Scientific Note

Ultrastructure of the egg of *Coquillettidia juxtamansonia* (Chagas, 1907) (Diptera: Culicidae)

Cecilia Ferreira de Mello[1], Jacenir Reis dos Santos-Mallet[2], Fernanda Morone[1], Anthony Érico Guimarães[1], Carlos Brisola Marcondes[3], and Jeronimo Alencar[1]✉

[1]*Instituto Oswaldo Cruz (Fiocruz), Laboratório de Diptera, Av. Brasil 4365, CEP: 21045-900 Manguinhos, Rio de Janeiro, Brasil, jalencar@ioc.fiocruz.br*

[2] *Instituto Oswaldo Cruz (Fiocruz), Laboratório de Transmissores de Leishmanioses, Av. Brasil 4365, CEP: 21045-900 Manguinhos, Rio de Janeiro, Brasil*

[3] *Universidade Federal de Santa Catarina, Departmento de Microbiologia, Immunologia e Parasitologia, Centro de Ciências Biológicas, 88040-900 Florianópolis (SC), Brasil*

Received 6 August 2013; Accepted 22 September 2014

Mosquitoes in the tribe Mansoniini are distributed into two genera: *Mansonia* Blanchard (27 spp.) and *Coquillettidia* Dyar (57 spp.). Adults are large, aggressive biters and mostly zoophilic, but opportunistic in their preferences (Consoli and Lourenço-de-Oliveira 1994). Eggs are usually adhered to aquatic plants and larvae get air from them (Forattini 1965). Some species have been incriminated as vectors of arboviruses (Forattini 1965, Hervé et al. 1986). Microfilariae of *Wuchereria bancrofti* were found in the proboscis of experimentally infected *Cq. juxtamansonia* (Chagas, 1907) (Davis 1935).

Eggs of 19 species of *Coquillettidia* have been described (Reinert 2010), all of them only by optical microscopy. Adequate differentiation of eggs is useful for correct identification in their breeding places and possibly for the study of connection among species in larger groups. A description and morphometric study of *Coquillettidia juxtamansonia* (Chagas) eggs by Scanning Electron Microscopy is developed.

Five blood fed females of *Cq. juxtamansonia* were obtained from Reserva Ecológica do Guapiaçu (REGUA), in Municipality of Cachoeiras de Macacu, in the Brazilian state of Rio de Janeiro (22º25'23.9" S 42º44'30.0" W). Mosquitoes were identified by keys provided by Consoli and Lourenço-de-Oliveira (1994) and Forattini (2002). From 30 eggs obtained, 11 were utilized for this study. The females were fed during the collection, utilizing suction tubes (Marcondes et al. 2007) and individually maintained in glass tubes 25 mm wide and 50 mm high, having wet cotton covered by a filter paper, in the bottom, for egg-laying (Bates and Roca-Garcia 1945).

Eggs were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide, both buffered with 0.1M sodium cacodylate at pH 7.2. After washing in the same buffer, the eggs were dehydrated in a series of increasing concentrations of ethanol and subjected to the critical-point drying method using superdry CO2 in a Balzers apparatus. The eggs were then mounted on metal supports, gold plated and observed and photographed in a JEOL JSM 6390LV scanning electron microscope (Akishima, Tokyo, Japan), at magnifications of 200 to 5,000 times. Measurements were made directly on the images obtained, with the aid of the Semafore digital slow scan image recording system, version 3.1© (Insinooritoi misto J. Rimppi Oy, Finland) and analyzed using a SEM Control User Interface version 8.24© (JEOL TECHNICHS LTD), coupled to the microscope. The parameters measured were: total length, total width, micropylar collar thickness, and chorionic cell diameter and circumference and size of tubercles.

The terminology for describing the eggs was in accordance with Harbach and Knight (1980). The genera and subgenera are abbreviated as proposed by Reinert (2009).

Eleven eggs were obtained, with six in a raft and five separated. There was no difference in the exochorion eggs adhered and non-adhered. Eggs are black in color and conic, and the posterior extremity is more tapered than the anterior end. Total length is 573±6.44 µm and width in central part is 156±2.5µm. Width in the anterior pole is 153.2±1.26 µm and in the posterior one 89±4.5 µm (Figures 1A, B). Egg length/width ratio is 3.66±0.08. Dorsal surface is covered by an outer chorionic reticulum with diversified chorionic cells (hexagonal, pentagonal and rectangular) (Figure 1C).

Tubercles in the dorsal surface are composed of a wide hemispherical basis (7.5± 1.0 µm, n =10) and a superior spherical part (4.9± 1.1 µm, n =10). Chorionic tubercles of ventral surface are irregular and similar to those of dorsal surface, but smaller. Micropyle (3.5±0.26 µm) is raised and surrounded by a smooth micropylar disc (10.6±1.8 µm diameter) and by a regular collar (3.5±0.5 µm width) (Figure 1D). The posterior extremity is more flattened (Figure 1E), and no structures related to adhesion were evident.

Coquillettidia juxtamansonia was described from Juiz de Fora, in the Brazilian state of Minas Gerais, and its known distribution is on seven South American countries (Argentina, Bolivia, Brazil, Colombia, Paraguay, Peru and Venezuela (http://mosquitocatalog.org/taxon_descr.aspx?ID=16420).

Egg rafts of this species have been found in the water surface and not in plants, in two paralleled files of eggs, and cemented together forming rafts with 77.5±23.7 eggs (Davis 1933). An egg of *Cq. juxtamansonia* was represented (Davis 1933- Plate II), referring to "minute conical air cells" for adherence between eggs. These structures are probably the tubercles seen in the present study (Figure 1C). No structures related to fixation to plants, like those observed in other species of Mansoniini (Davis 1933, Mattingly 1971, 1972) were noticed in *Cq. juxtamansonia* eggs. Although

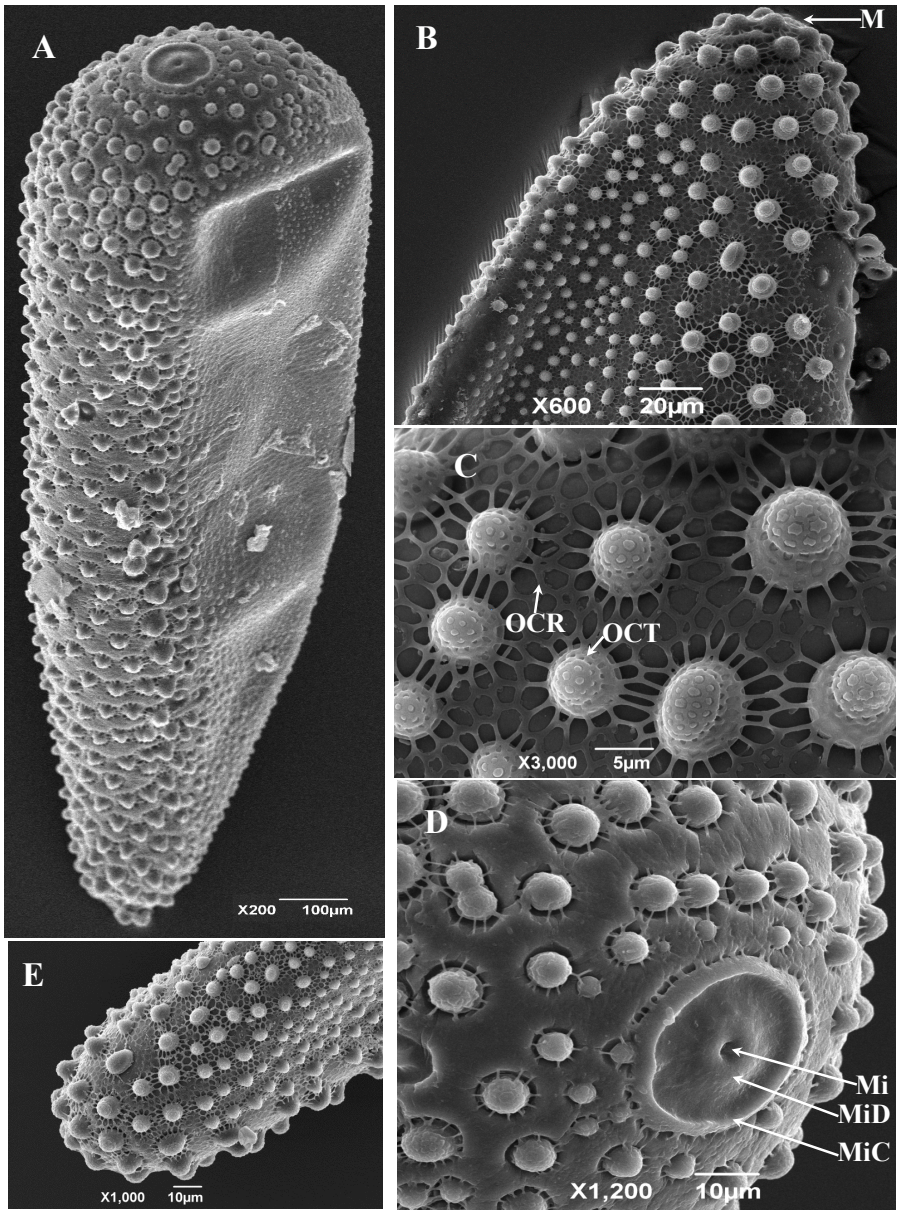

Figure 1. External morphology of egg of *Coquillettidia juxtamansonia*: A. Ventral (upper) view, anterior end at top of entire egg. B. Micropylar apparatus (M), located in the anterior area of the egg is formed by a collar with a well evidenced frame. C. Ornamentation of the outer chronionic reticulum (OCR) showing outer chorionic tubercles (OCT) constituted by two parts. D. Micropyle (Mi), Micropylar disk (MiD), Micropylar collar (MiC). E. Lateral view of posterior pole.

Mansoniini immature forms are usually associated to aquatic plants, 239 immature forms of *Cq. venezuelensis* (Theobald) were obtained from a pond without plants in the Brazilian state of Goiás (Alencar et al. 2011).

Only five of 13 species in *Coquillettidia* (*Rhynchotaenia*) have had their eggs described (Reinert 2010), and none of them by SEM. The general shape of egg of *Cq. juxtamansonia* is similar to that of *Coquillettidia* (*Coquillettidia*) *aurites* (Theobald) and *Cq.* (*Coq.*) *linealis* (Skuse), from other continents (Mattingly (1971, Figures 2g, h), but a more accurate comparison would need a detailed description of these eggs. *Mansonia titillans* (Walker), *Cq. venezuelensis*, *Cq. albicosta* (Peryassu) and *Mn. pseudotitillans* (Theobald), collected with *Cq. juxtamansonia* (Suárez et al. 1994, Lopes and Lozovei, 1996, Dantur Jury et al. 2012, D'Ávila and Gomes 2013), and possibly sharing the same breeding places, have quite different eggs (Mattingly 1971) or do not have their eggs described.

Acknowledgments

We thank FAPERJ (contract 112.076/2012) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (690143/01-0) for financial support To Dr. Ralph Harbach (NHM, London), for information on nomenclature of *Cq. juxtamansonia*. To Dr. Richard Wilkerson (WRAIR, Silver Spring), for help on bibliography. We thank the Microscopy Electronic Platform of the Instituto Oswaldo Cruz, Fiocruz

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