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THE EGGS OF *ANOPHELES GALVAOI* AND *ANOPHELES EVANSAE,* TWO SPECIES OF THE SUBGENUS *NYSSORHYNCHUS*

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ABSTRACT. The ultrastructure of the eggs of *Anopheles (Nyssorhynchus) galvaoi* Causey, Deane, and Deane and *Anopheles (Nyssorhynchus) evansae* (Brethes) are described and illustrated with scanning electron micrographs. The eggs of these species are similar to those of *Anopheles (Nyssorhynchus) aquasalis* Curry, *Anopheles (Nyssorhynchus) oswaldoi* (Peryassu), and *Anopheles (Nyssorhynchus) konderi* Galvão and Damasceno in having floats long, widely joined posteriorly on the dorsal surface, the frill encircling the anterior end of the egg, and the crown absent. A few distinctive characters to distinguish *An. evansae* from *An. galvaoi* are given.

KEY WORDS *Anopheles, Nyssorhynchus,* egg, ultrastructure

INTRODUCTION

The subgenus *Nyssorhynchus* Blanchard of *Anopheles* Meigen includes 29 species that have been traditionally subdivided into 3 sections: Albimanus (16 species), Argyritarsis (9 species), and Myzorhynchella (4 species). Cladistic morphological analysis of the subfamily Anophelinae demonstrated the 3 series to be paraphyletic groups (Sallum et al. 2000). In addition to the 2 species described in this article, eggs of 20 species of the subgenus *Nyssorhynchus* have been described and compared with scanning electron micrographs (Rosa-Freitas and Deane 1989; Linley 1992; Rodriguez et al. 1992; Linley et al. 1993, 1996; Linley and Lounibos 1993; Marucci 1996; Forattini et al. 1997, 1998; Lounibos et al. 1997, 1998; Rubio-Palis 1998). Many species of *Nyssorhynchus* are difficult to distinguish by adult female morphological characters. As a consequence, species recognition is most often based on male genitalic characters (Faran 1980, Linthicum 1988), molecular characters (Wilkerson et al. 1995), and also multiple sources of characters, including egg morphological characters (Lounibos et al. 1998). However, intraspecific egg variants may diminish the utility of these characters for species recognition. Intraspecific egg variants have been noted in *An. strodei* Root (Causey et al. 1944), *An. albimanus* Wiedeman (Rodriguez et al. 1992), *An. triannulatus* Neiva and Pinto (Lounibos et al. 1997), and *An. lutzii* Cruz (Forattini et al. 1998). The utility of scanning electron microscopy (SEM) of eggs for phylogenetic studies of the subgenus *Anopheles (Nyssorhynchus)* has been tested by Danoff-Burg and Conn (unpublished data), who also used morphological data from adult males and females as well as nuclear (ITS2) and mitochondrial DNA (COII, ND2, and ND6) sequence data. Egg data seem to be phylogenetically informative because they contributed many unambiguous synapomorphies. In a recent study on mosquito eggs with laser scanning microcopy and SEM, the proposal was made that the flattened side of the egg is the dorsal surface (Valle et al. 1999). This side traditionally has been considered to be the ventral surface (Hinton 1968, Harbach and Knight 1980). Scanning electron micrographs of eggs of *An. galvaoi* Causey, Deane and Deane and *An. evansae* (Brethes) are described and compared in the current paper.

MATERIALS AND METHODS

Eggs were obtained from 7 females of *An. galvaoi* and 5 of *An. evansae* that were collected from human bait in Dourado, Jacaré Pepira River (22°05′00″S, 48°26′33″W), State of São Paulo, Brazil. The procedures adopted to obtain eggs for SEM were described by Forattini et al. (1997). Eggs were allowed 36 h to embryonate, then 10–20 eggs of each oviposition were transferred to vials containing Bouin's fixative. The remaining eggs of each female were allowed to hatch and immatures were raised to get progeny for morphological identification. Lengths and widths of living eggs were measured with a stereomicroscope and digital length-measuring set. Eggs were examined in a Jeol JSM P15 scanning electron microscope (Akishima, Tokyo, Japan). Voucher specimens are at the Entomological Collection of Faculdade de Saúde Pública, Universidade de São Paulo.

RESULTS

Anopheles (Nyssorhynchus) galvaoi
(Figs. 1 and 2)

Size: Width 190–268 μm (mean 234 ± 0.01 μm), length 481–551 μm (mean 509 ± 0.02 μm),

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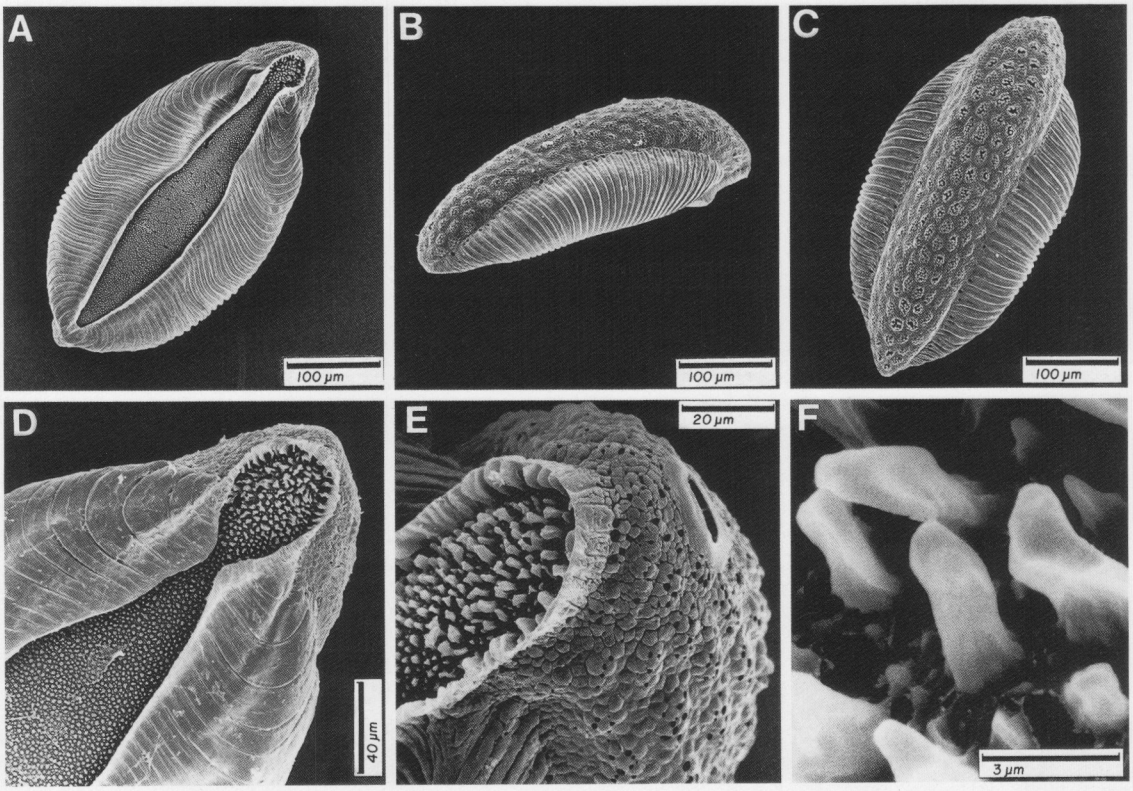

Fig. 1. Egg of *Anopheles* (*Nyssorhynchus*) *galvaoi.* A. Entire egg, anterior end at top, dorsal view. B. Entire egg, lateral view. C. Entire egg, anterior end at top, ventral view. D. Anterior end, dorsal view. E. Anterior end, dorsolateral view. F. Anterior end, showing details of deck tubercles.

length to width ratio 1.91–2.59 (mean 2.16 ± 0.1) (n = 72 eggs from 7 females).

Overall appearance: Black in color; boat-shaped in dorsal and lateral views (Figs. 1A, 1B); in lateral view the contour is slightly concave dorsally, curved ventrally (Fig. 1B). Floats lateral in position, long, well developed (Figs. 1A–1C), frill positioned anterior to the floats (Fig. 1D).

Dorsal surface: Deck in middle region of egg wide, narrow at both anterior and posterior ends; deck completely enclosed by floats and frill (Fig. 1A). Deck tubercles irregularly shaped with tiny tubercles intermixed with larger, more prominent tubercles (Fig. 2B); tubercles present on most anterior part of deck larger than those on middle and posterior parts (Figs. 1D, 2A).

Anterior end: Frill encircling anterior end of egg (Figs. 1D, 1E). Micropyle situated in the center of a low mound (Figs. 2C, 2D), micropylar collar separated from anterior margin of frill by narrow area (Fig. 2C). Collar surface smooth, inner boundary nearly straight between sectors and 7 short rays, connecting with micropylar disk about midway to the micropyle; micropylar disk with a continuous ring within sectors limited by short rays (Fig. 2D).

Posterior end: Slightly narrower than anterior end; pointed, only tip of egg visible beyond margin of floats; floats widely joined on posterior end (Fig. 2A); lobed tubercles absent. Posterior deck covered with tubercles similar to those present on middle deck (Fig. 2A).

Ventral and lateral surfaces: Plastron composed of chorionic cells with well-defined boundaries, cell surfaces covered with approximately elliptical, bumpy mounds that are perforated by pores irregular in size (Figs. 2E, 2F). Floats long, well developed, extending from anterior to posterior end of egg; ribs about 36 in number, slightly divided into lobes (Fig. 2E).

Anopheles (*Nyssorhynchus*) *evansae*
(Figs. 3 and 4)

Size: Width 190–266 µm (mean 238 ± 0.02 µm), length 455–533 µm (mean 492 ± 0.02 µm), length to width ratio 1.86–2.49 (mean 2.07 ± 0.13) (n = 36 eggs from 5 females).

Overall appearance: Black in color. Boat-shaped in dorsal and lateral views (Figs. 3A–3C). Floats wide, long, well developed, lateral in position, broadly joined posteriorly (Figs. 3A, 3F).

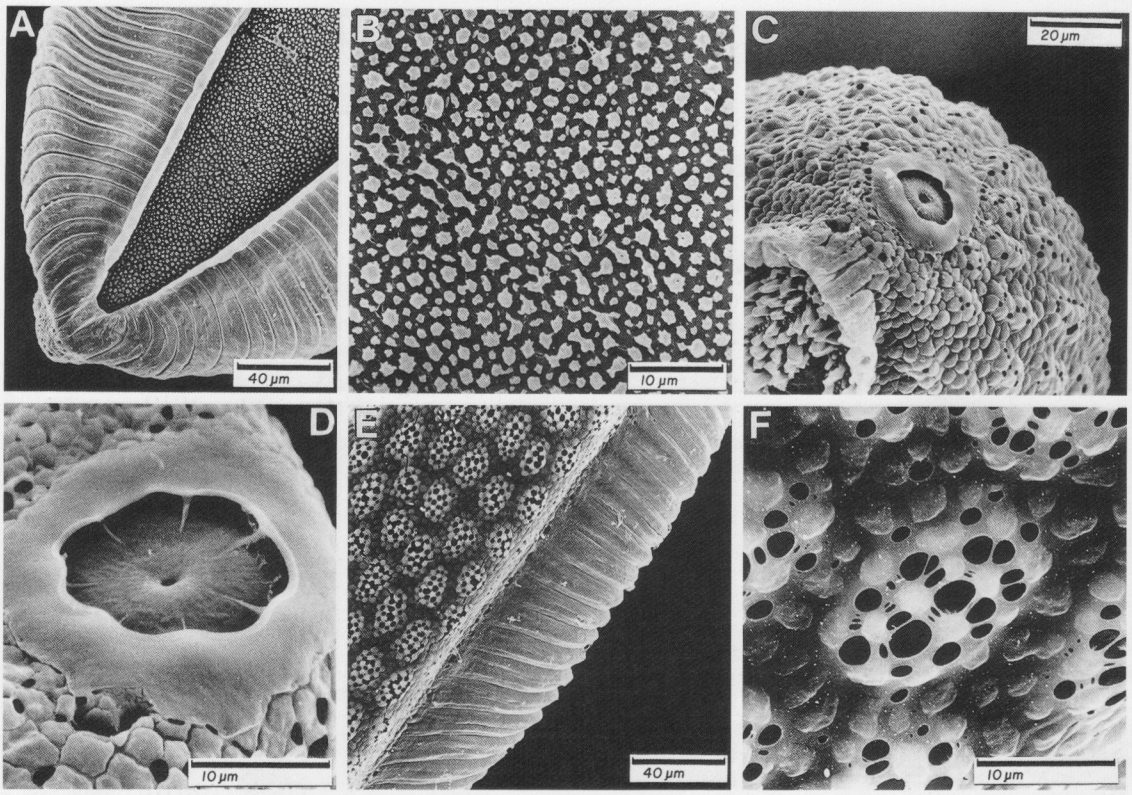

Fig. 2. Egg of *Anopheles* (*Nyssorhynchus*) *galvaoi*. A. Posterior end, dorsal view. B. Deck tubercles, middle region. C. Anterior pole showing micropyle. D. Micropyle. E. Outer chorion and float, ventral view. F. Outer chorion, ventral view.

Frill positioned anterior to the floats (Figs. 3A, 3D).

Dorsal surface: Deck in middle region of egg wide, narrower at posterior end, deck completely enclosed by floats and frill (Fig. 3A). Deck tubercles irregularly shaped with tiny tubercles intermixed with larger more prominent tubercles (Figs. 3D–3F, 4A); tubercles present on most anterior part of deck larger than those on middle and posterior parts (Figs. 3D, 3E).

Anterior end: Frill well developed, encircling anterior end of egg (Figs. 3A, 3D). Micropyle situated in the center of a low mound, micropylar collar smooth, inner boundary nearly straight between sectors and 8 short rays, extending from collar to about midway of micropylar disk that contains the micropyle (Figs. 4B, 4C); micropylar disk with a continuous ring within sectors limited by short rays (Fig. 4C).

Posterior end: Slightly narrower than anterior end, pointed, only tip of egg visible beyond margin of float; floats widely joined on posterior end (Fig. 3F); lobed tubercles absent. Posterior deck covered with tubercles similar to those present on middle deck (Fig. 3F).

Ventral and lateral surfaces: Plastron composed of chorionic cells with well-defined boundaries, cell surfaces covered with approximately round, bumpy mounds that are perforated by pores irregular in size (Figs. 4D–4F). Floats long, well developed, extending from anterior to posterior end of egg, ribs about 29–32 in number, slightly divided into lobes (Figs. 3B, 4E).

DISCUSSION

The ultrastructure of the eggs of 20 species of the subgenus *Anopheles* (*Nyssorhynchus*) has been described and compared using scanning electron micrographs (Rosa-Freitas and Deane 1989; Linley 1992; Rodriguez et al. 1992; Linley et al. 1993, 1996; Linley and Lounibos 1993; Marucci 1996; Forattini et al. 1997, 1998; Lounibos et al. 1997, 1998; Rubio-Palis 1998). Based on ultrastructure morphology, eggs of *An. galvaoi* and *An. evansae* are similar to those of *An. konderi* Galvão and Damasceno, *An. oswaldoi* (Peryassu), and *An. aquasalis* Curry in having very large, ventral floats, subequal to length of egg; anterior and posterior crowns absent; anterior frill open, encircling anterior end of egg without interior fusion; plastron covering posterior end of the egg; anterior

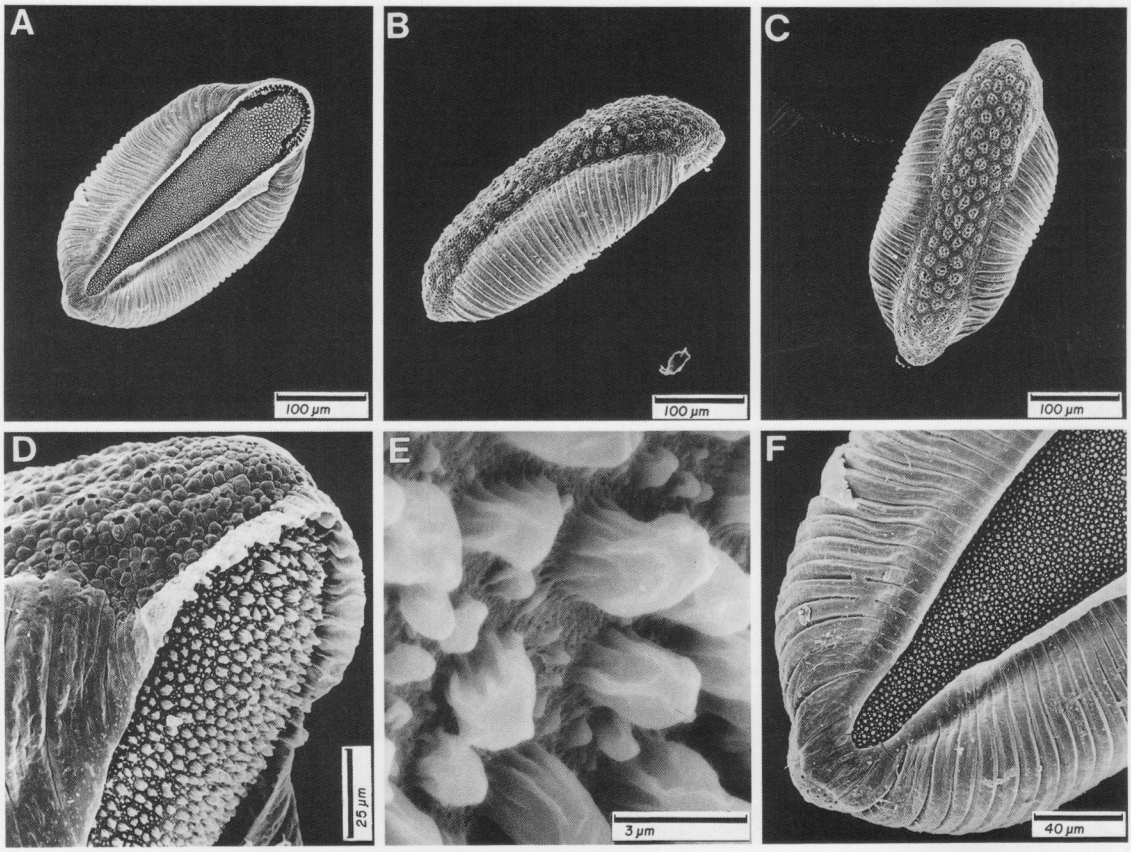

Fig. 3. Egg of *Anopheles* (*Nyssorhynchus*) *evansae.* A. Entire egg, anterior end at top, dorsal view. B. Entire egg, lateral view. C. Entire egg, anterior end at top, ventral view. D. Anterior end, dorsolateral view. E. Anterior end, showing details of deck tubercles. F. Posterior end, dorsal view.

float not fused closed, posterior region of float fused closed; chorionic cell ultrastructure bubbled, chorionic cell boundaries well defined, chorion covered with many mounds that are perforated by pores.

The separation of *An. evansae, An. galvaoi, An. oswaldoi, An. konderi,* and *An. aquasalis* by egg morphology can be problematic because few morphological characters exist that may have limited usefulness for distinguishing these species, and intraspecific egg variants also possibly exist among geographically distinct populations. Currently, eggs of *An. evansae, An. oswaldoi,* and *An. konderi* may be distinguished from that of *An. galvaoi* by the tubercles on the anterior end of the deck area that are shorter and broader than those present on *An. galvaoi.* Also, in *An. evansae, An. oswaldoi,* and *An. konderi* those tubercles have deeply buttressed walls and approximately flat tips (Fig. 3E), whereas in *An. galvaoi* they are taller than broad, tapering to apex with nearly smooth walls (Fig. 1F).

Finally, in *An. aquasalis* the anterior, middle, and posterior deck tubercles are similar in shape and development, and the ventral plastron is made up of hexagonal or pentagonal outer chorionic cells with indistinct boundaries (see Linley et al. 1993). In *An. galvaoi, An. evansae, An. konderi,* and *An. oswaldoi,* the chorionic cells of the ventral plastron have distinct boundaries. Also, the inner boundary of the micropylar collar is significantly concave between sectors in *An. aquasalis, An. oswaldoi,* and *An. konderi,* whereas it is approximately straight in *An. galvaoi* and *An. evansae.* Except for the boundary of the micropylar collar character, distinguishing *An. evansae* from *An. oswaldoi* and *An. konderi* by egg morphology is not an easy task because of the absence of species-specific characters.

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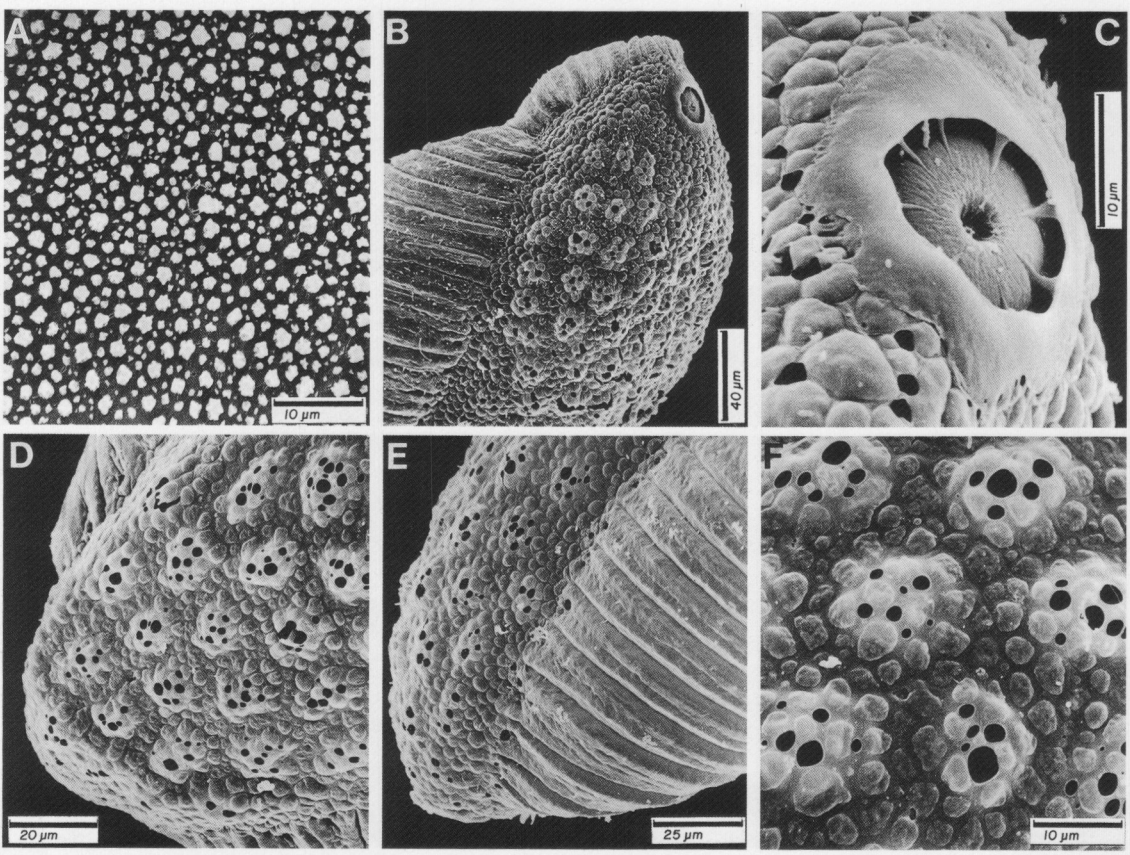

Fig. 4. Egg of *Anopheles* (*Nyssorhynchus*) *evansae*. A. Deck tubercles, middle region. B. Anterior pole, ventrolateral view, showing micropyle. C. Micropyle. D. Posterior end, ventral view. E. Outer chorion and float, lateral view. F. Outer chorion, ventral view.

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