

# Scanning Electron Microscopy Study of the Egg of *Haemagogus* (*Haemagogus*) *capricornii* Lutz, 1904 (Diptera: Culicidae)

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**ABSTRACT** Morphological details are provided for the dorsal and ventral surfaces of both extremities and the micropylar area of eggs of *Haemagogus* (*Haemagogus*) *capricornii* Lutz, captured in the Biological Reserve of Tinguá, State of Rio de Janeiro, Brazil. The eggs were observed by scanning electron microscopy with a morphometrical analysis of the main structures. The outer chorionic cells on the ventral surface were extremely regular, such as those observed in *Hg. equinus* and *Hg. janthinomys*. The tubercles present differences in form, size, and distribution. Filaments to attach to the substrate were observed in this species.

**KEY WORDS** Insecta, mosquito, eggs, ultrastructure, scanning electron microscopy

THE GENUS *Haemagogus* presents great specific diversity and includes 28 valid species, all neotropical in distribution except *Haemagogus* (*Haemagogus*) *equinus* Theobald, which reaches some southern points of the nearctic area (Forattini 1965). Some species are of epidemiological importance because of their involvement in the transmission of the sylvatic yellow fever (SYF) virus and other arboreal viruses and are important in the natural cycle of these zoonoses (Dégallier et al. 1992).

*Haemagogus* (*Haemagogus*) *capricornii* Lutz, a species from southern Brazil, is distributed from the south of the state of Bahia to the north of Rio Grande do Sul and reaches the territory of the Missões in Argentina (Forattini 2002). Its habits are mainly acrodendrofilic, according to the region and the season. However, during the rainy season, it can be found at ground level (Forattini and Gomes 1988). It is an efficient SYF vector in Brazil (Waddell and Kumm 1948, Waddell 1949), and according to Arnell (1973), it is possibly the only species linked to the transmission of SYF in south-east Brazil.

A considerable part of the available information about its biology refers to other congeneric species, because it was confused with *Hg. janthinomys* Dyar (Consoli and Lourenço-de-Oliveira 1994) for a long time. Therefore, we are in need of investigations that will clarify questions on biological aspects, geographical distribution, vectorial capacity, and diagnostic morphological characters of both species. *Hg. capricornii* females and fourth-stage larvae are practically

indistinguishable from those of *Hg. janthinomys*, and the two species can be identified only through examination of the male genitalia. Because eggs from few species of this genus have been described, the aim of this study was to observe morphologic details of the eggs of *Hg. capricornii*, with the purpose of completing the characterization of this species.

## Materials and Methods

The eggs of *Hg. capricornii* used in this study were laid by females from the Biological Reserve of Tinguá (BRT), located in the city of Nova Iguaçu, State of Rio de Janeiro (latitude 22°28'–22°39' S; longitude 43°13'–43°34' W). Females already blood fed in nature were captured, using an oral suction tube, and brought to the laboratory on the same day. They were used only if in a perfect state and were isolated individually inside a glass tube with a flat bottom that was 25 mm wide and 50 mm high and contained cotton soaked in tap water and covered with filter paper in the bottom to provide a substrate for oviposition (Bates and Roca-Garcia 1945). Approximately 20 females were captured, from which 30 eggs were obtained; 10 of these were submitted for morphometrical analysis. The specimens were identified by associating with male specimens obtained from the same egg batches and using the key published by Arnell (1973).

Shortly after being laid, the eggs were taken from the filter paper with a brush, fixed in 2.5% glutaraldehyde and post-fixed in osmium tetroxide 1% in 0.1 M sodium cacodylate buffer (pH 7.2). After being washed in the same buffer, the eggs were dehydrated in a series of increasing ethanol concentrations and submitted to the critical point drying method using superdried CO<sub>2</sub> in a Balzers apparatus. They were mounted on metallic stubs, covered with gold, and

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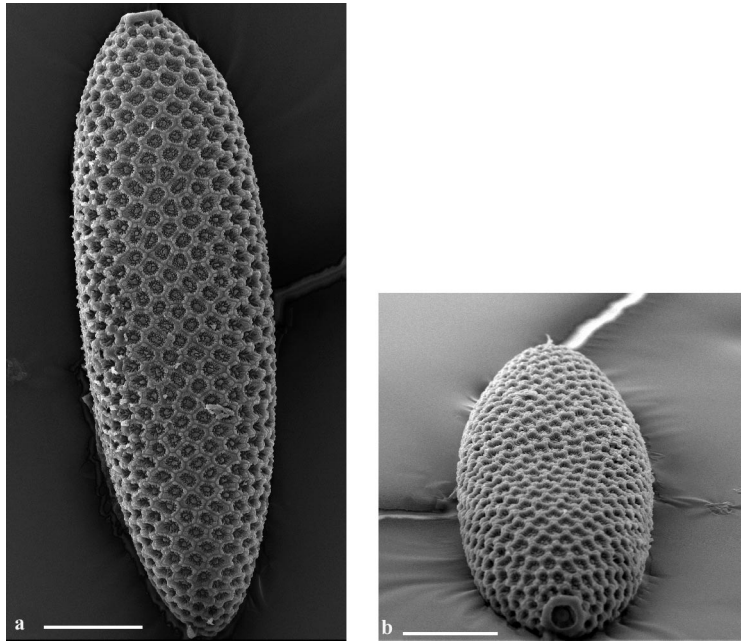


Fig. 1. Egg of *Hg. capricornii*. (a) Ventral (top) view, anterior end at top, showing micropylar collar. Scale, 100  $\mu\text{m}$ . (b) Frontal view showing micropylar apparatus. Scale, 100  $\mu\text{m}$ .

observed through a Jeol 5310 scanning electron microscope (SEM, Jeol Ltd., Tokyo, Japan).

The measurements were carried out directly on the SEM images, with Semafore analysis software, which is coupled to the microscope. The measured parameters were total length, total width, thickness of the micropylar collar, and size of the micropyle. The terminology used for the description of the eggs follows Harbach and Knight (1980).

### Results

The eggs are black, elliptic in contour,  $\approx 622 \mu\text{m}$  in total length, and  $192 \mu\text{m}$  wide in their central region (Fig. 1, a and b). Their total length/width mean ratio in the central area was  $3.24 \mu\text{m}$ . Averages of these measurements are shown in Table 1. The anterior extremity tapers off abruptly at one-fifth of its length, beginning to taper in the posterior extremity at one-third its length.

The ventral surface (dorsal in natural position) of the exochorion shows extremely regular chorionic cells even at very low power, with hexagonal and sometimes pentagonal ornamentations (Fig. 2a). Each chorionic cell has a high outer chorionic reticulum, which are solid with irregular borders (Fig. 2c). They are  $22.5 \pm 1.2$  (SD)  $\mu\text{m}$  ( $n = 10$ ) in their longitudinal diameter and  $44.7 \pm 2.9 \mu\text{m}$  ( $n = 10$ ) in circumference. The chorionic cell has a porous aspect, with elongated tubercles disposed symmetrically in the periphery, and big rounded tubercles, disposed in groups of three in each vertex of the chorionic cells, giving the set a very regular pattern (Fig. 2b). Small tubercles, the surfaces of which are wrinkled are observed inside of

each chorionic cell. Some of them are isolated while others are connected in a greater or lesser extent (Fig. 2c). The isolated tubercles are  $0.8\text{--}2.22 \mu\text{m}$  ( $1.6 \pm 0.36 \mu\text{m}$ ,  $n = 10$ ) in diameter, disposed mainly in the periphery, with a mean density of 11.3/cell. In the central area of each chorionic cell, we found a group of connected tubercles that cannot be characterized as a single central tubercle because their coalition is not total. It was not possible to measure the central tubercles or the small fused groups because of the irregular pattern resulting from that fusion.

In the anterior extremity, the chorionic cells present tubercles that are progressively smaller to-

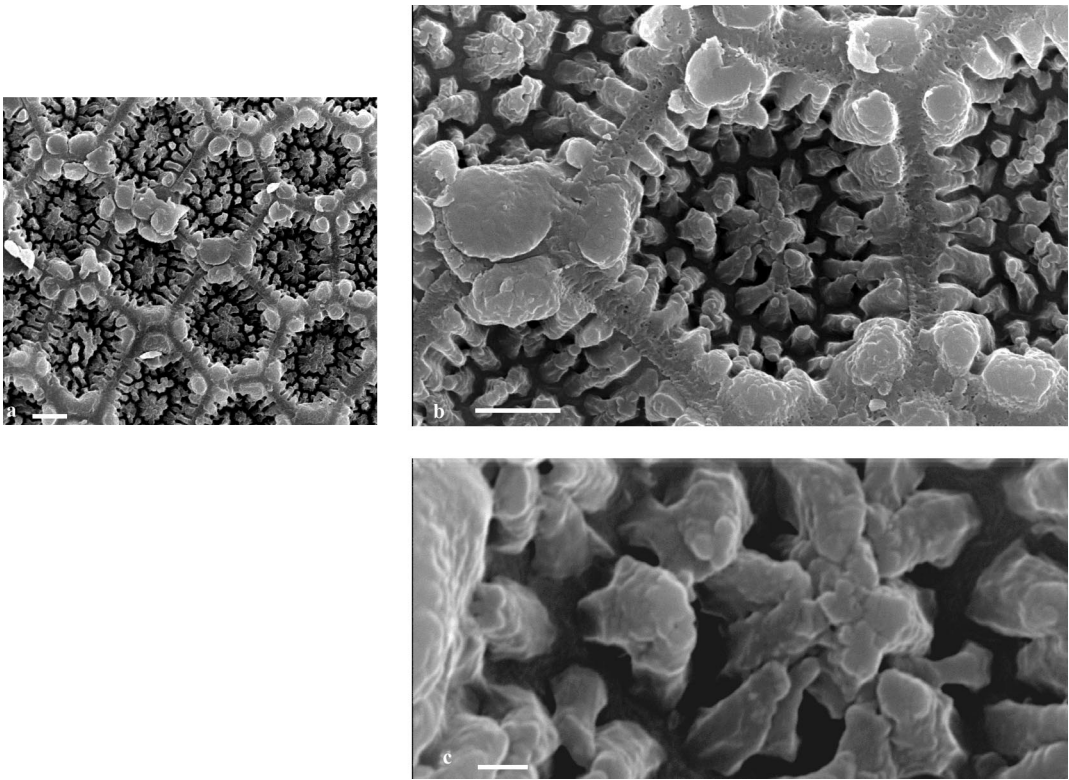
Table 1. Measurements of the eggs of two species of *Haemagogus* ( $n = 10$ )

	Species	
	<i>Hg. capricornii</i>	<i>Hg. janthinomya</i> <sup>a</sup>
	Total length, $\mu\text{m}^b$	
Mean $\pm$ SE	600.4 $\pm$ 35.0	759.4 $\pm$ 6.9
Range	581.0–631.0	730.4–794.2
	Maximum width <sup>b</sup>	
Mean $\pm$ SE	172.1 $\pm$ 17.5	207.5 $\pm$ 4.7
Range	154.0–197.0	179.7–226.1
	L/W ratio <sup>c</sup>	
Mean $\pm$ SE	3.52 $\pm$ 0.27	3.68 $\pm$ 0.09
Range	3.21–4.07	3.37–4.19
	Cell exochorium diameter ( $\mu\text{m}$ ) <sup>a</sup>	
Mean $\pm$ SE	15.2 $\pm$ 0.8	21.7 $\pm$ 0.4

<sup>a</sup> After Linley and Chadee (1991).

<sup>b</sup> Significantly greater ( $P < 0.01$ , *t*-test).

<sup>c</sup> Not significant.



**Fig. 2.** Egg of *Hg. capricornii*. (a) Ventral surface of the egg with typical outer chorionic cells. Scale, 10  $\mu\text{m}$ . (b) Pentagonal ornamentation of outer chorionic cells showing tubercles disposed in groups of three in the outer chorionic reticulum. Scale, 5  $\mu\text{m}$ . (c) Detail of tubercles in the center of a chorionic cell. Scale, 1  $\mu\text{m}$ .

ward the ventral side (Fig. 3a). The micropylar apparatus ( $\approx 10.0 \mu\text{m}$  thick), located in the anterior area of the egg, had a quite prominent continuous collar, with defined borders and rounded margins. The internal diameter of this apparatus measures  $\approx 25.8 \mu\text{m}$ . Centrally, the micropylar disc is  $\approx 15.0 \mu\text{m}$  wide and is separated from the other structures by a nearly continuous groove that is  $\approx 4.10 \mu\text{m}$  wide and only has a few connecting points with the adjacent area. In the center of the disk, there is the micropyle that is  $\approx 2.37 \mu\text{m}$  wide (Fig. 3b). The outer chorionic cells are slightly smaller toward their posterior extremity.

Some eggs of *Hg. capricornii* were joined to each other, always by their dorsal surface (Fig. 4, a and b). In this region, the chorionic cells are composed of filaments fused in groups (Fig. 4, d and e); some of these filaments present extremely large development, being observed from one-third of the anterior dorsal area, and connected as nodules, forming an agglomerate (Fig. 4c) that was 0.4–2.0  $\mu\text{m}$  wide and 15.6–31.1  $\mu\text{m}$  long. The lateral chorionic surface presents small cells fused in group; many of these cells clearly showed a pentagonal ornamentation, with agglomerated filaments that are linked to the tubercles, some with aspect of small nodules (Fig. 4, c and d).

## Discussion

Based on current knowledge, it remains difficult to separate *Hg. capricornii* from *Hg. janthinomys*, especially in areas where the two species are sympatric (Forattini 1965, Arnell 1973).

Mattingly (1973) was the first author to describe, although with little detail, the eggs of two *Haemagogus* species: *Hg. spegazzinii* Brethes and *Hg. Lucifer* Howard, Dyar, and Knab, 1913. In that study, the description of the egg structures did not allow the separation of *Hg. capricornii* from those two. In 1991, Linley and Chadee described, in an SEM study, the eggs of *Hg. equinus* and *Hg. janthinomys*. In these species, the eggs taper abruptly at both extremities, the tapering beginning at the anterior one-third and the posterior one-fifth. On the contrary, we have shown that in *Hg. capricornii*, the egg tapers at its anterior and its posterior one-third. According to their total length and width, the eggs of *Hg. capricornii* are less elongated than those of *Hg. janthinomys* (Table 1). Linley and Chadee (1991) based their morphometric study on *Hg. janthinomys* specimens from Trinidad, the eggs of which seem to be longer than those of the only specimen collected in the State of Mato Grosso, Brazil, and measured by us. The length and width of the former were  $759.4 \pm 6.9 \mu\text{m}$  and  $207.5 \pm 4.7 \mu\text{m}$ ,

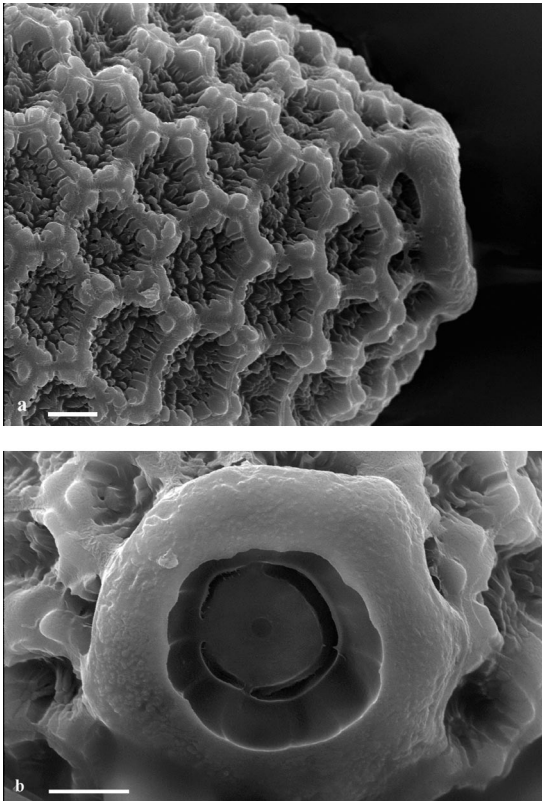


Fig. 3. Egg of *Hg. capricornii*. (a) Collar located in the anterior extremity of the egg. Scale, 10  $\mu\text{m}$ . (b) Micropylar apparatus showing a continuous collar, a micropylar disc, and a micropyle. Scale, 10  $\mu\text{m}$ .

respectively, whereas our specimen was 641.0  $\mu\text{m}$  long and 166.0  $\mu\text{m}$  wide. The eggs of *Hg. capricornii* were shorter ( $600.4 \pm 35.0$   $\mu\text{m}$  long and  $172.1 \pm 17.5$   $\mu\text{m}$  wide). Using the Student *t*-test, we compared the average of length and width obtained in *Hg. capricornii* eggs with the results obtained in *Hg. janthinomys* (data from Linley and Chadee 1991). These results were highly significant ( $P < 0.01$ ). These measures may provide good diagnostic characters to separate the two species. Likewise, the average diameter of chorionic cells obtained in our measures in *Hg. capricornii* ( $P < 0.01$ ) was also significant compared with that of *Hg. janthinomys* obtained by Linley and Chadee (1991). However, using the same test, the difference between the averages in relation to the length/width ratio is not significant. The chorionic surfaces of *Hg. equinus* and *Hg. janthinomys* eggs present cells with regular aspects, usually hexagonal and sometimes pentagonal. However, the tubercles inside the chorionic cells of *Hg. capricornii* are different, and the internal aspect of the cells may be of diagnostic value to separate the eggs of these three species. In *Hg. equinus*, the disposition of the small tubercles inside the cells is uniform, and in *Hg. janthinomys*, there are small elevations in the cells' center. However, these elevations are not

Table 2. Characters that may be useful for the separation of the eggs of species of *Haemagogus* mosquitoes

	<i>Hg. capricornii</i>	<i>Hg. janthinomys</i> <sup>a</sup>	<i>Hg. equinus</i> <sup>a</sup>	<i>Hg. spegazzinii</i> <sup>b</sup>	<i>Hg. lucifer</i> <sup>b</sup>	<i>Hg. leucoclaeus</i>
Total length range ( $\mu\text{m}$ )	581.0–631.0	730.4–794.2	561.2–700.8	NA <sup>c</sup>	NA	551.0–574.0
Maximum width range ( $\mu\text{m}$ )	154.0–197.0	179.7–226.1	165.2–209.8	NA	NA	151–169.0
L/W ratio range	3.21–4.07	3.37–4.19	3.04–3.80	NA	NA	NA
Vertex of the chorionic cells	With three tubercles	Without tubercles	Without tubercles	NA	NA	One or more tubercles
Width range of chorionic cells ( $\mu\text{m}$ )	22.5	21.7	15.3	NA	NA	13.7
Fusion of tubercles	yes	yes	?	NA	NA	No
Disposition of the cells internal tubercles	Irregularly fused in the center	Forming elevations	Uniform	NA	NA	No individualized tubercles
Mean density of tubercles (mg per cell)	11.3	?	40.5	NA	NA	21.1
Micropylar collar	Prominent and continuous	Prominent and continuous	Low, with small excavations	Prominent and continuous	Absent	Prominent and continuous
Width of micropylar disk ( $\mu\text{m}$ )	15.0	13.0	NA	NA	NA	7.3
Micropylar orifice	2.3	2.9	2.9	NA	NA	1.6

<sup>a</sup> After Linley and Chadee (1991).

<sup>b</sup> After Mattingly (1973).

<sup>c</sup> Not available.

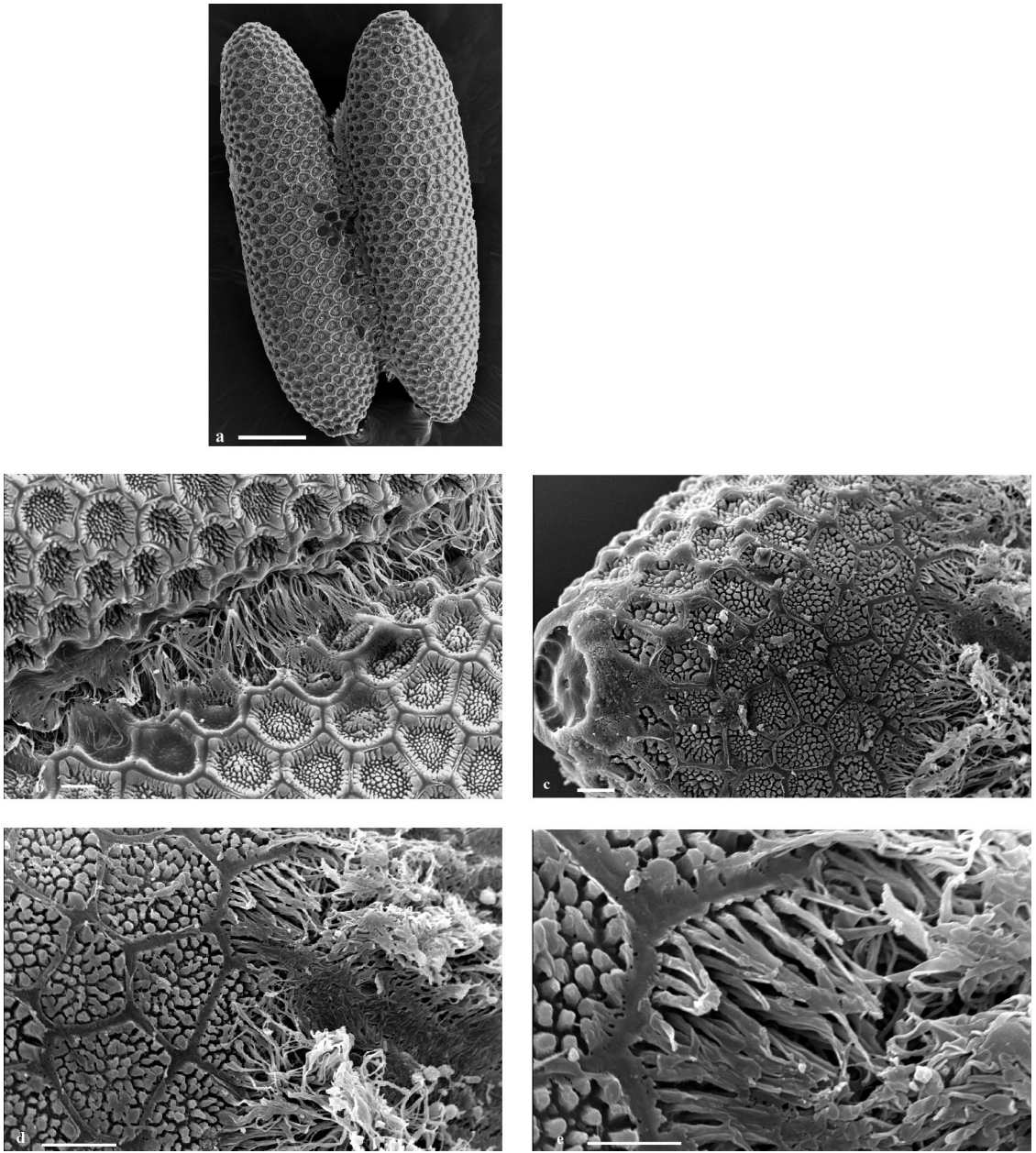


Fig. 4. Egg of *Hg. capricornii*. (a) Eggs united in the dorsal area through filaments. Scale, 100  $\mu\text{m}$ . (b) Details of filaments. Scale, 10  $\mu\text{m}$ . (c) Aspect of dorsal chorionic cells in the anterior region. Scale, 10  $\mu\text{m}$ . (d) Filaments fused in groups. Scale, 10  $\mu\text{m}$ . (e) Details of fused filaments. Scale, 5  $\mu\text{m}$ .

fused like the central tubercles found in some species of *Aedes* and *Ochlerotatus*. In *Hg. capricornii*, these tubercles form groups of varying size. The chorionic cells of the ventral surface of the egg of *Hg. janthinomya* measured in the middle of the egg are larger than those of *Hg. capricornii*. The filaments in the external dorsal surface, which have been observed in the two species studied by Linley and Chadee (1991) and in *Hg. capricornii* (current study), possibly help the egg to adhere to the substrate. The ornamentation of the

exochorion of *Hg. capricornii* reveals significant differences to the egg of *Hg. leucocelaenus* Dyar and Shannon, 1924. Tubercles observed on the margins of the chorionic cells are symmetrically arranged in relation to the longitudinal axis as well as the internal smaller and individualized tubercles. Some of them are arranged peripherally, and others are grouped in the center of the cell (Alencar et al. 2003). Among the species of *Haemagogus* whose eggs were described, *Hg. capricornii* is the only one where three tubercles

were observed at the extremity of each vertex of the chorionic cells.

The exochorion of the eggs of some other genera of *Aedini* mosquitoes have also been studied. Matsuo et al. (1974) described structures similar to those described in *Hg. capricornii* on the ventral (=superior) surface of *Ochlerotatus (Finlaya) albolaralis* (Theobald) and *Oc. (Fin.) melanopterus* (Giles). The same author showed that in *Aedes aegypti* (Linnaeus), *Ae. albopictus* (Skuse), *Ae. pseudoalbopictus* (Borel), and *Ae. alcasidi* Huang, the exochorionic cells have a great papilla in their center and small tubercles accompanying the pentagonal or hexagonal pattern in their periphery. In *Oc. togoi* (Theobald), they reported small papillae in an irregular fashion and the lack of a central tubercle.

The micropylar collar of *Hg. janthinomys* is slightly prominent, continuous, and has a clearly defined micropylar disc (Linley and Chadee 1991), in part similar to the one observed by Linley (1989) in *Ae. aegypti* and by us in *Hg. capricornii*. These structures are different in *Ae. albopictus*, where the collar is moderately prominent and not continuous. In *Hg. equinus*, this structure is not very evident, although continuous, and has small excavations. The collar is present and conspicuous in *Hg. spegazzinii* but absent in *Hg. lucifer* (Mattingly 1973). In our observations in *Hg. leuco-celaenus* (Alencar et al. 2003), the same prominent and continuous aspect of the collar was noted, differing in only the form of membranous creases arranged uniformly in a helical manner seen in this species. All the characteristics that may be useful for the separation of the eggs of species of *Haemagogus* mosquitoes are shown in Table 2. Based in the morphometrical and morphological differences visualized by SEM, we conclude that is possible to distinguish *Hg. capricornii* from *Hg. janthinomys*, as well as the other studied species, *Hg. equinus*, *Hg. lucifer*, and *Hg. spegazzinii*.

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