Scanning Electron Microscopy of the Egg of *Ochlerotatus albifasciatus* (Diptera: Culicidae)

JACENIR REIS DOS SANTOS-MALLET,[1] RAQUEL M. GLEISER,[2] JERONIMO ALENCAR,[1] WILLIAM DE ALMEIDA MARQUES,[1] JULIANA SOARES SARMENTO,[1] GERSON AZULIM MÜLLER,[3,4] AND CARLOS BRISOLA MARCONDES[3,5]

J. Med. Entomol. 46(5): 980–985 (2009)

ABSTRACT *Ochlerotatus albifasciatus* (Macquart) (Diptera: Culicidae) is a mosquito species of medical and veterinary importance, usually associated with temporary groundwater habitats. Eggs obtained from females collected in Córdoba city, in the center of Argentina, were studied using scanning electron microscopy. The eggs are elliptical in outline, measuring ≈615.7 ± 37.24 μm in length (l) and 180.7 ± 22.87 μm in width (w), with an egg index (l/w ratio) of 2.9–3.7. The outer chorionic sculpture has racquet-shaped and wristwatch-shaped cells consisting of a small central tubercle surrounded by larger tubercles and followed by a row of smaller tubercles on one or both sides, respectively, although variations between regions of the egg were observed. The micropylar apparatus has a collar with evident molding and edges of determined length, albeit irregular, with defined margins for the transition area and a thickness of ≈10.7 μm. The margins of the micropylar disc are raised and the disc measures ≈21.1 μm in diameter. The micropyle is distinct.

KEY WORDS Culicidae, Aedini, egg, scanning electron microscopy, *Ochlerotatus albifasciatus*

Ochlerotatus (*Ochlerotatus*) *albifasciatus* (Macquart) (=*Aedes albifasciatus;* see Reinert et al. 2000) is a South American (Neotropical Region) floodwater mosquito that occurs from Brazil and Bolivia to as far south as Tierra del Fuego (Mitchell and Darsie 1985). It is the main vector of the western equine encephalomyelitis virus in Argentina (Mitchell et al. 1987, Avilés et al. 1992) and possibly other arboviruses (Sabattini et al. 1985). A combination of field findings and experimental research suggests it is a potential vector of *Dirofilaria immitis* (Vezzani et al. 2006). In addition, as this species attacks humans and domestic animals, in vast zones it can be a nuisance due to allergic reactions from its bites (Docena et al. 1999). In central Argentina, losses in dairy production have been attributed to peak densities of this mosquito (Ludueña Almeida 1994).

Several characteristics of mosquitoes have been used for their identification, but only a small number of species have had their eggs studied. The eggs of ≈84% of the species of the tribe Aedini are unknown (Reinert 2005). Descriptions of mosquito eggs based on scanning electron microscopy (SEM) can aid species identification and be used for immature mosquito habitat studies, being particularly relevant for medically important species such as *Oc. albifasciatus.* Because the systematics of the tribe has been intensely studied, with proposals of reclassification (Reinert 2000; Reinert et al. 2004, 2006, 2008), information on the egg morphology could constitute an important addition.

Although characteristics of the eggs of *Oc. albifasciatus* such as drought resistance and installment hatching (Campos and Sy 2006, Campos 2008) have been studied, the morphology of the eggs has not been described in detail yet. The current study had the objectives of describing the eggs of *Oc. albifasciatus* by means of SEM and measurements of the main structures. *Ochlerotatus* is accepted as a genus according to Reinert (2000) and Marcondes (2007).

Materials and Methods

The *Oc. albifasciatus* eggs used in this study came from females collected in the neighborhood of the city of Córdoba, Córdoba Province, in the center of Argentina. In total, 15 females were caught and 49 eggs were obtained. Ten eggs were used for morphological study. The species was identified by morphology, using the key of Darsie (1985). Immediately after oviposition, the eggs were removed from the filter paper by using a paintbrush. They were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide,

[1] Instituto Oswaldo Cruz (Fiocruz), Av. Brasil 4365, 21045-900 Rio de Janeiro, Brazil.
[2] CREAN, National University of Córdoba, Córdoba, Argentina.
[3] Department of Microbiology and Parasitology, Center of Biological Sciences, Federal University of Santa Catarina, 88040-900 Florianópolis, Santa Catarina 5000, Brazil.
[4] Department of Zoology, Sector of Biological Sciences, Federal University of Paraná, Curitiba, Santa Catarina 81531-980, Brazil.
[5] Corresponding author, e-mail: cbrisola@mbox1.ufsc.br.

0022-2585/09/0980–0985$04.00/0

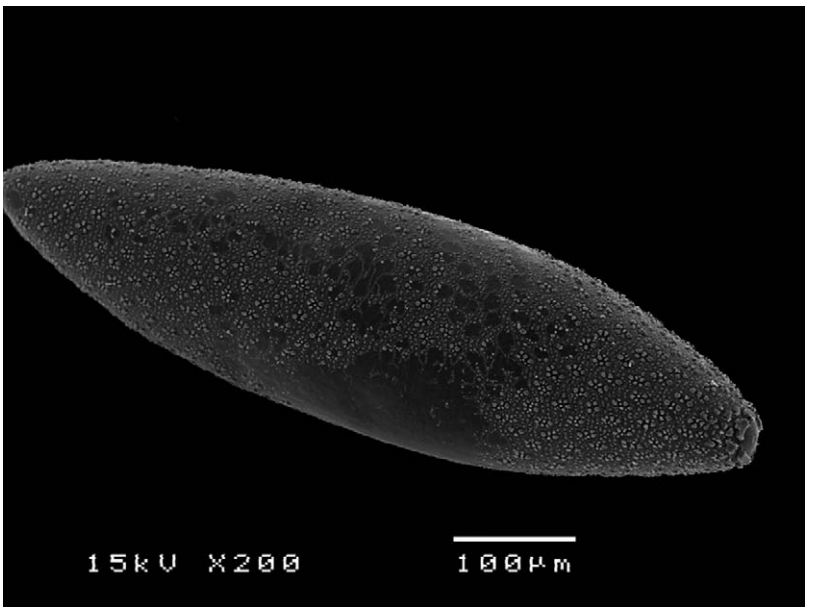

Fig. 1. Egg of *Oc. albifasciatus*. Ventral surface view; posterior end located at top left of image. Scale bar = 100 μm.

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 critical-point dried using superdry CO_2. Next, the eggs were mounted on gold-plated metal supports and observed in a JEOL 5310 scanning electron microscope (Akishima, Tokyo, Japan). The eggs were photographed at magnifications of 200–5,000× in dorsal and ventral positions to view the outer chorion and the micropyle.

The measurements were made directly on the images obtained, with the aid of the Semafore digital slow scan image recording system, version 3.1 (Insinooritoimisto J. Rimppi Oy, Finland), analysis software coupled to the JEOL microscope (Joel [Skandinoviska] AB, Soppentuma, Sweden). The parameters measured include total length, total width, micropylar collar thickness, and chorionic cell diameter and circumference. Only the means have been cited. The terminology used for describing the eggs follows Harbach and Knight (1980). Two-letter abbreviations for mosquito genera are those proposed by Reinert (1975, 2000) and Reinert et al. (2006).

Results

The eggs, laid singly, are black and elliptical in outline, with a length of ≈615.7 ± 37.24 μm and a width of 180.7 ± 22.87 μm at their central region (Fig. 1), with an egg index (l/w ratio) of 2.9–3.7. The anterior extremity (Figs. 1 and 2B) tapers abruptly from midlength, whereas such tapering is more gradual at the posterior extremity (Figs. 1 and 2B). The ventral surface (upper surface in the natural position) of the outer chorion has irregular cells shaped as "racquets" and others shaped like wristwatches. The larger part of the racquet-shaped cells has a small central tubercle surrounded by five to eight larger tubercles, all of them rounded, with a longitudinal diameter of 1.95–4.10 μm. A row of five to six smaller tubercles is present in the "handle of the racquet". The other cell type, which resembles a wristwatch, also has a central small tubercle in the central area surrounded by other four or five larger tubercles. The two lateral arms are composed of a row of three to five tubercles (Fig. 3). These are present in most of the cells observed and comprise a very regular pattern. The same pattern of cells types is observed in the dorsal region of the egg. In this region, the outer chorionic reticulum has a porous appearance and a thickness ranging from 2.5 to 4.1 μm at the anterior extremity close to the micropylar apparatus and in the more medial area. The isolated tubercles exhibit wide variation per cell (Fig. 4). Tubercles of greater diameter in the central region of some cells are characterized as central tubercles. The micropylar apparatus, located in the anterior region of the egg, bears a collar with very evident molding and edges of determined length, albeit irregular, with defined margins for the transition area and a thickness of ≈9.6 μm. The micropylar disc has raised margins and measures ≈21.1 μm in diameter. The micropyle is evident, with a diameter of ≈2.1 μm (Fig. 5).

Discussion

The eggs of a very small number of South American species of the genus *Ochlerotatus* have been described by means of SEM (Reinert 2000, 2005). The descriptions of these eggs can be accepted as aids for specific characterization, because of the degree of dissimilarity present in the structure of the outer chorion of these species. Most *Ochlerotatus* species that have

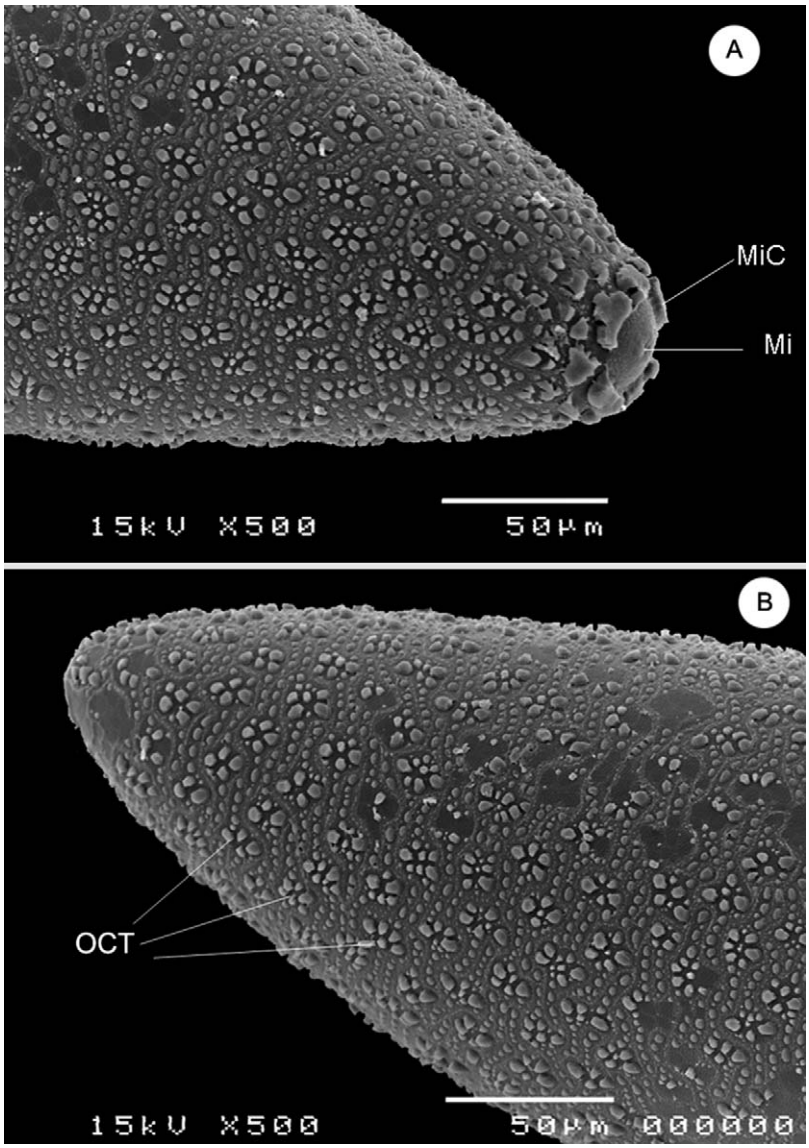

Fig. 2. Egg of *Oc. albifasciatus.* (A) Anterior region showing the micropylar apparatus (scale bare = 50 μm). (B) Surface near posterior end (scale bar = 50 μm). MiC, micropylar collar; Mi, micropyle; OCT, outer chorionic tubercle.

been described exhibit a well developed pattern of sculptures (Reinert et al. 2008), as also is observed in *Oc. albifasciatus.* In this species, there is relatively little differentiation between the ventral and dorsal surfaces of the egg, as observed in other floodwater mosquitoes, such as *Aedes vexans* (Meigen) (Linley 1990). However, container (or phytotelmata) species show more contrasting patterns (Alencar et al. 2005a,b), such as larger tubercles and a more intricate reticulum on the ventral surface and small, less prominent tubercles on the dorsal surface, probably linked to some degree of cementing to the substrate (Linley and Service 1994).

The presence of a central papilla or tubercle in the chorionic cells has been described in several species of Aedini. Matsuo et al. (1974) observed that the chorionic cells of *Stegomyia aegypti* (L.) and *Stegomyia pseudalbopicta* Borel have a large papilla in the central area and small tubercles on the periphery. In *Haemagogus tropicalis* Cerqueira & Antunes, there are always one or two tubercles of greater diameter in the central area, surrounded by smaller tubercles on the periphery, a characteristic that differentiates this species from others in the genus *Hemagogus* (Alencar et al. 2008). *Oc. albifasciatus* has a contrasting pattern because most chorionic cells have a small central tubercle surrounded by larger tubercles.

Structures such as filaments seem to have the function of maintaining the adhesion and protecting eggs from removal by predatory insects. Anchorage

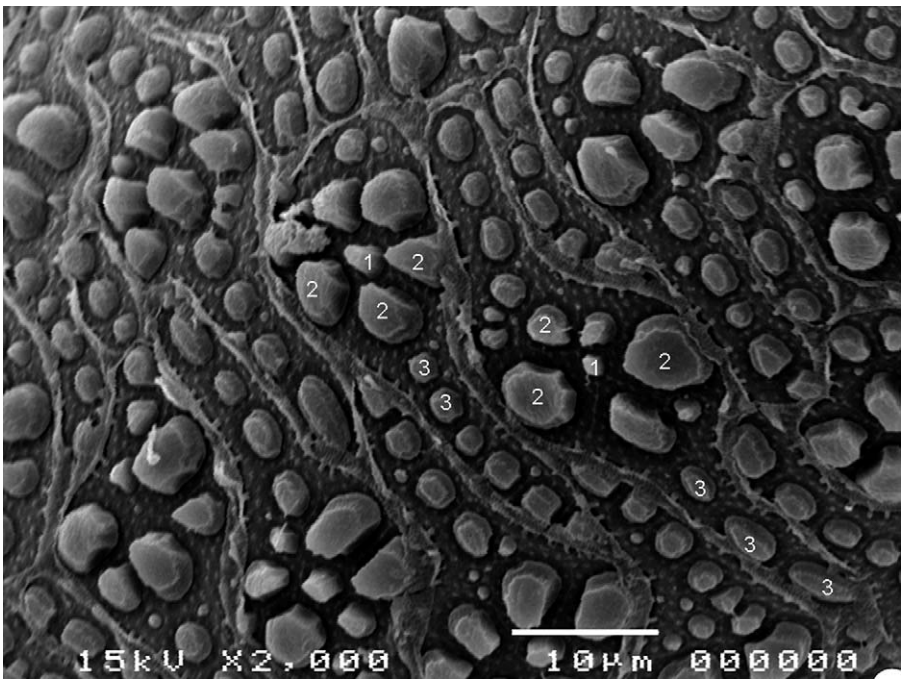

Fig. 3. Typical ornamentation of the outer chorionic reticulum. Detail of the racquet- and wristwatch-shaped cells in the ventral region. (1) Central tubercle. (2) Peripheral tubercles. (3) Tubercles in the handle of the racquet. Scale bare = 10 μm.

of the eggs may also grant protection against flushing by rain water. Filaments are observed on the dorsal surface of eggs of *Hemagogus equinus* Theobald, *Hemagogus janthinomys* Dyar, and *Hemagogus capricornii* Lutz and seem to increase the adhesion surface area at the time of oviposition (Linley and Chadee 1991, Alencar et al. 2005b). The chorionic cells in the dorsal region of the egg of *Ochlerotatus terrens* (Walker) have elongated tubercles with a very regular pattern (Alencar et al. 2005a). They are larger on the periphery and are sometimes fused into groups at the vertices. These

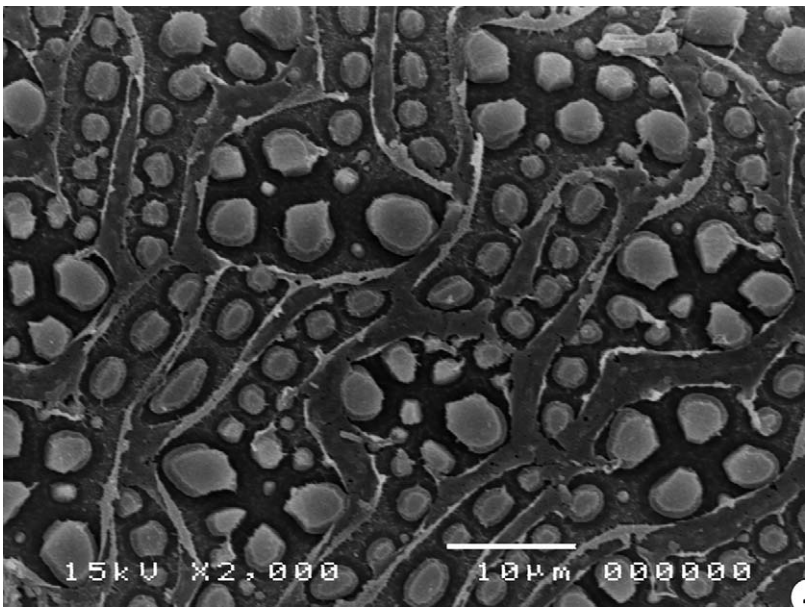

Fig. 4. Dorsal region showing tubercles of different shapes. Scale bare = 10 μm.

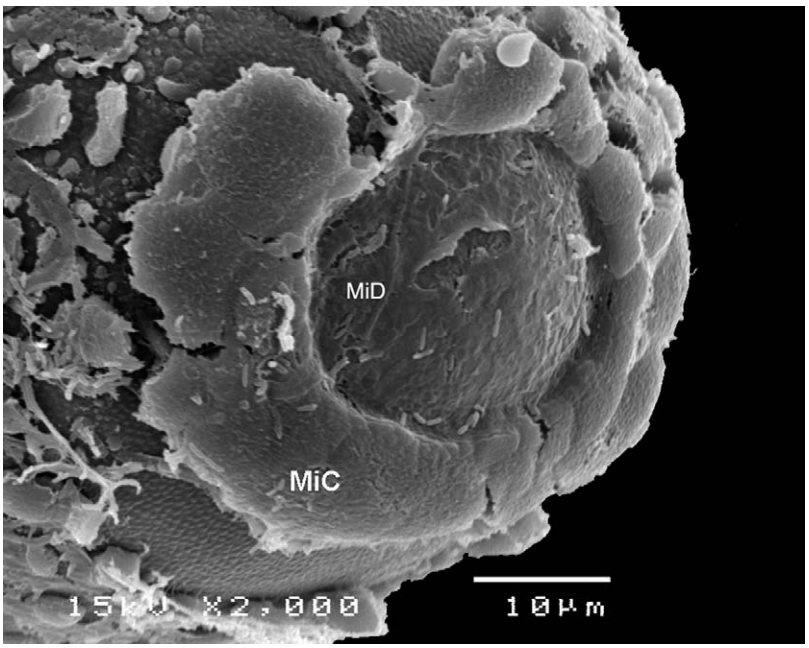

Fig. 5. Anterior region of the egg showing the micropylar disc (MiD) framed by a well-defined micropylar collar (MiC). Scale bar = 10 μm.

tubercles also seem to have the function of maintaining the adhesion of the egg to the substrate. Such structures could not be seen on the egg of *Oc. albifasciatus.* Because this species lays drought-resistant eggs on damp soils (Gleiser and Gorla 2007), the eggs may be washed to depressions where water accumulates and thus may not need anchoring structures. Eggs of *Ochlerotatus* were considered as deposited singly (Reinert et al. 2004), but Reinert et al. (2008) considered the deposition unknown for this species.

The sculptural appearance of the outer chorion exhibits notable variety when examined with scanning electron microscopy, boosting the power of morphological studies that were previously limited to optical microscopy. Scanning electron microscopy has opened up the possibility of performing detailed morphological studies with the objective, among others, of contributing to the identification of mosquitoes (Forattini 2002). Descriptions of eggs have proved useful to distinguish closely related species, such as the *Anopheles quadrimaculatus* complex (Reinert et al. 1997), *Hemagogus* species (Alencar et al. 2008), and *Toxorhynchytes* (Chadee et al. 1987) species. Similar results may be obtained for species of genus *Ochlerotatus*, which includes some very difficult to differentiate species, except by male genitalia, such as *Ochlerotatus hastatus* (Dyar) and *Ochlerotatus oligopistus* (Dyar). By recording the morphological characteristics of the eggs of each species, it may be possible to construct keys for identifying mosquito eggs after their collection from egg traps or soil (or debris) samples.

Acknowledgments

We thank Hertha Mayer Cell Laboratory (Federal University of Rio de Janeiro-UFRJ) for allowing the use of the scanning electron microscope and Adalberto José da Silva for technical support. We thank Ralph Harbach and an anonymous reviewer for suggesting modifications in the text. R.M.G. is a Career Member of the National Council of Scientific and Technical Research (Consejo Nacional de Investigaciones Científicas y Técnicas). This study is part of the Project "Internal dynamics of rain forest: specificity of animal-plant interaction" within the Brazilian-German program "Mata Atlântica," and we acknowledge the financial support by BMBF (01LB0205) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (690143/01-0).

References Cited

Alencar, J., A. E. Guimarães, H. R. Gil-Santana, and J. R. Santos-Mallet. 2005a. Scanning electron microscopy of eggs of *Ochlerotatus* (*Protomacleaya*) *terrens* Walker. J. Am. Mosq. Control Assoc. 21: 355–359.

Alencar, J., A. E. Guimarães, R. P. Mello, C. M. Lopes, N. Degallier, and J. R. Santos-Mallet. 2005b. Scanning electron microscopy study of the egg of *Haemagogus* (*Haemagogus*) *capricornii* Lutz, 1904 (Diptera: Culicidae). J. Med. Entomol. 42: 1–6.

Alencar, J., N. Degallier, A. E. Guimarães, J. M. Costa, W. A. Marques, V. C. Silva, and J. R. Santos-Mallet. 2008. Scanning electron microscopy of the egg of *Haemagogus tropicalis.* J. Am. Mosq. Control Assoc. 24: 16–20.

Avilés, G., M. S. Sabattini, and C. J. Mitchell. 1992. Transmission of Western equine encephalomyelitis virus by Argentine *Aedes albifasciatus* (Diptera: Culicidae). J. Med. Entomol. 29: 850–853.

Campos, R. E. 2008. Long-term storage and viability of *Ochlerotatus albifasciatus* eggs (Diptera: Culicidae). Mem. Inst. Oswaldo Cruz 103: 115–117.

Campos, R. E., and V. E. Sy. 2006. Variation in the hatching response of *Ochlerotatus albifasciatus* egg batches (Diptera: Culicidae) in temperate Argentina. Mem. Inst. Oswaldo Cruz 101: 47–53.

Chadee, D. D., G. J. Small, and S.L.C. O'Malley. 1987. The description of the eggs of *Toxorhynchites moctezuma* (Diptera: Culicidae) from Trinidad, West Indies with notes on other eggs. Mosq. Syst. 19: 1987–237.

Darsie, R. F., Jr. 1985. Mosquitoes of Argentina. Part I. Keys for identification of adult females and fourth stages larvae in English and Spanish (Diptera: Culicidae). Mosq. Syst. 17: 153–253.

Docena, G. H., P. Benítez, R. E. Campos, A. Macia, R. Fernández, and C. A. Fossati. 1999. Detection of allergens in *Aedes albifasciatus* mosquito (Diptera: Culicidae) extracts by immunological methods. J. Invest. Allerg. Clin. Immunol. 9: 165–171.

Forattini, O. P. 2002. Culicidologia médica. Editora da Universidade de São Paulo, São Paulo, Brazil.

Gleiser, R. M., and D. E. Gorla. 2007. Predicting the spatial distribution of *Ochlerotatus albifasciatus* (Diptera: Culicidae) abundance with NOAA imagery. Bull. Entomol. Res. 97: 607–612.

Harbach, R. E., and K. L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Plexus Publishing, Marlton, NJ.

Linley, J. R. 1990. Scanning electron Microscopy of the eggs of *Aedes vexans* and *Aedes infirmatus* (Diptera: Culicidae). Proc. Entomol. Soc. Wash. 92: 685–693.

Linley, J. R., and D. D. Chadee. 1991. Fine structure of the eggs of *Haemagogus equinus* and *Hg. janthinomys* (Diptera: Culicidae). J. Med. Entomol. 28: 434–445.

Linley, J. R., and M. W. Service. 1994. Fine structure of the eggs of *Aedes durbanensis*, *Aedes woodi*, and *Eretmapodites quinquevittatus* (Diptera: Culicidae). Mosq. Syst. 26: 59–72.

Ludueña Almeida, F. F. 1994. Ecología poblacional de *Aedes* (*Ochlerotatus*) *albifasciatus* Macquart, 1838 (Diptera: Culicidae) en el arco sur de Mar Chiquita (Córdoba). Doctoral dissertation, Universidad Nacional de Córdoba, Córdoba, Argentina.

Marcondes, C. B. 2007. Taxonomic changes: disprove or accept them. Trends Parasitol. 23: 302–303.

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

Mitchell, C. J., and R. F. Darsie, Jr. 1985. Mosquitoes of Argentina. Part II. Geographic distribution and bibliography (Diptera: Culicidae). Mosq. Syst. 17: 279–360.

Mitchell, C. J., T. P. Monath, M. S. Sabattini, J. F. Daffner, C. B. Cropp, C. H. Calisher, R. F. Darsie, Jr., and W. L. Jakob. 1987. Arbovirus isolations from mosquitoes collected during and after the 1982–1983 epizootic of western equine encephalitis in Argentina. Am. J. Trop. Med. Hyg. 36: 107–113.

Reinert, J. F. 1975. Mosquito generic and subgeneric abbreviations (Diptera: Culicidae). Mosq. Syst. 7: 105–110.

Reinert, J. F. 2000. New classification for the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera, and notes on certain subgenera and species. J. Am. Mosq. Control Assoc. 16: 175–188.

Reinert, J. F. 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.

Reinert, J. F., P. E. Kaiser, and J. A. Seawright. 1997. Analysis of the *Anopheles* (*Anopheles*) *quadrimaculatus* complex of sibling species (Diptera: Culicidae) using morphological, cytological, molecular, genetic, biochemical, and ecological techniques in an integrated approach. J. Am. Mosq. Control Assoc. Suppl. 13: 1–102.

Reinert, J. F., R. Harbach, and I. J. Kitching. 2004. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zool. J. Linn. Soc. 142: 289–368.

Reinert, J. F., R. E. Harbach, and I. J. Kitching. 2006. Phylogeny and classification of *Finlaya* and allied taxa (Diptera: Culicidae: Aedini) based on morphological data from all life stages. Zool. J. Linn. Soc. 148: 1–101.

Reinert, J. F., R. E. Harbach, and I. J. Kitching. 2008. Phylogeny and classification of *Ochlerotatus* and allied taxa (Diptera: Culicidae: Aedini) based on morphological data from all life stages. Zool. J. Linn. Soc. 153: 29–114.

Sabattini, M. S., T. P. Monath, C. J. Mitchell, J. F. Daffner, G. S. Bowen, R. Pauli, and M. S. Contigiani. 1985. Arbovirus investigations in Argentina, 1977–1980. I. Historical aspects and description of study sites. Am. J. Trop. Med. Hyg. 34: 937–944.

Vezzani, D., D. F. Eiras, and C. Wisnivesky. 2006. Dirofilariasis in Argentina: historical review and first report of *Dirofilaria immitis* in a natural mosquito population. Vet. Parasitol. 136: 259–273.

Received 19 March 2009; accepted 5 May 2009.