# DESCRIPTION OF THE EGGS OF ANOPHELES (KERTESZIA) LANEANUS AND ANOPHELES (NYSSORHYNCHUS) ANTUNESI (DIPTERA: CULICIDAE) BY SCANNING ELECTRON MICROSCOPY

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ABSTRACT. The eggs of Anopheles laneanus Correa and Cerqueira and Anopheles antunesi Galvão and Amaral are described and illustrated with scanning electron micrographs. The egg of An. laneanus is compared with those of Anopheles cruzii Dyar and Knab and Anopheles bellator Dyar and Knab, and An. antunesi is compared with Anopheles lutzii Cruz and other Nyssorhynchus species.

#### **INTRODUCTION**

Anopheles subgenus Kerteszia includes several species that are acknowledged as vectors of human malaria in several countries throughout South America. This list includes Anopheles neivai Howard, Dyar and Knab, An. bellator Dyar and Knab, An. homunculus Komp, and An. cruzii Dyar and Knab. In addition, Anopheles laneanus Correa and Cerqueira is suspected to be a vector of human malaria in Bolivia (Martinez and Prosen 1953).

Despite its public health importance, the subgenus *Kerteszia* is still poorly known systematically and needs a more comprehensive taxonomic study. Since its description, *An. laneanus* has been the subject of several taxonomic changes before being definitively elevated to specific rank (Forattini 1962, Zavortink 1973). The immature stages of this species are not adequately described, and no information on the egg has been published previously. Further, *An. cruzii* and *An. bellator* are the only 2 species of the *Kerteszia* subgenus for which the ultrastructure of eggs was described with scanning electron microscopy (SEM) (Forattini and Marucci 1993).

The Myzorhynchella Section of An. (Nyssorhynchus) includes 4 incompletely described species: An. antunesi Galvão and Amaral, An. lutzii Cruz, An. parvus (Chagas), and An. nigritarsis (Chagas). The egg, adult male, and immature stages of An. nigritarsis remain unknown. The eggs of An. antunesi, An. lutzii, and An. parvus are incompletely known and based on examinations by light microscopy: Galvão and Amaral (1940), Galvão (1941), and Forattini (1962) for An. antunesi, Galvão (1941) for An. lutzii, and Cova Garcia (1961) for An. parvus. The present study provides descriptions of the eggs of An. laneanus and An. antunesi with scanning electron micrographs.

# MATERIALS AND METHODS

Eggs were obtained from ovipositions of 10 females of An. laneanus and 3 females of An. antunesi collected in Campos do Jordão, State of São Paulo, Brazil, the type locality of both species. The females were blood fed and traumatized by removal of a wing in order to induce oviposition. Eggs were fixed in alcoholic Bouin's solution 24 h after oviposition. Fixed eggs were prepared for SEM following methodology described by Forattini and Marucci (1993) and examined in a JEOL JSM-P15 scanning electron microscope immediately after sputter-coating with gold/carbon. Measurements of An. laneanus and An. antunesi were made on 43 eggs from 5 females and 11 eggs from 3 females, respectively. Eggs were measured under light microscope using a digital length-measuring set WILD MMS 235. Terminology follows that of Harbach and Knight (1980).

## RESULTS

## Anopheles (Kerteszia) laneanus Correa and Cerqueira (Figs. 1–3)

Size: Width 0.13–0.17 mm (mean 0.16  $\pm$  0.009 mm), length 0.41–0.45 mm (mean 0.43  $\pm$  0.01 mm), length/width ratio 2.56–3.37 (mean 2.78  $\pm$ 0.16) (n = 43 eggs from 5 females). Color: Black. Overall appearance: Broadly boat-shaped in lateral and ventral views (Figs. 1A, 1B and 2A); in lateral view the contour is straight ventrally, concave on dorsal surface (Fig. 2H). Floats are lateral in position, closer to ventral than dorsal surface, well developed, long (Fig. 1A, 1B); frill positioned anterior and posterior to the floats (Figs. 1A, 1B). Ventral surface: Deck narrow (Figs. 1B and 2A), slightly wider at anterior and posterior 0.2, with slightly expanded areas at anterior and posterior ends both bearing a row of 3-5 lobed tubercles (Figs. 1B and 2D–G). The lobed tubercles are large with fingerlike extensions like spokes of a cartwheel, about 9 in number. The deck is completely enclosed by floats and frill and covered with fine tubercles. Deck tubercles irregularly shaped (Figs. 2B, 2C) with several shallow cavities in the tuber-

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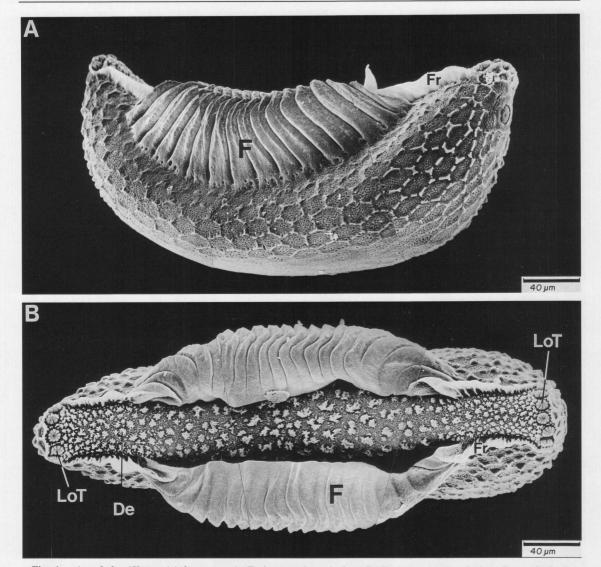


Fig. 1. Anopheles (Kerteszia) laneanus. A. Entire egg, lateral view. B. Entire egg, ventral view. De = deck, F = float, Fr = frill, LoT = lobed tubercle.

cles, and smaller, variable shaped tubercles intermixed with larger tubercles. Dorsal and lateral surfaces: Outer chorionic cells of dorsal (Figs. 3A-3F) and lateral (Fig. 2H) surfaces usually hexagonal in shape, sometimes pentagonal. Float large, almost two-thirds of the egg length, ribs 15-17 (n = 5) in number (Figs. 1A, 1B and 2I). Anterior end, micropyle: Anterior end slightly wider than posterior (Fig. 2A), frill more developed laterally, less developed around lobed tubercles (Figs. 1A and 2D, 2F), deck tubercles on this area similar in general aspect but smaller and denser than those of middle deck (Fig. 1B). Micropyle situated in the center of micropylar collar (Figs. 3G-3I). Micropylar collar separated from anterior margin of frill, collar surface smooth, inner boundary with shallow excavations between short rays that connect with the micropylar disk about midway to the micropyle (Figs. 3G–3I). Chorionic cells around micropylar collar well developed, mostly pentagonal in shape (Fig. 3H). Micropyle situated in a low mound (Fig. 3I). *Posterior end:* Similar to anterior end with respect to frill, tubercles, conformation of plastron, and deck (Figs. 1B and 2A).

## Anopheles (Nyssorhynchus) antunesi Galvão and Amaral (Figs. 4 and 5)

Size: Width 0.20–0.23 mm (mean 0.22  $\pm$  0.01 mm), length 0.53–0.62 mm (mean 0.60  $\pm$  0.04 mm), length/width ratio 2.38–3.05 (mean 2.77  $\pm$ 

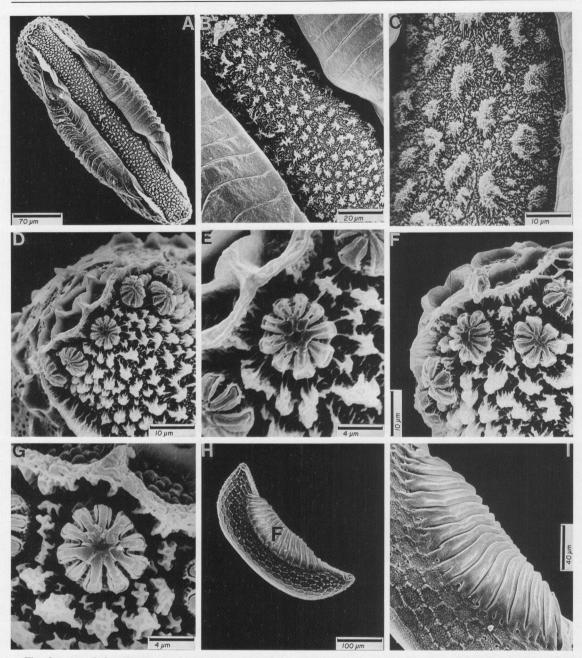


Fig. 2. Anopheles (Kerteszia) laneanus. A. Entire egg, ventral view, anterior end at top. B, C. Deck tubercles, central area. D, E. Lobed tubercles, anterior end. F, G. Lobed tubercles, posterior end. H. Entire egg, lateral view. I. Float. F =float.

0.22) (n = 11 eggs from 3 females). Color: Black. Overall appearance (Figs. 4A-4C): Broadly boatshaped in lateral view (Fig. 1B), spindle-shaped in ventral view (Figs. 1A, 1C), ventral surface slightly concave or nearly flat, and dorsal surface convex in lateral view. Float lateral in position, covering part of ventral surface, distance between floats variable, sometimes floats nearly in contact with each other in middle region of ventral surface; frill absent (Fig. 4A). *Ventral surface:* Deck narrow, narrower at anterior and posterior areas (Fig. 4A), lobed tubercles absent on anterior and posterior ends (Figs. 5B, 5E). Deck covered uniformly with fine tubercles (Figs. 4A and 5G), irregular in shape (Fig. 5H). *Dorsal and lateral surfaces:* Outer chorionic cells of dorsal plastron with poorly marked boundaries (Figs. 4D-4F). Floats long, extending nearly total length of egg, very deep at middle, ribs



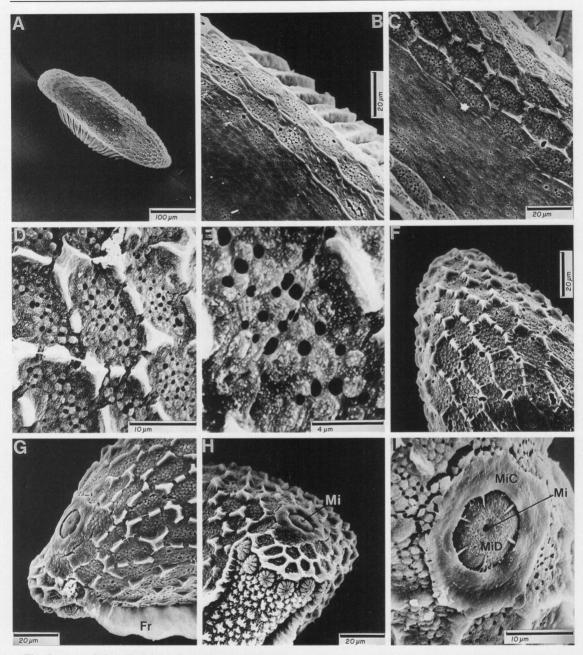


Fig. 3: Anopheles (Kerteszia) laneanus. A. Entire egg, dorsal view, anterior end at top, on the left. B, C. Outer chorion, dorsal view. D, E. Outer chorion, anterior end, dorsal view. F. Posterior end, dorsal view. G. Anterior end, lateral view. H. Anterior end, dorsoventral view. I. Micropylar apparatus. Mi = micropyle, MiC = micropylar collar, MiD = micropylar disc.

30-40 (n = 3) in number, ribs weakly divided into lobes (Fig. 5I), lobes less distinguishable at anterior and posterior ends. *Anterior end, micropyle:* Anterior end more blunt than posterior (Figs. 5B, 5E), frill not developed at anterior end, floats folded over anterior end (Figs. 5A, 5B), deck tubercles adjacent to anterior area similar to those on middle deck (Fig. 5B), micropylar collar separated from anterior margin of float, collar surface smooth, exterior outline irregular, inner boundary with shallow excavations between short rays that divide disk into 8 sectors (Fig. 5C). Chorionic cells around micropylar collar similar to those of dorsal surface in having blisterlike aspect (Fig. 5C). *Posterior end:* Narrower than anterior end (Fig. 5E), somewhat similar in ventral view with respect to conformation

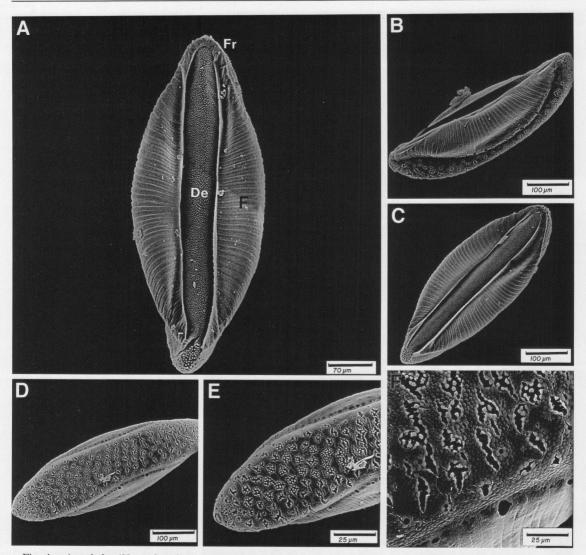


Fig. 4. Anopheles (Nyssorhynchus) antunesi. A. Entire egg, ventral view, anterior end at top. B. Entire egg, lateral view. C. Entire egg, ventral view, anterior end at top, on the right. D. Entire egg, dorsal view. E. Anterior end, dorsal view. F. Outer chorion, dorsal surface. De = deck, F = float, Fr = frill.

of plastron and deck, tubercles larger than those of middle deck, frill absent (Figs. 5D-5F).

#### DISCUSSION

The egg of An. (Kerteszia) laneanus is similar to those of An. cruzii Dyar and Knab and An. bellator Dyar and Knab, as described and illustrated by Forattini and Marucci (1993), with respect to overall appearance, conformation of plastron and deck, frills, floats, micropyle, and lobed tubercles. However, An. laneanus seems to differ from An. cruzii and An. bellator in deck tubercles, which are uniform in An. cruzii and An. bellator but are sparser and larger in the middle deck in An. laneanus.

By comparing the egg of An. antunesi with those

of other Nyssorhynchus species examined with SEM, it was possible to find that the egg of An. antunesi is structurally distinct from all other Nyssorhynchus, such as Anopheles darlingi Root (Linley 1992), An. albimanus Wiedemann (Rodriguez et al. 1992), An. aquasalis Curry (Linley et al, 1993), An. rangeli Gabaldon, Cova Garcia and Lopez, and An. dunhami Causey (Linley and Lounibos 1993). The main differences involve characters of the deck, floats, and frill. Anopheles antunesi can be unmistakably recognized by the absence of frill on anterior and posterior ends and the presence of a small posterior deck area supporting larger tubercles than those found on the main deck. This unusual posterior area is barely isolated from the main part of the deck, and the floats are long, extending

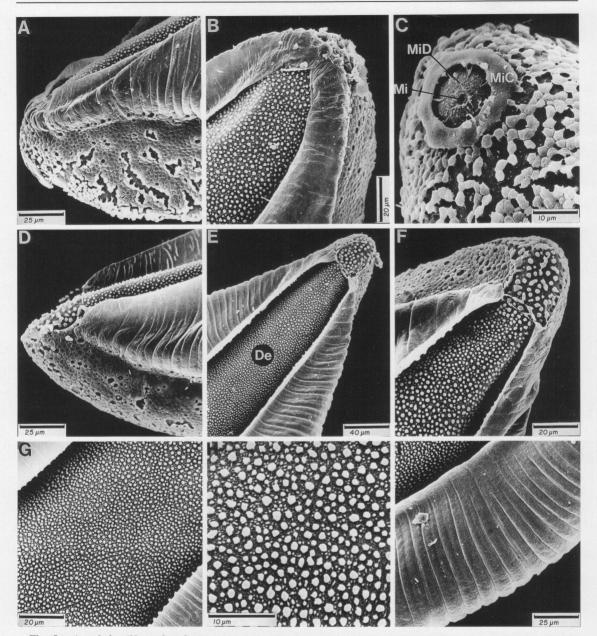


Fig. 5. Anopheles (Nyssorhynchus) antunesi. A. Anterior end, lateral view. B. Anterior end, ventral view. C. Micropylar apparatus. D. Posterior end, lateral view. E, F. Posterior end, ventral view. G, H. Deck tubercles, central area. I. Float. De = deck, Mi = micropyle, MiC = micropylar collar, MiD = micropylar disc.

almost the entire egg, widely joined anteriorly on the ventral surface, and the anterior end is barely visible beyond the anterior margin of the floats. Further, *An. antunesi* can be easily distinguished from *An. lutzii*, whose egg has a small anterior deck area surrounded by the frill, and the floats are widely joined posteriorly on the ventral surface (Galvão 1941). Finally, the eggs of *An. darlingi, An. rangeli,* and *An. dunhami* are clearly distinct from that of An. antunesi by having a deep micropylar crown (Linley and Lounibos 1993).

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