molding and edges, albeit irregular, with defined margins for the transition area and a thickness of ~10 μm. The micropyle disc has raised margins and a diameter of ~20 μm. The micropyle orifice was very evident, with a diameter of ~2.0 μm (Fig. 6).

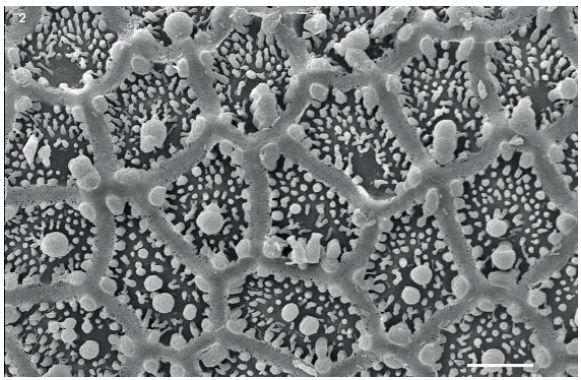

Fig. 2. Typical ornamentation of the outer chorionic reticulum showing 2 types of tubercles. Scale, 10 μm.

DISCUSSION

The eggs of 5 species of the genus *Haemagogus* have been described with scanning electron microscopy. *Haemagogus capricornii* Lutz, Alencar et al. (2005b); *Haemagogus celeste* Dyar and Nunez Tovar, Chadee and Bennet (1990); *Haemagogus equinus* Theobald, Chadee and Bennet (1990), Linley and Chadee (1991); *Haemagogus janthinomys* Dyar, Linley and Chadee (1991); and *Haemagogus leucocelaenus* (Dyar and Shannon), Alencar et al. (2003). The exocorium ornamenta- tion of these eggs can be used to identify species (Table 1).

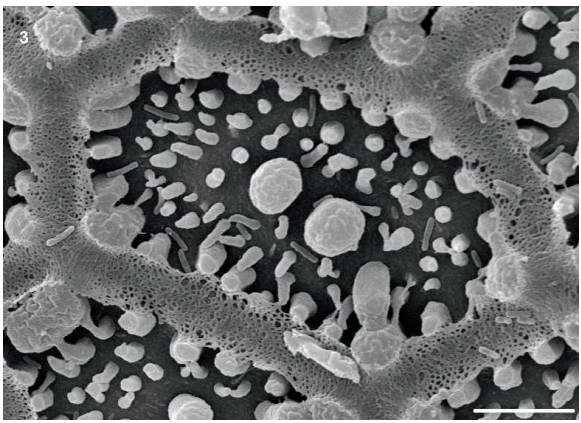

Fig. 3. Detail of the central region of chorionic cell showing round tubercles. Scale, 5 μm.

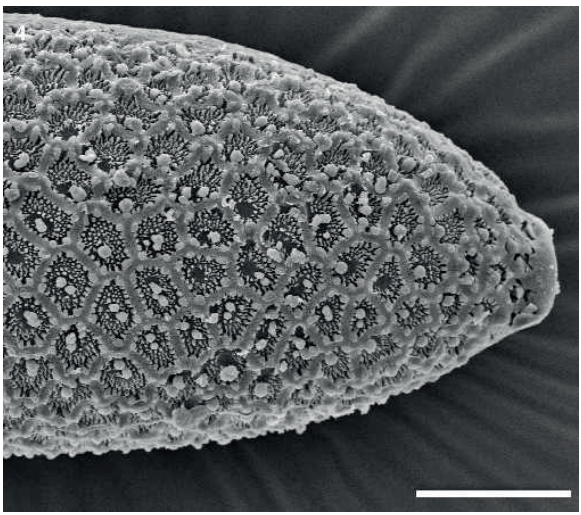

Fig. 4. Ventral region showing tubercles of various types with different shapes and the micropylar collar.

Linley and Chadee (1991) and Alencar et al. (2005b), in studies on the eggs of *Hg. equinus, Hg. janthinomys,* and *Hg. capricornii,* observed filaments on the dorsal surface of eggs. These structures may maintain the adhesion of the egg and protect it against predatory insects. Such structures on the dorsal surface were not seen on the egg of *Hg. tropicalis.* Alencar et al. (2003) showed that *Hg. leucocelaenus* (subgenus *Conospostegus*) had no rugosity on the chorionic reticulum. This structure was extremely rugose in *Hg. tropicalis*.

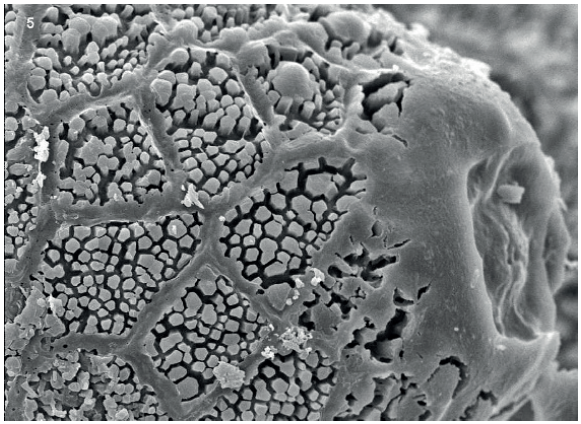

Fig. 5. Micropylar apparatus, located in the anterior area of the egg (dorsal view) formed by a collar with a defined frame. Scale, 10 μm.

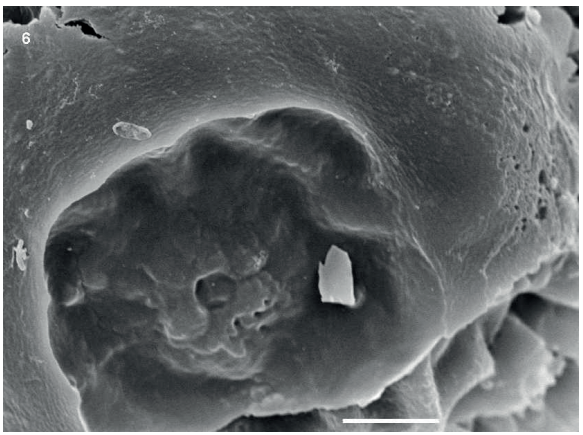

Fig. 6. The micropylar disk centrally located. Scale, 5 μm.

Matsuo et al. (1974) observed that the chorionic cells of *Aedes aegypti* L. and *Aedes pseudoalbopictus* Borel had a large papilla in the central area and small tubercles on the periphery. In the observation by Alencar et al. (2005a) on *Ochlerotatus terrens,* Walker, the chorionic cells had elongated tubercles with a very regular pattern in the central region. They were bigger on the periphery and were sometimes fused into groups at the vertices. In *Hg. tropicalis* this pattern was not observed. There were always 1 or 2 tubercles of greater diameter in the central area, surrounded by smaller tubercles on the periphery. This characteristic differentiates this species from the others in the genus *Haemagogus*.

The micropyle collar observed in *Hg. janthinomys* (Linley and Chadee 1991) was prominent and continuous, with a defined micropyle disk that was similar to what was observed by Alencar et al. (2005b) in *Hg. capricornii*. The layout of this assemblage differed in *Hg. tropicalis,* in which although the micropyle collar was evident, it did not have a transition area with a well-defined chorionic reticulum but, rather, it was completely irregular.

Using the information collected in this paper, scanning electron microscopy may facilitate identification of species in the *Haemagogus* complex (Forattini 2002).

Table 1. Comparative dimension of eggs of *Haemagogus* species.

Species	Length (mm)		Width (mm)		L/W ratio		Fusioned tubercles	Disposition of internal tubercles	Micropylar collar	Width of micropylar disc (mm)	Micropylar orifice
	x ¯ 6SE	Range	x ¯ 6SE	Range	x ¯ 6SE	Range					
Haemagogus equinus	627.5 ± 13.3	561.2-700.8	183.7 ± 4.2	165.2-209.8	3.43 ± 0.08	3.04-3.80	Yes	Individualized	Low, with small excavations	NA	2.9
Haemagogus janthinomys	759.4 ± 6.9	730.4-794.2	207.5 ± 4.7	179.7-226.1	3.68 ± 0.09	3.37-4.19	Yes	Periphery fused	Prominent and continuous	13.0	2.9
Haemagogus leucocelaenus	563.0 ± 7.5	551.0-574.0	158.5 ± 5.0	151.0-169.0	3.56 ± 0.13	3.26-3.8	NA1	No individualized	Prominent and continuous	7.3	1.6
Haemagogus cacricornii	618.29 ± 5.3	615.0-625.0	129.0 ± 4.8	119.0-133.0	4.79 ± 0.17	4.65-5.14	Yes	Irregularly fused in the center	Prominent and continuous	15.0	2.3
Haemagogus tropicalis	580.5 ± 4.09	574.0-585.0	143.0 ± 2.6	139.0-146.0	4.07 ± 0.06	3.98-4.17	NA	Individualized	Prominent and continuous	19.4	2.0

ACKNOWLEDGMENTS

We thank Francisco Correa Castro and Helio Cardoso Saraiva (Evandro Chagas Institute, Belém, Pará) for their help in collecting the females and obtaining the eggs of *Hg. tropicalis,* thanks to their perfect knowledge of the amazonian environment, and to Hertha Meyer Cell Laboratory of the Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, for allowing the use of the scanning electron microscope.

REFERENCES CITED

Alencar J, Guimarães AE, Gil-Santana HR, Santos- Mallet JR. 2005a. Scanning electron microscopy of eggs of *Ochlerotatus* (*Protomacleaya*) *terrens* Walker, 1856 (Diptera: Culicidae). *J Am Mosq Control Assoc* 21:355–359.

Alencar J, Guimarães AE, Mello RP, Lopes CL, Dégallier N, Santos-Mallet JR. 2003. Microscopia eletrônica de varredura de ovos de *Haemagogus leucocelaenus* (Diptera: Culicidae). *Rev Saúde Pública* 37:658–661.

Alencar J, Guimarães AE, Mello RP, Lopes CL, Dégallier N, Santos-Mallet JR. 2005b. A scanning electron microscopy study of the egg of *Haemagogus* (*Haemagogus*) *capricornii* Lutz, 1904 (Diptera: Culicidae). *J Med Entomol* 42:1–6.

Arnell JH. 1973. Mosquito studies (Diptera, Culicidae). XXXII. A revision of the genus *Haemagogus*. *Contrib Am Entomol Inst* 10:1–174.

Bates M, Roca-Garcia M. 1945. Laboratory studies of the Saimiri-Haemagogus cycle of jungle yellow fever. *Am J Trop Med* 25:203–216.

Chadee DD, H. Bennett.. 1990. Scanning electron microscopy of the eggs of *Haemagogus celeste* and *Haemagogus equines* (Diptera: Culicidae) from Trinidad, West Indies. *Mosq Syst* 22:11–14.

Forattini OP. 2002. *Culicidologia Médica*. São Paulo, Brasil: Editora da Universidade de São Paulo.

Harbach RE, Knight KL. 1980. *Taxonomists' glossary of mosquito anatomy*. Marlton, NJ: Plexus Publish- ing.

Linley JR, Chadee DD. 1991. Fine structure of the eggs of *Haemagogus equinus* and *Hg. janthinomys* (Dip- tera: Culicidae). *J Med Entomol* 28:434–445.

Matsuo K, Yoshida Y, Lien JC. 1974. Scanning electron microscopy of mosquitoes II. The egg surface structure of 13 species of *Aedes* from Taiwan. *J Med Entomol* 11:179–188.

Reinert JF. 2005. List of species described in the egg stage of tribe Aedini (Diptera: Culicidae) with their literature citations. *J Am Mosq Control Assoc* 21:252–262.

Sioli H. 1985. Amazônia—Fundamentos da ecologia da maior região de florestas tropicais. Rio de Janeiro, Vozes: Trad J Becker.