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SCIENTIFIC NOTE

SCANNING ELECTRON MICROSCOPY OF THE EGGS OF *AEDES SCAPULARIS* FROM SOUTHERN SOUTH AMERICA

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ABSTRACT. The eggs of *Aedes scapularis* analyzed by scanning electron microscopy are black and elliptical in outline, measuring approximately 620.4 ± 16.74 μm long and 163.7 ± 16.90 μm ($n = 10$) wide, with an egg index (length/width ratio) of 3.79. The anterior extremity tapered abruptly from a width of 51.6 μm, while such tapering was more gradual at the posterior extremity, from a width of 61.4 μm. The ventral surface of the chorionic coating presented cells with a tubular aspect containing tubercles in rows at a density of 5 to 9 per cell with 2 different sizes, the largest measuring 7.23 ± 0.98 μm in a longitudinal diameter and the smallest 4.15 ± 0.53 μm ($n = 30$). In the dorsal region, the external chorionic reticulum had a porous appearance, and its thickness ranged from 2.5 to 4.1 μm. Isolated tubercles presented wide variation per cell. In the central region of some chorionic cells were tubercles of greater diameter, characterized as central tubercles of 8.45 ± 0.67 μm, and around them 3 to 5 smaller tubercles measuring 2.57 ± 0.26 μm. The micropylar apparatus presented a collar with a very evident molding and edges with defined margins for the transition area and a thickness of around 11.1 μm. The micropyle disc margins were raised, measuring around 17.8 μm in diameter and 229 μm in circumference. The micropyle orifice was very evident, with a diameter of 1.41 μm.

KEY WORDS Culicidae, Aedini, egg, ultrastructure, scanning electron microscopy, *Aedes scapularis*

Aedes scapularis (Rondani) is a Neotropical flood water mosquito widely distributed in tropical and subtropical America, from Texas and Florida in the United States to Río Negro Province in Argentina (Arnell 1976, Mitchell and Darsie 1985). Throughout South America, it is distributed east of the Andes mountain range. *Aedes scapularis* has been found infected with several arboviruses (Rocio, Melão, Ilhéus, Mayaro, Venezuelan equine encephalitiditis) (Forattini 2002), in addition to Yellow Fever, which infected it more easily than *Aedes aegypti* L. (Soper et al. 1933). It is a suspected vector of Rocio encephalitis in Southeastern Brazil based on field and laboratory evidence (Forattini et al. 1995) and is a good vector of *Dirofilaria immitis* Leidy in Rio de Janeiro State (Labarthe et al. 1998). Because it has a tendency to adapt to residential areas (Forattini et al. 1989), *Ae. scapularis* is potentially very important for the transmission of pathogens to humans.

Several characteristics of mosquitoes have been utilized for their identification, but only a small number of species have had their eggs studied. The morphological characteristics of the eggs of most (ca. 84%) species of Aedini are still unknown (Reinert 2005). The description of mosquito eggs by scanning electron microscopy (SEM) can be used for the identification of breeding places, being particularly important for medically important species such as *Ae. scapularis*. Because the systematics of the tribe has been intensely studied with new proposals of reclassification (e.g., Reinert et al. 2004), the information on the egg morphology could constitute an important addition.

Although a few illustrations of *Ae. scapularis* eggs have been published (Forattini 1965, 1996, 2002), no detailed descriptions are available so far. In the present study, we analyze and describe the eggs of *Ae. scapularis* from 2 different geographic localities by means of SEM.

The *Ae. scapularis* eggs used in this study came from females collected in the Environmental Conservation Unit Desterro, at Florianópolis, Santa Catarina State, Brazil, and from the neighborhood of the city of Córdoba, Córdoba Province, in the central part of Argentina. The first area has secondary Atlantic forest in recuperation and humid subtropical climate

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(Cfa) according to the Köppen classification. Córdoba City has a mid-latitude winter dry climate (CWa) with an average summer temperature of 24°C and winter temperature of 11°C.

A total of 10 engorged females were isolated individually to obtain 50 eggs, laid on plaster of Paris or wet filter paper. The species was identified by morphology (Arnell 1976, Consoli and Oliveira 1994). Eggs were fixed in 2.5% glutaraldehyde and post-fixed 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 and photographed under a Jeol JSM 6390LV scanning electron microscope (Akishima, Tokyo, Japan), at magnifications of 200–5,000×.

The measurements were made directly on the images obtained, with the aid of the Semafore digital slow scan image-recording system and version 3.1 analysis software (Insinooritoimisto J. Rimppi Oy, Finland) coupled to the JEOL microscope. The parameters measured were length, width, micropylar collar thickness, and chorionic cell diameter and circumference. Only the means are cited. The terminology for description used was in accordance with Harbach and Knight (1980).

The singly laid eggs were black and elliptical in outline and measured 620.4 ± 16.74 μm long and 163.7 ± 16.90 μm wide (n = 10), with an egg index (length/width ratio) of 3.79. The anterior extremity tapered abruptly from a width of 51.6 μm, while such tapering from a width of 61.4 μm was more gradual at the posterior extremity. The ventral surface (upper surface in the natural position) of the chorionic coating presented cells with a tubular aspect containing tubercles set in rows at a density of 5–9 per cell (Fig. 1.1). In this region, the tubercles inside the chorionic cells presented 2 different sizes, the largest measuring 7.23 ± 0.98 in a longitudinal diameter and the smallest 4.15 ± 0.53 μm (n = 30) (Fig. 1.2).

The micropylar apparatus showed a collar with clear molding and edges of determined length, albeit irregular, with defined margins for the transition area and a thickness of around 11.1 μm. The micropyle disc margins were raised, measuring around 17.8 μm in diameter and 229 μm in circumference. The micropyle orifice was very evident, with a diameter of around 1.41 μm (Fig. 1.3).

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 apparatus and in the more medial area. The isolated tubercles presented wide variations per cell. In the central region of some chorionic cells, there were tubercles of larger diameter that were characterized as 8.45 ± 0.67 μm (n = 30) central tubercles surrounded by 3 to 5 smaller tubercles measuring 2.57 ± 0.26 μm (n = 30) (Fig. 1.4).

Based on variations described in the morphology of *Ae. scapularis*, it has been proposed that it is either a complex of species or at an early phenotypical differentiation stage (Forattini 2002), contrary to the opinion of Arnell (1976), who called it a single species. The observations of 2 populations showed no significant morphological differences between the eggs of *Ae. scapularis* from the wet areas of Brazil and dry areas of Argentina. However, more detailed studies on populations from other areas are needed to accept or reject the complex species hypothesis.

The ventral and dorsal surfaces of the egg of *Ae. scapularis* showed relatively little differentiation, as was observed in other floodwater mosquitoes such as *Aedes albifasciatus* Macquart, recently described (Santos-Mallet et al. 2009). Otherwise, the egg of *Ae. scapularis* is quite different of those of *Ae. albifasciatus*, because these present racquet-shaped cells. Both species are found in the same larval habitats, at least in Argentina (Campos et al. 2004). *Aedes scapularis* was never found in tree holes, but the species is adapted to anthropic environmental changes with a strong tendency toward human establishments (Silva and Menezes 1996), and thus the use of containers as larval habitats could become more frequent. *Aedes scapularis* was collected along with *Aedes albopictus* (Skuse) and other species of the genus (*Aedes hastatus*/*oligopistus*) from containers (Forattini et al. 1997) and a ground pool (Forattini et al. 1998). The fine structure of the eggs of *Ae. albopictus* and *Ae. aegypti* were described by Linley (1989), and Haddow et al. (2009) compared eggs of 3 tree-hole species (*Aedes japonicus* (Theobald), *Aedes triseriatus* Say, and *Ae. albopictus*). The dimensions of *Ae. scapularis* eggs are intermediate to those of the other species, but the shape of tubercles and of the micropylar apparatus are quite distinct (see also Matsuo and Kunou 1972). Because *Ae. japonicus* has been frequently found in rock pools in the United States (Andreadis and Wolfe 2010) and this mosquito can be introduced in South America, differences between its egg tubercles and those of *Ae. scapularis* should be emphasized. In *Ae. japonicus*, the ventral chorionic cells are primarily hexagonal and variable in size. Within the cell fields, tubercles range from 3 to 6 in number, are typically irregular in size, and appear trapezoidal in shape (Haddow et al. 2009). In *Ae. scapularis* cells have a tubular aspect with tubercles in rows. Differences are also observed

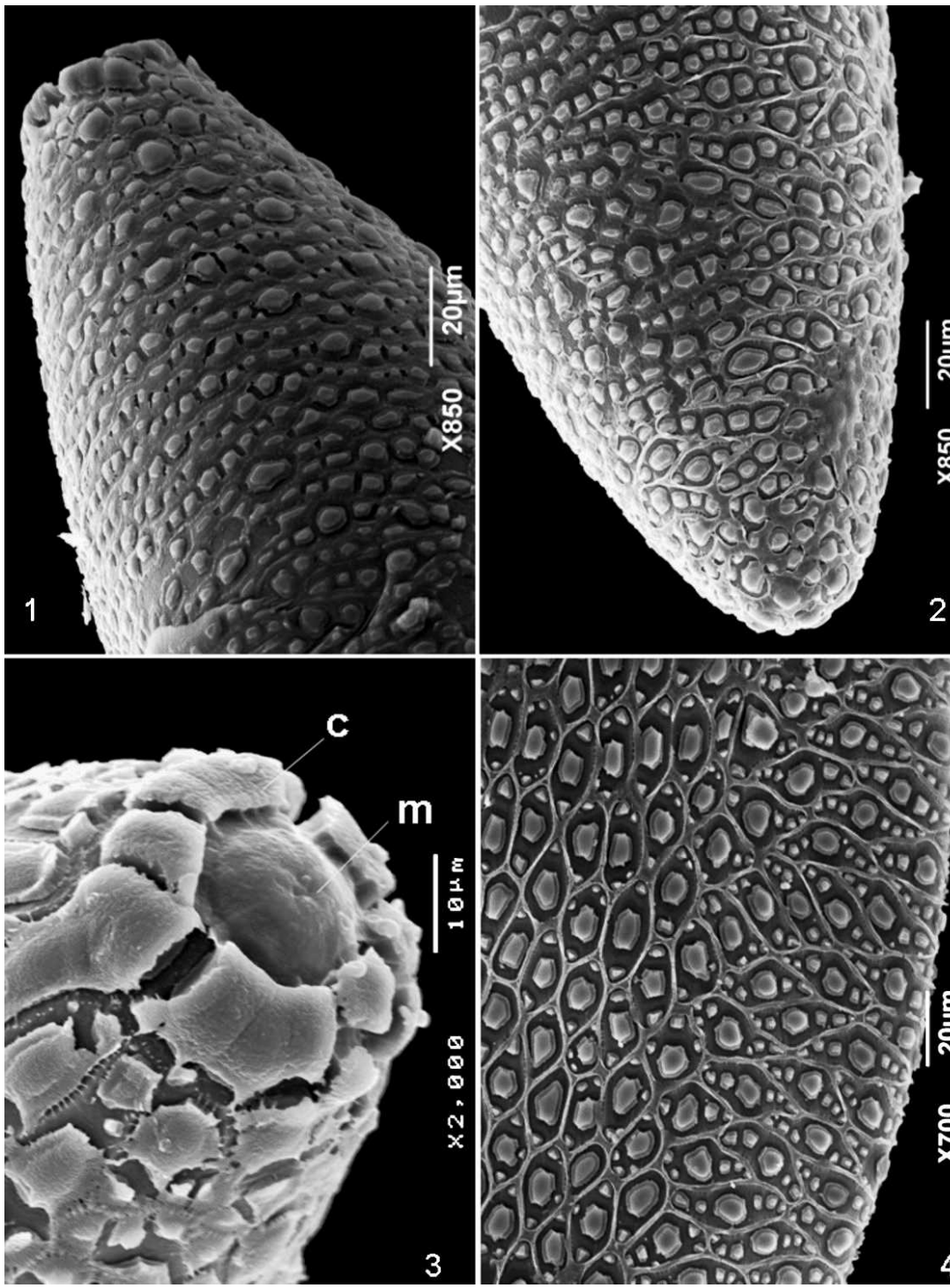

Fig. 1. Electron micrographs of the eggs of *Aedes scapularis* showing: (1) anterior region—micropylar collar and typical ornamentation of the outer chorionic reticulum with 2 types of tubercles, (2) posterior region of the egg showing tubercles, (3) detail of the micropylar apparatus in the anterior region showing the collar (c) and the micropyle (m), and (4) lateral region of the egg showing ornamentation of the dorsal (right) and ventral (left) regions.

on the dorsal surface, where the external chorionic reticulum of *Ae. scapularis* has a porous appearance, tubercles are isolated and presenting a wide variation in each cell. In *Ae. japonicus*, the outer chorionic cells become extremely irregular in shape, each containing 2 large tubercles with small oval-shaped tubercles grouped around them, and a large thread-shaped tubercle extending from this grouping.

In several species of Aedini, the presence of a central papilla or tubercle in the chorionic cells has been described. The chorionic cells of the eggs in *Ae. albifasciatus* have a small central tubercle surrounded by larger tubercles (Santos-Mallet et al. 2009), whereas in *Aedes terrens* there are elongated tubercles with a very regular pattern in the central region. They are bigger on the periphery and sometimes fused into groups at the vertices (Alencar et al. 2005). Another frequent pattern is a large papilla in the central area and small tubercles on the periphery, as described for *Ae. albopictus* and *Ae. aegypti* (Matsuo and Kunou 1972) and *Aedes excrucians* and *Aedes hexodontus* (Matsuo 1975), in addition to some species of the *Haemagogus* genus (Alencar et al. 2008), and this is also the case for *Ae. scapularis*.

The sculptural appearance of the exocorium presents notable variety that makes it possible to draw up descriptions using scanning electron microscopy. This especially boosts the power of morphological studies, which previously were limited to optical microscopy (e.g., Horsfall and Craig 1956). This tool has opened up the possibility of performing detailed morphological studies with the objective, among others, of contributing toward resolving the identification of members of the *Haemagogus* complex (Forattini 2002).

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