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Original Article

Comparing the egg ultrastructure of three *Psorophora ferox* (Diptera: Culicidae) populations

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(With 1 figure)

Abstract

Characterising the external morphology of mosquito eggs is important, since it facilitates the identification of material from breeding sites and contributes to the understanding of species biology and group systematics. Therefore, was to compare eggs from different *Psorophora ferox* populations using scanning electron microscopy (SEM). Eggs were obtained from adult female of *Ps. ferox* collected in the Poços das Antas Biological Reserve (Reserva Biológica de Poços das Antas, Rio de Janeiro, Brazil). From each female, one portion of eggs (n = 5) was reared for identification purposes, and the second portion (n = 10) was examined using SEM for morphometric analysis. The egg morphology was then compared to that of eggs from *Ps. ferox* populations in Florida (USA) and Arena (Trinidad). The exochorion ornamentation of the populations differs considerably in the morphology of the tubercles of the chorionic cells, external chorionic reticulum, micropylar collar, and micropyle.

Keywords: scanning electron microscopy, mosquitoes, Aedini, morphometry, egg.

Comparação da ultraestrutura de ovos de três populações de *Psorophora ferox* (Diptera: Culicidae)

Resumo

Caracterização da morfologia externa dos ovos do mosquito é importante, uma vez que facilita a identificação de materiais a partir de locais de reprodução e contribui para a compreensão da biologia das espécies e sistemática do grupo. O objetivo do presente estudo foi comparar os ovos de diferentes populações *Psorophora ferox* usando MEV. Os ovos foram obtidos a partir de fêmeas de *Ps. ferox* que foram coletadas na Reserva Biológica de Poço das Antas (Reserva Biológica de Poço das Antas, Rio de Janeiro, Brasil). A partir de cada fêmea, uma parte dos ovos (n = 5) foi criado para fins de identificação, e uma segunda parte (n = 10) foi examinado usando MEV para análise morfométrica. Foi então comparada a morfologia dos ovos de populações de *Ps.ferox* da Flórida (EUA) e Arena (Trinidad). A ornamentação do exocório das populações difere consideravelmente na morfologia dos tubérculos das células coriônicas, retículo coriônico externo, colar micropilar e micrópila.

Palavras-chave: microscopia eletrônica de varredura, mosquitos, Aedini, morfometria, ovo.

1. Introduction

The tribe Aedini (Culicidae) includes 1,255 species, of which several are medically important vectors of yellow fever, dengue, and other arboviruses. Several morphological aspects of these species have been investigated; however, the eggs of only 16% of the species in tribe Aedini have been studied, mostly by light microscopy (Reinert, 2005). Approximately 30% of the species of *Psorophora* have their eggs described and illustrated (Reinert, 2010; Pacheco et al., 2012). The characterisation of insect eggs using SEM facilitates the direct identification of insect species and eliminates the need to foster the development of eggs into identifiable phases *via* laboratory rearing, which is usually laborious, costly, and carries certain risks if the insect is a disease vector. In addition, the information obtained using SEM can also be used for phylogenetic studies.

Psorophora spp., which are commonly known as flood mosquitos, lay their eggs in ground water, much like other Aedini mosquitoes, and frequently wait for flooding before laying eggs. Most species of the genus are widely distributed, and *Psorophora* (*Janthinosoma*) *ferox* (Humboldt 1819) has been reported to occur in the three Americas from Ontario (south Canada) to northern Argentina and Uruguay, with a distribution that spreads east of the Andes Cordillera and includes the central, eastern, and southeastern areas of the USA, Mexico, Central America, the Antilles, Colombia, Venezuela and Brazil (Forattini, 2002; Orlandin et al., 2017). *Ps. ferox* has been reported to harbour several arboviruses, namely Rocio (Lopes et al., 1981), West Nile virus (Kulasekera et al., 2001), eastern equine encephalitis (Cupp et al., 2004), and Ilheus virus (Turell et al., 2005), and were also shown to transmit Rocio experimentally (Mitchell et al., 1986).

Although Ligth microscope (LM) has been used since the beginning of the 20th century and continues to be used sporadically, Scanning electron microscopy (SEM) has facilitated more detailed descriptions of egg surfaces since the 1960s. Linley and Chadee (1990) used SEM to determine the exochorion patterns of eggs from several *Psorophora ferox* populations. They reported that the exochorion patterns of *Ps. ferox* from Florida (USA) were clearly different from those of *Ps. ferox* from Trinidad, in both the number and shape of the external chorionic tubers in each chorionic cell.

The present study aimed to compare *Ps. ferox* eggs from different geographical regions, using SEM to perform a morphometric analysis of the main exochorion structures.

2. Material and Methods

To obtain eggs, female *Ps. ferox* were collected from Poço das Antas Biological Reserve (Reserva Biológica Poço das Antas; REBIO-PA), which and includes 5,226 ha (perimeter of 44 km) and is located in the central part of the Silva Jardim municipality, Rio de Janeiro, Brazil (22°33'11.4" S and 42°17'49.8" W.). Five blood-fed females were captured, sampling was conducted over 2 months (march and april 2015), using an oral suction tube, and taken to the laboratory, where they were maintained in 30 ml plastic vials until they laid eggs (4-5 d).

Immediately after oviposition, 15 eggs were taken from each female: five eggs were reared in a total of 25, and ten were submitted to morphometric analyses in a total of 50. The species was identified using Forratini dichotomous keys (2002).

For the SEM analysis, the eggs were removed from the filter paper using a brush, fixed in 2.5% glutaraldehyde, and post-fixed in 1% osmium tetroxide, both in 0.1 M sodium cacodylate buffer at 7.2 pH. After being washed in the buffer, the eggs were dehydrated in an increasing ethanol series, and critical-point dried using super-dry CO^2 in a Balzers device (Hayat, 1970). The eggs were then mounted on metal supports, coated with gold, and observed using a JEOL JSM 6390LV scanning electron microscope (JEOL, Ltd., Akishima, Tokyo, Japan) at 200-5,000× magnification. The eggs were also photomicrographed in both dorsal and ventral positions in order to observe both the exochorion and micropyle.

Morphological measurements were directly performed by measuring the features on the photomicrographs, using Semafore digital slow scan image recording system, version 3.1 (Insinooritoimisto J. Rimppi Oy, Ojakkala, Finland), and analysed using SEM Control User Interface version 8.24 (JEOL, Ltd.), which was coupled to the microscope. The measured parameters included total length, total width, chorionic tubercle diameter, micropyle, and micropyle annexes, for comparision with the data obtained by Linley and Chadee (1990). The terminology used to describe the eggs followed Harbach and Knight (1980), and the genera have been abbreviated as proposed by Reinert (2009).

3. Results

The *Ps. ferox* eggs are deep black in colour and separately, strongly adherent to the surface. When observed by SEM, the eggs were elliptical, with a mean length and width of 816.8 and 205.6 µm, respectively, and egg index (length/width ratio) was 3.97. (Figure 1A). The exochorion presented a regular distribution of chorionic reticulum, with hexagonal and sometimes pentagonal chorionic cells internally coated by small tubercles (length: 12.9-17.9 µm; width: 5.14-6.22 µm). The small tubercles were irregularly shaped, being rectangular, rounded, square, or tubular, and their densities ranged from 18 to 22 tubercles per cell (16.2 ± 3.21; $n = 10$). In addition, the exochorion also presented large, conical tubercles (length 13-18 µm; width 5.1-6.2 µm) at one extremity of the chorionic cells (Figure 1B), which contrasted with the patterns observed in the eggs of other *Psorophora* spp. (Table 1), thus distinguishing the eggs of *Ps. ferox* from those of its congeners. Meanwhile, the tubercles exhibited a porous appearance, without any nodules on their surface, and the surface of the chorionic reticulum in the anterior region of the egg was not rough. The micropylar collar was prominent and continuous and presented a conspicuous micropylar disc (diameter: 20.4 µm, thickness: 7.96 µm; Figure 1C) at its centre; and the micropyle (diameter: 2.4 µm) was observed in the centre of this disc (Figure 1D). The characteristics of the tubercles of specimens of *Psorophora* (*Janthinosoma*) *ferox* of three different regions are shown in the table 2.

4. Discussion

The measurements of the *Ps. ferox* eggs obtained here differed from those eggs from populations in Florida and Trinidad, which were described by Linley and Chadee (1990).

In general, the eggs from REBIO-PA were smaller, although some were within the size range observed in eggs from Florida. The size and number of tubercles also differed among the three locations (Table 1), which

suggested intraspecific differences. The mean length and width of *Ps. ferox* eggs from Illinois (Horsfall et al., 1952) were lower than those of eggs from Florida, Trinidad, and REBIO-PA, but it was not possible to compare any of the other features of eggs from Illinois, since the study was based on light microscope observations. In comparison to *Psorophora varipes* (Coquillett 1904) (Horsfall et al., 1952) and *Ps. albigenu* (Lutz 1908) (Pacheco et al., 2012), the *Ps. ferox* eggs also presented a longer cylinder-shaped tubercle at one extremity and were distinctly longer, thus characterising the eggs of *Ps. ferox* from those of other species.

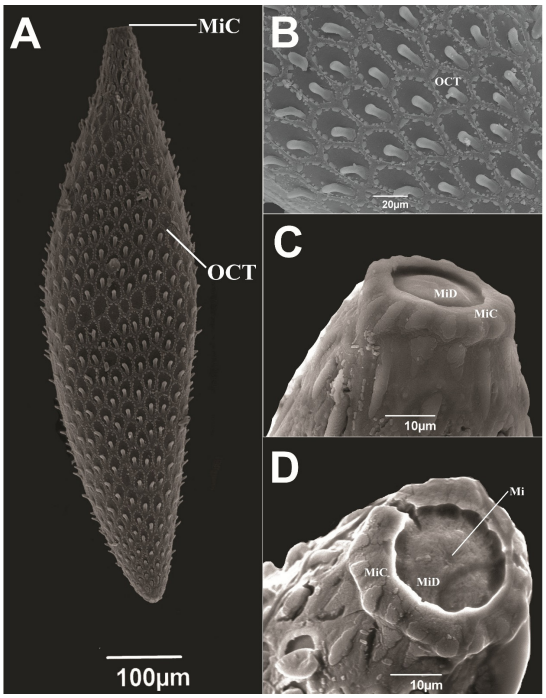

Figure 1. Egg of *Psorophora (Janthinosoma) ferox*. (A) ventral (upper) view; (B) typical ornamentation of the outer chorionic reticulum showing two types of tubercles; (C) anterior region of the egg showing micropylar apparatus, formed by a micropilar disc with a well evidenced frame; (D) anterior pole of egg, with micropyle. Mi, micropyle; MiC, micropylar collar; MiD, micropylar disc; OCT, outer chorionic tubercle.

Exochorion structures exhibit remarkable variation, and characterising these structures using SEM greatly enhances morphological studies that were previously limited to LM and facilitates the identification of Culicidae taxa from different species complexes (Forattini, 2002). Although Horsfall et al. (1952) devised a taxonomic key for several North American *Psorophora* species, the key was based on LM and could potentially be refined using information obtained by SEM. However, of the 17 *Psorophora* species (of 52 species) for which egg morphology has been reported, most descriptions are based on LM (Reinert, 2005), and of the 21 *Psorophora* species in Brazil, egg descriptions have only been provided for nine species, with only three species described using SEM: *Ps. cingulata* (Fabricius 1805), *Ps. albigenu*, and *Ps. ferox*. This scarcity of information, along with the wide distribution of most species, emphasises the need for investigating the egg morphology of other species.

In the present study, the SEM-based description of *Ps. ferox* eggs from REBIO-PA and comparison to other *Ps. ferox* populations revealed few dissimilarities in the exochorion morphology. However, it was verified difference in the measurements of the eggs of Trinidad and Florida, when compared to Brazil in relation to the lenght, width and characteristics of the tubercles being the samples of Brazil the most disparate, thus contributing to inter- and intraspecific taxonomy of Culicidae species.

Table 1. Dimensions of eggs of specimens of *Psorophora* (*Janthinosoma*) *ferox* of three different regions.

Specimen	Lenght µm		Widht µm		Lenght/Widht ratio	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Ps ferox (*Fl)	904.5 ± 13.7	845.5 - 963.6	250.2 ± 3.6	227.9 - 265.5	3.62 ± 0.03	3.44 - 3.75
Ps ferox (*Tr.)	918.3 ± 1.9	911.0 - 927.7	316.7 ± 1.7	311.1 - 322.2	2.90 ± 0.02	2.83 - 3.04
Ps ferox (Br)	816.8 ± 19.4	760.3 - 844.7	205.6 ± 5.5	190.3 - 216.7	3.97 ± 0.04	3.88 - 4.08

(Fl.): Vero Beach-Florida (EUA); (Tr.): Arena (Trinidad); (Br.) Poço das Antas Biological Reserve-Rio de Janeiro, Brazil. *From Linley and Chadee (1990).

Table 2. Characteristics of the tubercles of specimens of *Psorophora* (*Janthinosoma*) *ferox* of three different regions.

Specimen	Small tubercles			Higher tubercles		
	Amount	Mean ± SE	Range	Amount	Length	Width
Ps ferox (*Fl)	36	32.9 ± 1.7	17-51	36	13-25	5.5-7
Ps ferox (*Tr.)	28	27.5 ± 0.6	23-35	28	13-25	5.5-7
Ps ferox (Br)	26	16.3 ± 0.6	10-22	26	13-18	5.1- 6.2

(Fl.): Vero Beach-Florida (EUA); (Tr.): Arena (Trinidad); (Br.) Poço das Antas Biological Reserve-Rio de Janeiro, Brazil. *From Linley and Chadee (1990).

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