

ULTRASTRUCTURE OF EGGS OF *ANOPHELES RONDONI*, *ANOPHELES LUTZII*, AND *ANOPHELES PARVUS*, THREE SPECIES OF THE SUBGENUS *NYSSORHYNCHUS*

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ABSTRACT. The ultrastructures of the eggs of *Anopheles* (*Nyssorhynchus*) *rondoni* (Neiva & Pinto), *Anopheles* (*Nyssorhynchus*) *lutzii* Cruz, and *Anopheles* (*Nyssorhynchus*) *parvus* (Chagas) are described and illustrated with scanning electron micrographs. The egg of *Anopheles rondoni* is similar in several respects to those of other species of the *Argyritarsis* Section. The egg of *An. lutzii* is similar to that of *Anopheles antunesi* Galvão and Amaral in having floats widely joined anteriorly on the ventral side, and the anterior end barely visible beyond the floats. The egg of *An. parvus* is remarkable in possessing an anterior fingerlike structure that bears several lobed tubercles at the apex. The fingerlike structure and the micropyle are within the prominent anterior crown formed by the frill. The egg of *An. parvus* has floats with the anterior pole uppermost, which is an unusual position for *Anopheles*.

KEY WORDS *Anopheles*, *Anopheles rondoni*, *Anopheles lutzii*, *Nyssorhynchus*, egg ultrastructure, malaria vectors, mosquito taxonomy

INTRODUCTION

Anopheles rondoni (Neiva and Pinto) is the sister species of *Anopheles strodei* Root and *Anopheles benarrochi* Gabaldón, Cova-Garcia, and Lopez in the Strodei Complex of the Albimanus Section of *Anopheles* (*Nyssorhynchus*) (Faran 1980). *Anopheles lutzii* Cruz and *Anopheles parvus* (Chagas) belong to the Myzorhynchella Section of the subgenus *Nyssorhynchus*. *Anopheles rondoni*, *An. lutzii*, and *An. parvus* are widely distributed in the southern Neotropical region. Except for eggs of *An. rondoni* and *Anopheles ininii* Senevet and Abonnenc, the eggs of only a few species of the Albimanus Section have been described using a scanning microscope: *Anopheles albimanus* Wiedemann, (Rodriguez et al. 1992); *Anopheles darlingi* Root (Linley 1992); *Anopheles rangeli* Gabaldón, Cova-Garcia, and Lopez and *Anopheles dunhami* Causey (Linley and Lounibos 1993); and *Anopheles aquasalis* Curry (Linley et al. 1993). The eggs of other species have been described using a light microscope (Causey et al. 1944). Within the Myzorhynchella Section the eggs of *An. lutzii* and *An.*

parvus have been described using a stereomicroscope: *An. lutzii* (Galvão 1941) and *An. parvus* (Causey et al. 1944, and Cova-Garcia 1961). The egg of *Anopheles antunesi* has also been studied with a scanning electron microscope (Forattini et al. 1997). Remarkable morphological differences are found among the eggs of the species of the subgenus *Nyssorhynchus*.

Eggs from gravid females of *An. rondoni*, *An. lutzii*, and *An. parvus* were obtained from field collections made in Dourado and Pariquera-Açu, State of São Paulo, Brazil. These eggs were examined with light microscopy and scanning electron microscopy and are described in this paper.

MATERIALS AND METHODS

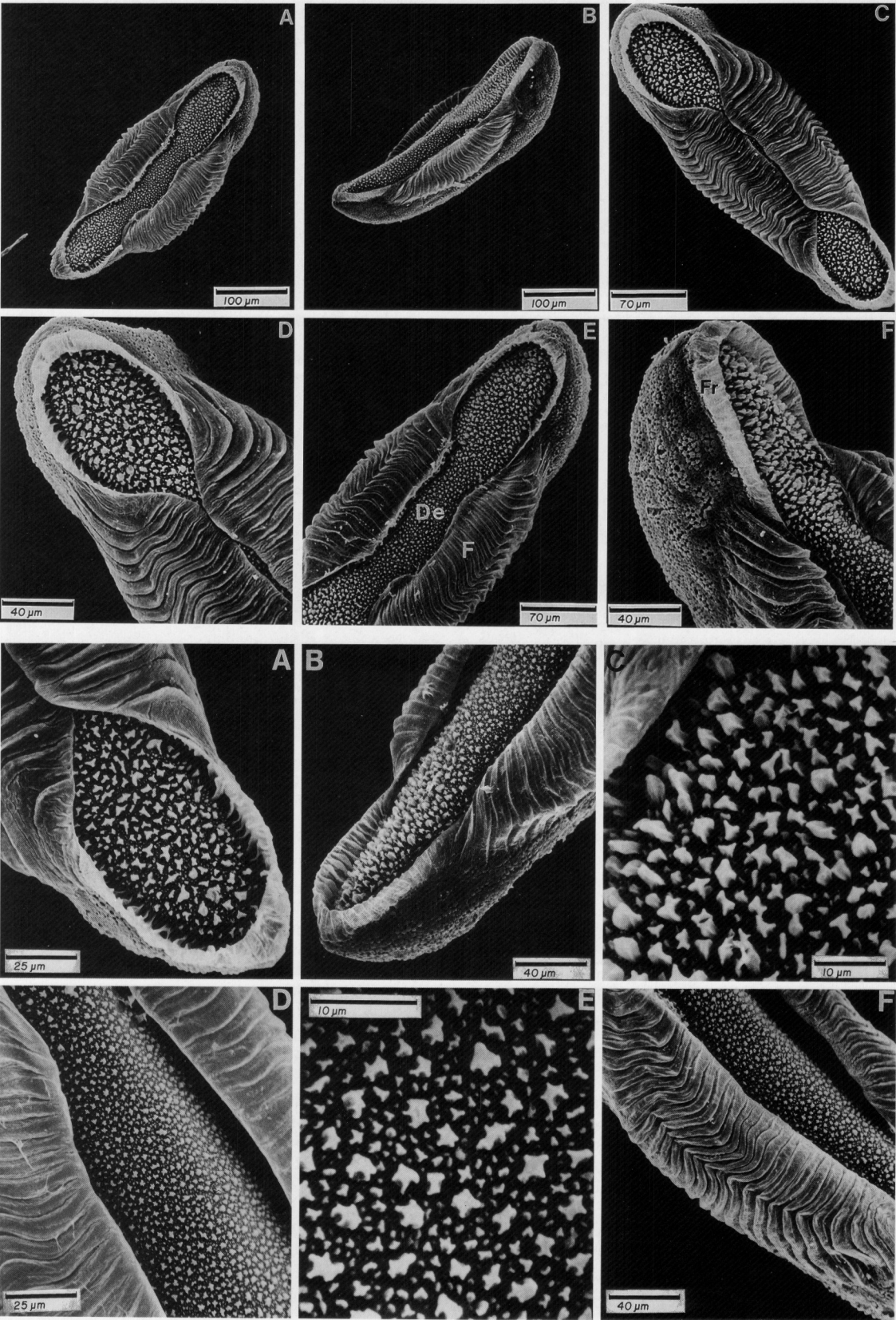
Eggs were obtained from ovipositions of 10 females of *An. rondoni*, 3 of *An. lutzii*, and 3 of *An. parvus*. Females of the 3 species were taken with a human bait in Dourado, SP 255, road km 124 (22°5'S, 48°26'33"W), and one female of *An. lutzii* was collected with a Shannon trap, in Pariquera-Açu; both sites are in the State of São Paulo, Brazil. The preparative procedures to obtain eggs for scanning electron microscopy were as described by Forattini et al. (1997). Lengths and widths of living eggs were measured with a stereomicroscope and a digital length-measuring set. Eggs were examined using a scanning electron microscope. Terminology follows Harbach and Knight (1980).

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Fig. 1. Egg of *Anopheles* (*Nyssorhynchus*) *rondoni*. A. Entire egg, anterior end at top, on the right, ventral view. B. Entire egg, lateral view. C. Entire egg, anterior end at top, on the left, ventral view. D. Anterior end, ventral view. E. Anterior end and floats, ventral view. F. Anterior end, lateral view. De, deck; F, float; Fr, frill.

Fig. 2. Egg of *Anopheles* (*Nyssorhynchus*) *rondoni*. A. Posterior end, ventral view. B. Posterior end, lateral view. C. Deck tubercles, anterior end. D, E. Deck tubercles, central area. F. Float, lateral view.



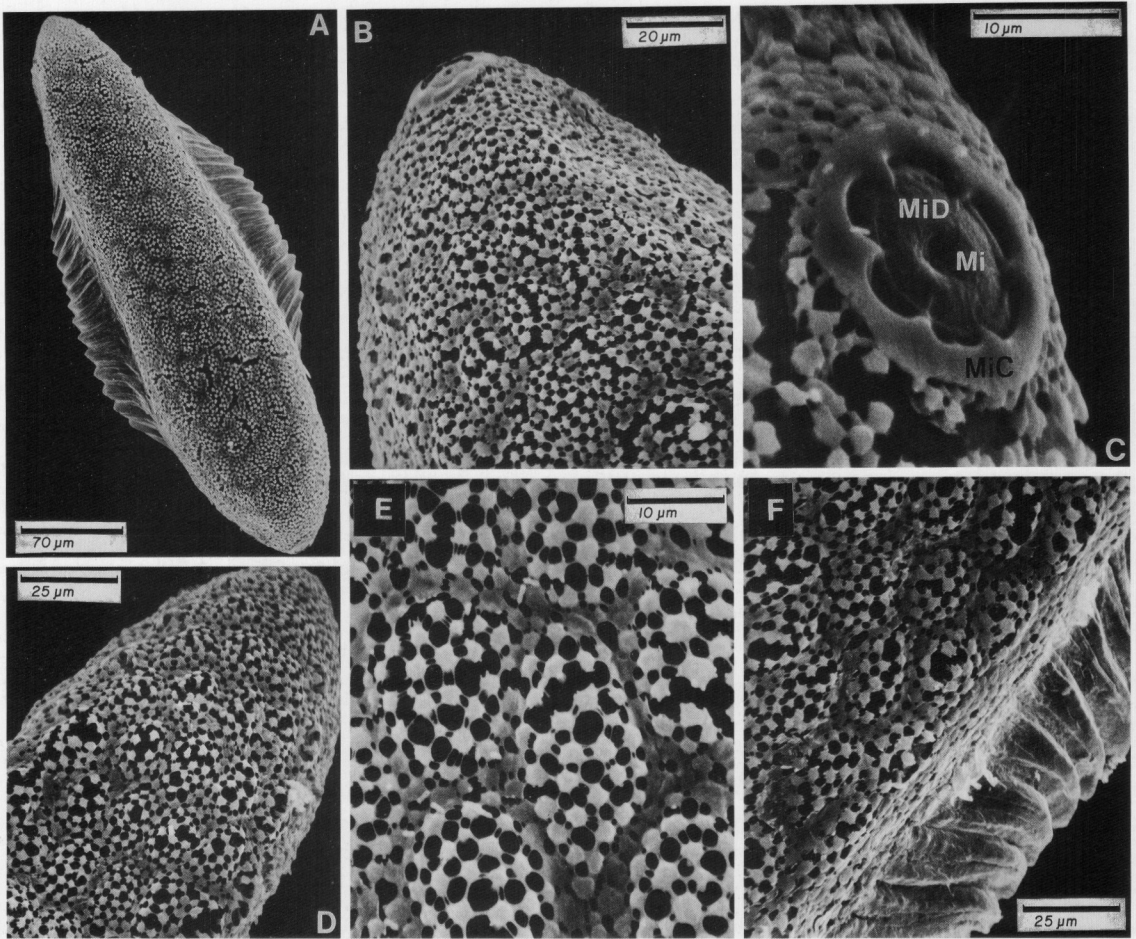


Fig. 3. Egg of *Anopheles (Nyssorhynchus) rondoni*. A. Entire egg, posterior end at top, on the left, dorsal view. B. Anterior end, dorsal view. C. Micropyle. D. Posterior end, dorsal view. E. Outer chorion, dorsal surface. F. Outer chorion and float, dorsal view. Mi, micropyle; Mic, micropylar collar; MiD, micropylar disc.

RESULTS

Anopheles (Nyssorhynchus) rondoni (Neiva and Pinto) (Figs. 1–3)

Size: Width 158–205 μm (mean $180 \pm 0.01 \mu\text{m}$), length 431–532 μm (mean $474 \pm 0.02 \mu\text{m}$), length to width ratio 2.37–2.98 (mean 2.64 ± 0.13) ($n = 61$ eggs from 8 females).

Color: Black.

Overall appearance: Broadly boat-shaped in ventral view (Figs. 1A, 1C); in lateral view the contour is slightly concave ventrally, curved dorsally (Fig. 1B). Floats lateral in position, closer to ventral than dorsal surface, well developed (Figs. 1A, 1B); frill positioned anterior and posterior to the floats (Fig. 1C).

Ventral surface: Deck in middle region of egg short, narrow, sometimes deck partly or completely obliterated by float (Fig. 1C), deck completely enclosed by floats and frill (Figs. 1A, 1C). Deck tu-

bercles irregularly shaped with tiny tubercles intermixed with larger, more prominent tubercles (Fig. 2D), each deck tubercle irregular in outline, walls with shallow indentation, top smooth (Fig. 2E), tubercles present on most anterior part of deck (Figs. 1D, 1F; and 2C) larger and more dense than those on most posterior part (Figs. 2A, 2B), tubercles on middle region of deck smallest (Fig. 2D). Frill present on anterior and posterior ends of egg (Figs. 1F and 2B); lobed tubercles absent.

Dorsal and lateral surfaces: Outer chorionic cells of dorsal surface with indistinct boundaries (Fig. 3A); area of each cell slightly raised, covered with small nodules interspersed with several pores (Figs. 3A, 3B, 3D), areas encircling chorionic cells (Fig. 3E) and those adjacent to floats with fewer pores (Fig. 3F). Floats moderately long, extending about 0.5 of length of egg, formed by 15–24 ($n = 6$) ribs (Figs. 1C, 1E; 2F; and 3A).

Anterior end: Anterior end slightly wider than posterior end (Figs. 1A and 3A), frill well devel-

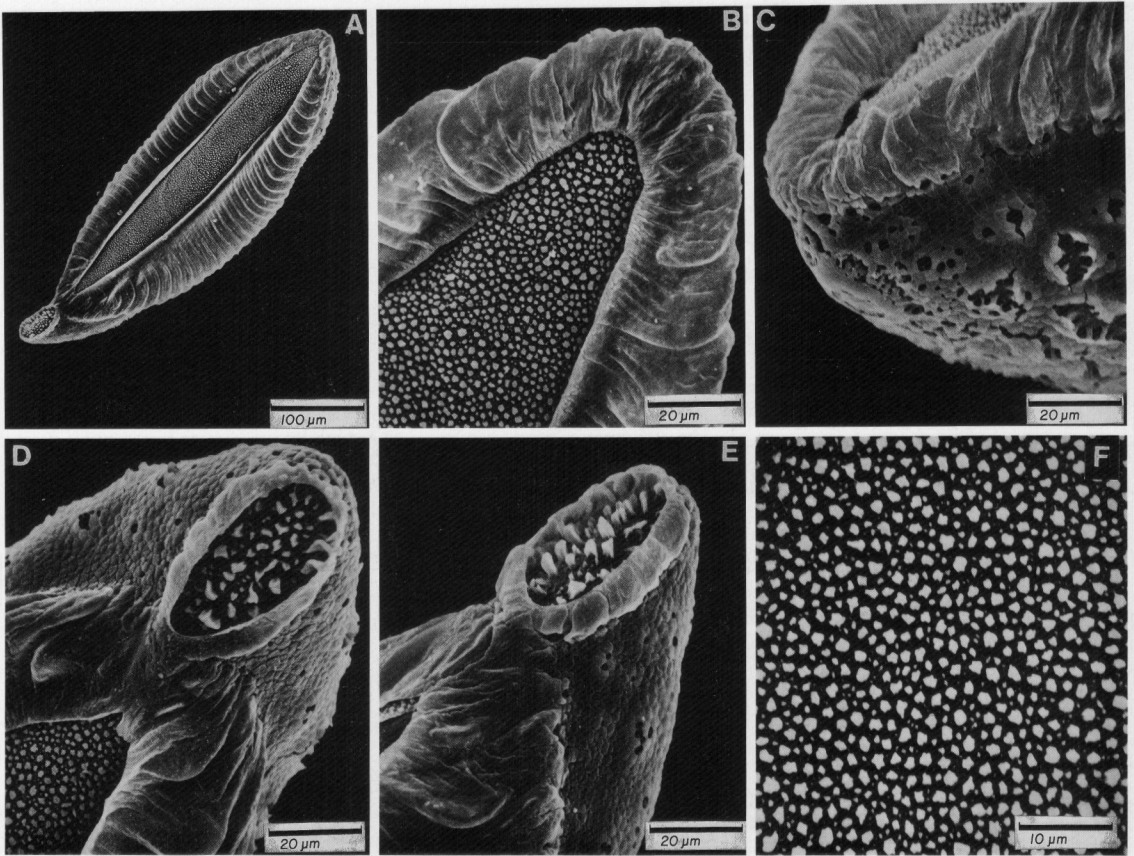


Fig. 4: Egg of *Anopheles (Nyssorhynchus) lutzii* (Dourado form). A. Entire egg, anterior end at top, on the right. B. Anterior end, ventral view. C. Anterior end, lateral view. D. Posterior end, ventral view. E. Posterior end, lateral view. F. Deck tubercles, central area.

oped (Figs. 1E, 1F). Micropyle situated in the center of a low mound, micropylar collar separated from anterior margin of frill by narrow area with few pores (Figs. 1F and 3C). Collar surface smooth, inner boundary with conspicuous excavations and 6 short rays that connect with micropylar disk about midway to the micropyle (Fig. 3C); micropylar disk with a continuous ring within sectors limited by short rays.

Posterior end: Similar to anterior end with respect to frill, tubercles, conformation of plastron and deck (Figs. 2A, 2B; and 3D).

Anopheles (Nyssorhynchus) lutzii Cruz (Figs. 4–6)

Size: Width 175–210 µm (mean 200 ± 0.01 µm), length 467–566 µm (mean 535 ± 0.03 µm), length to width ratio 2.30–3.14 (mean 2.68 ± 0.24) ($n = 22$ eggs from 3 females).

Color: Black.

Overall appearance: Broadly boat-shaped in ventral, lateral, and dorsal views (Figs. 4A; 5A, 5E; and 6), ventral surface nearly flat, dorsal surface

curved in lateral view. Floats lateral in position, closer to ventral than dorsal surface, well developed (Fig. 5E); frill present only on posterior part of egg (Figs. 4D, 4E).

Ventral surface: Deck in middle region of egg wide, tapering toward anterior and posterior parts of egg (Fig. 4A); frill reduced, present only on posterior end of egg, surrounding a small posterior deck area (Figs. 4D, 4E); floats widely joined on anterior end (Figs. 4B, 4C) and on posterior part of egg anteriorly to frill (Figs. 4D, 4E); lobed tubercles absent. Deck covered uniformly with larger, nearly globose tubercles intermixed with smaller tubercles (Fig. 4F).

Dorsal and lateral surfaces: Outer chorionic cell of dorsal plastron with indistinct boundary, plastron formed by small, flat, widely joined nodules interspersed with several irregularly shaped open areas (Figs. 5B, 5C), areas adjacent to floats and posterior end of egg with few small pores (Figs. 5B, 5D). Floats well developed, extending from anterior end to posterior 0.2 and widely joined both anteriorly and posteriorly on ventral surface (Figs. 4B, 4D), anterior end not visible beyond anterior margin of

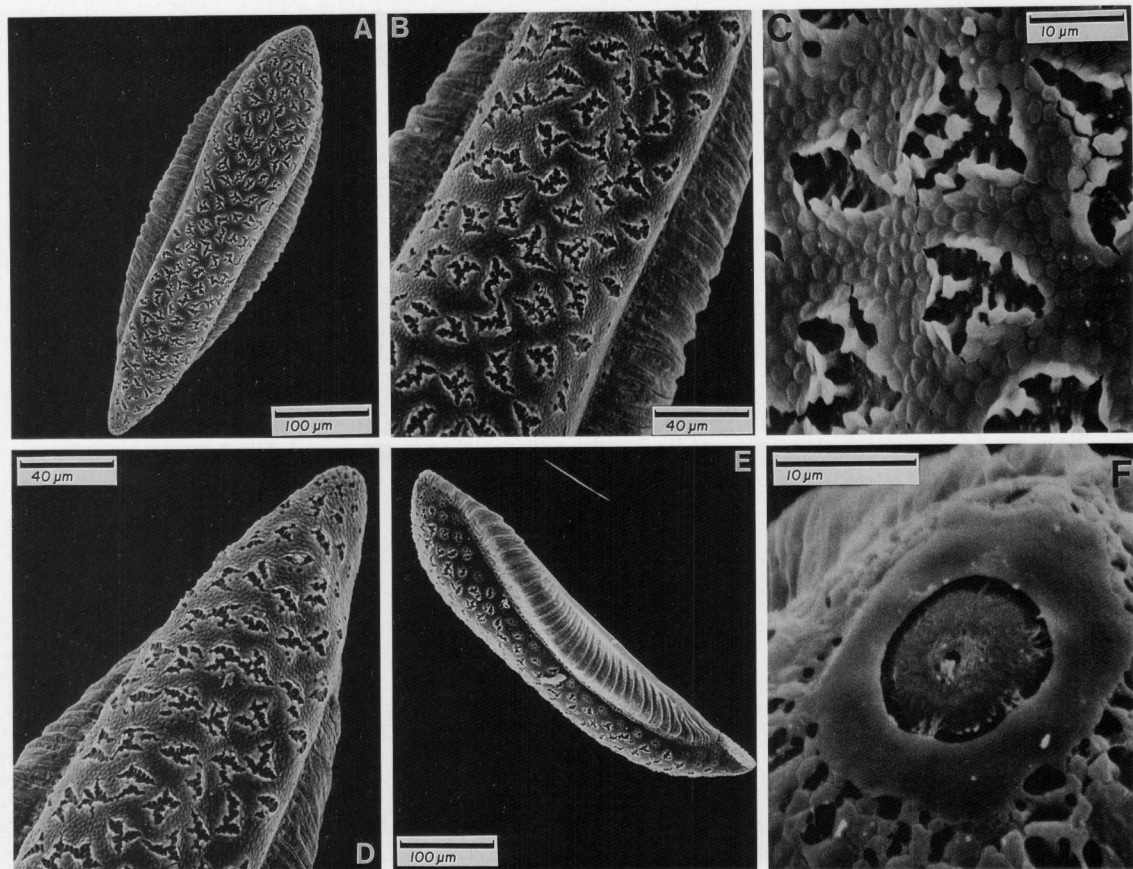


Fig. 5. Egg of *Anopheles (Nyssorhynchus) lutzii* (Dourado form). A. Entire egg, anterior end at top, on the right, dorsal view. B, C. Outer chorion, dorsal surface. D. Posterior end, dorsal view. E. Entire egg, anterior end at top, on the left, lateral view. F. Micropyle.

floats (Figs. 4B, 4C). Posterior float margin joined to anterior edge of posterior crown (Figs. 4D, 4E); ribs about 27–30 ($n = 3$) in number, ribs weakly divided into lobes (Fig. 5E).

Anterior end: Anterior end more blunt than posterior (Fig. 4A), frill absent, floats folded over this part (Fig. 4B), deck tubercles adjacent to anterior area similar to those on main deck (Fig. 4B). Micropylar collar separated from anterior margin of egg by narrow area (Fig. 5F), collar surface smooth, external boundary irregular, inner boundary of collar with shallow to deep excavations and thin to wide short rays that divide micropylar disk into about 6 sectors (Fig. 5F). Plastron around micropylar collar formed by small, flat nodules interspersed with several pores (Fig. 5D).

Posterior end: Narrower than anterior end (Figs. 4A and 5A) and with shallow crownlike structure formed by the frill (Fig. 4D). Deck area within crown with larger and more prominent tubercles than those on main deck (Fig. 4E). Lateral and dorsal plastron adjacent to this area with few pores.

Anopheles (Nyssorhynchus) parvus (Chagas) (Figs. 7–9)

Size: Width 424–511 μm (mean 463 ± 0.03 μm), length 106–143 μm (mean 130 ± 0.01 μm), length to width ratio 0.25–0.31 (mean 0.28 ± 0.02) ($n = 21$ eggs from 3 females).

Color: Black.

Overall appearance: Boat-shaped in ventral, lateral, and dorsal views (Figs. 7A and 9A); anterior end 0.3 wider than posterior end with conspicuous crownlike structure (Figs. 7B, 7D) surrounding the micropyle and fingerlike structure, which is ventral to micropyle (Figs. 7D; and 8A, 8D).

Ventral surface: Floats wide in ventral view, deck completely enclosed by floats. Deck somewhat elliptical in outline (Figs. 7A–7C), slightly wider at middle part of egg, and uniformly covered with tubercles. Tubercles uniform in appearance over deck region but irregular in outline and size, top surface domed (Fig. 7F).

Dorsal and lateral surfaces: Outer chorionic cells of dorsal plastron indistinct, dorsal surface

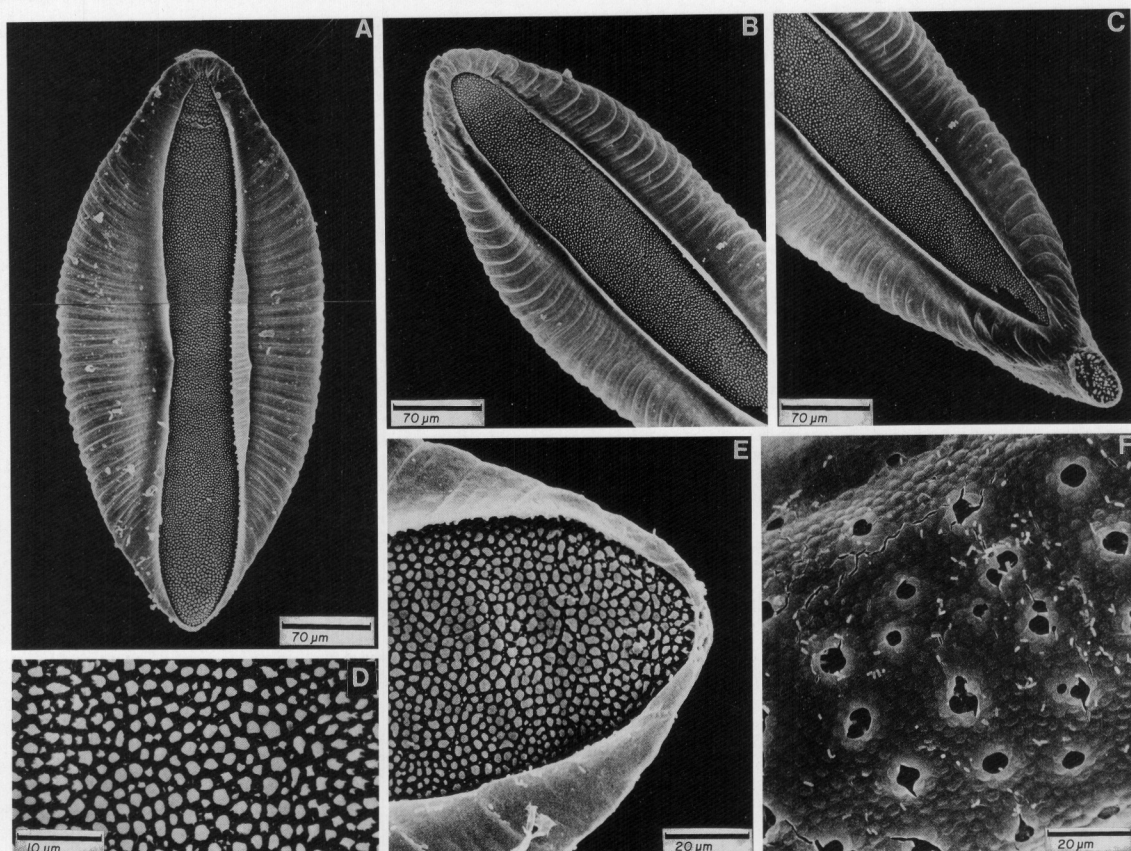


Fig. 6. Egg of *Anopheles (Nyssorhynchus) lutzii*. A. Entire egg, anterior end at top, ventral view. B. Anterior end, ventral view. C. Posterior end, ventral view. D. Deck tubercles, central area. E. Posterior end, ventral view. F. Outer chorion, dorsal view. A, D–F Ribeira Valley form. B, C. Dourado form.

uniformly covered with small, closely spaced, somewhat rounded tubercles usually connected by short bridges and separated by gaps (Figs. 9D–9F). Floats positioned well toward ventral side (Fig. 9A), floats barely visible in dorsal view; in lateral view, floats long and narrow, extending from anterior 0.25 to posterior 0.9, and widely joined both anteriorly and posteriorly on ventral surface (Figs. 7A–7C), float ribs not counted, ribs weakly divided into lobes (Figs. 9A, 9B), inner wall strongly ribbed (Fig. 9B). Anterior margin of float well separated from crown (Fig. 7B).

Anterior end: Anterior end wider than posterior (Figs. 7A–7C), frill developed at anterior pole into conspicuous crownlike structure (Figs. 7D; and 9D, 9E), surrounding micropyle and very prominent, fingerlike structure that bears several lobed tubercles (Figs. 8A, 8E), small deck area within crown with larger surface tubercles than those present on remainder deck area (Figs. 8D–8F), larger tubercles intermixed with smaller, variable-shaped tubercles. Crownlike tubercles less domed, more prominent, more irregularly shaped, and with deep cavities in the walls. Fingerlike structure within crown immediately ventral to micropyle (Fig. 8A). Lobed tu-

bercles large with about 6 or 7 fingerlike extensions arising from central area (Figs. 8B, 8C). Micropyle situated in low mound, in the center of micropylar disk (Fig. 8F). Micropylar collar raised, separated from posterior margin of fingerlike structure by narrow deck area (Fig. 8F); collar surface smooth; outer boundary of micropylar disc with small, prominent, spinelike structure on ventral margin; inner boundary smooth (Figs. 8E, 8F).

Posterior end: Narrower than anterior end, plas-tron covering posterior end formed by tubercles that are similar to those on lateral and dorsal plas-tron, but with fewer gaps. Posterior end well visible beyond posterior margin of floats (Figs. 7E and 9C).

Comparison of Dourado and Ribeira Valley forms of *An. lutzii*

Eggs of the 2 populations of *An. lutzii* studied differ in several morphological features. The Dourado form has a conspicuous crown formed by a shallow frill on the posterior end of the ventral surface (Fig. 6C), and the floats are widely joined both anteriorly and posteriorly on the ventral surface

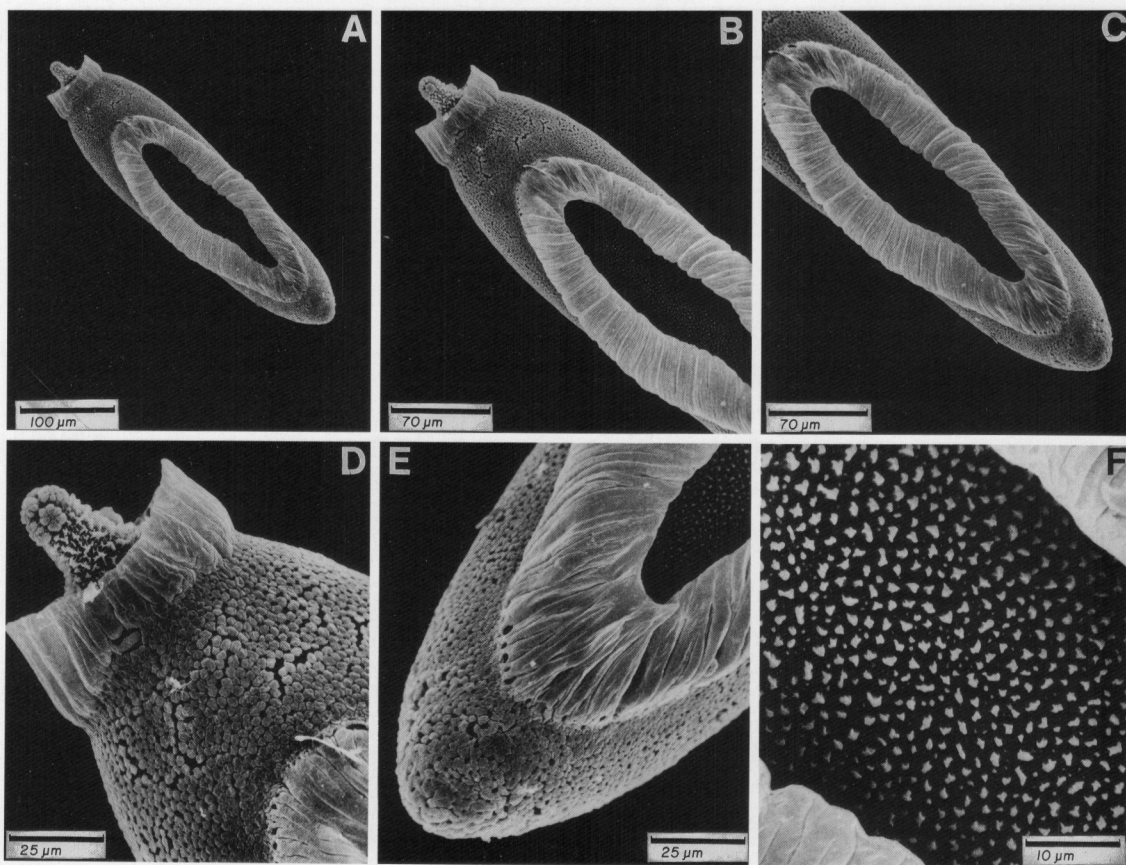


Fig. 7. Egg of *Anopheles (Nyssorhynchus) parvus*. A. Entire egg, anterior end at top, on the left, ventral view. B. Anterior two-thirds, ventral view. C. Posterior two-thirds, ventral view. D. Anterior end, ventral view. E. Posterior end, ventral view. F. Deck tubercles, central area.

(Figs. 6B, 6C), the posterior margin of the float is close to the posterior crownlike structure (Fig. 6C), tubercles of the deck area within the crown are different in aspect from those on the main deck (Figs. 4D, 4E), and the dorsal and lateral plastron have several irregularly shaped open areas (Fig. 5A). In the Ribeira Valley form, the frill is absent both anteriorly and posteriorly (Figs. 6A, 6E), and the deck tubercles are somewhat similar in aspect throughout the deck area, but slightly larger on both the anterior and posterior ends (Figs. 6A, 6D, 6E), the floats are narrowly joined on the posterior pole (Fig. 6E), and the dorsal and lateral plastron have several somewhat circular pores (Fig. 6F).

DISCUSSION

Except for *An. ininii*, whose egg remains unknown, the egg of *An. rondoni* is structurally distinct from those of most species of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. Within the Albimanus Section, eggs of most species have long floats that extend almost the entire length of the egg (*An. albimanus*, *An. aquas-*

alis, *An. rangeli*, *An. dunhami*, *Anopheles evansae* (Brèthes), *Anopheles oswaldoi* (Peryassú), *Anopheles triannulatus* (Neiva and Pinto), *Anopheles galvaoi* Causey, Deane, and Deane, *An. benarrochi*, the floats are usually widely joined posteriorly and the frill is present only on the anterior end (*An. evansae*, *An. oswaldoi*, *An. aquasalis*, *An. galvaoi*, *An. benarrochi*, *An. darlingi*, *An. rangeli*, *An. dunhami*), and the anterior part of the deck is usually separated from the main deck and is surrounded by a shallow crown (*An. evansae*, *An. oswaldoi*, *An. galvaoi*, *An. benarrochi*) or a deep crown (*An. darlingi*, *An. dunhami*, *An. rangeli*). Based on Causey et al. (1944), the egg of *An. rondoni* closely resembles that of "typical" *An. strodei*. However, *An. strodei* was observed to have morphological variations that might be indicative of several populations within the species (Lounibos et al. 1997). Furthermore, comparing the egg of *An. rondoni* with those of species of the Argyritarsis Section, it was found to be structurally similar to the eggs of *Anopheles argyritarsis* Robineau-Desvoidy (Causey et al. 1944), *Anopheles albitarsis* Lynch-Arriábalzaga (Rosa-Freitas and Deane 1989, Marucci

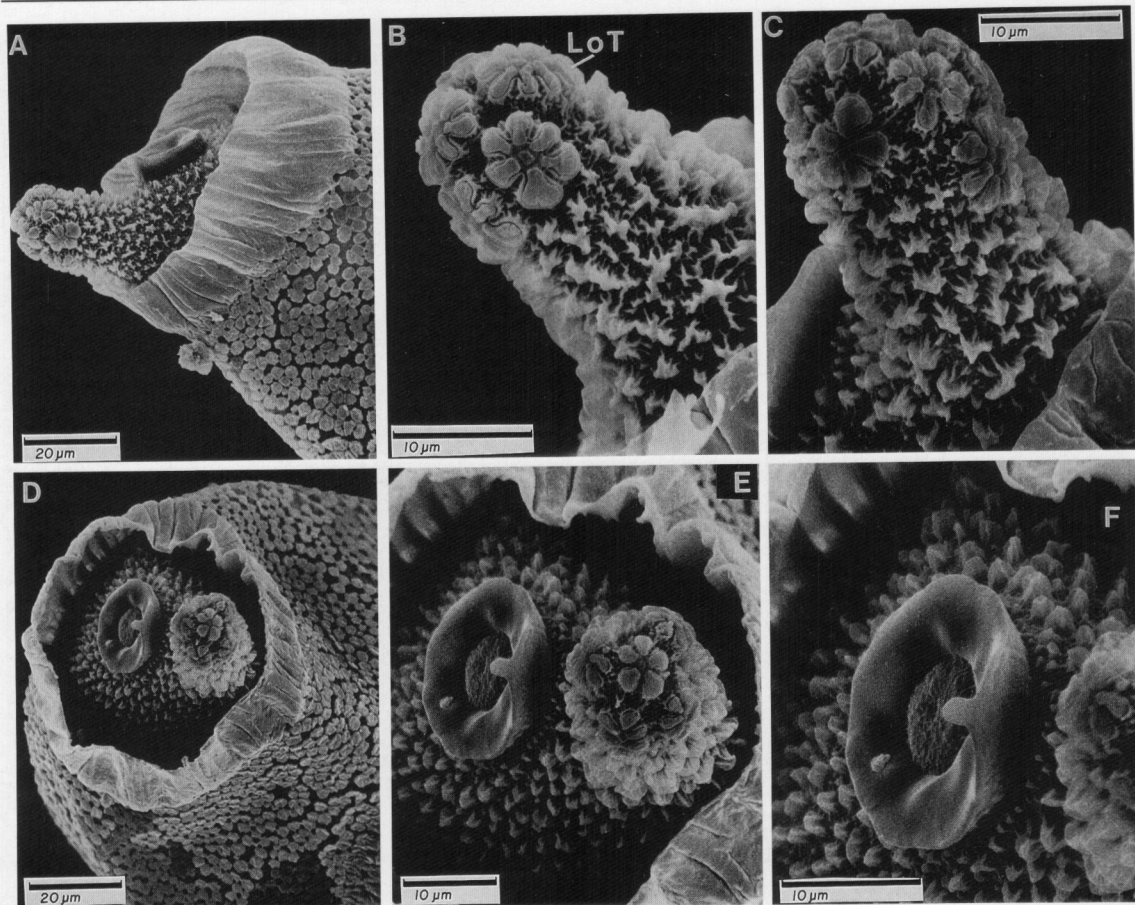


Fig. 8. Egg of *Anopheles (Nyssorhynchus) parvus*. A. Anterior end, lateral view, dorsal side up. B. Anterior fingerlike structure, lobed tubercles, ventral view. C. Anterior fingerlike structure, lateral view. D, E. Anterior fingerlike structure and micropyle within anterior crown, anterior view. F. Micropyle. LoT, lobed tubercles.

1996, as *An. albitarsis* A), *Anopheles deaneorum* Rosa-Freitas (Rosa-Freitas 1989), and *An. albitarsis* B of Wilkerson et al. (1995) (Marucci 1996). Among species of the Albimanus Section, the egg of *An. rondoni* most closely resembles that of *Anopheles nuneztovari* Gabaldón (as *Anopheles goeldii* Rozeboom and Gabaldón) (Causey et al. 1944), and *An. triannulatus* (Causey et al. 1944), even considering that *An. nuneztovari* is polymorphic within its vast distribution range (Linley et al. 1996). The eggs of these 6 species are similar in having the frill continuous with the floats at both the anterior and posterior ends of the egg, the anterior and posterior parts of deck confluent with the main deck area, and the floats moderately long, extending nearly 0.5 of the length of the egg. However, the egg of *An. rondoni* can be easily separated from those of *An. albitarsis*, *An. deaneorum*, and *An. albitarsis* B by the pattern of slightly raised cells formed by small nodules interspersed with pores on the dorsal plastron, the irregularly shaped deck tubercles, the tubercles larger and more prominent on the anterior and posterior parts of the deck,

the micropylar collar that is well separated from the dorsal frill margin, the deck that is narrower on the middle region, the float ribs that are sinuous, and the floats that are laterally swollen. The eggs of *An. albitarsis*, *An. albitarsis* B, and *An. deaneorum* have the dorsal plastron uniformly covered with small, closely spaced tubercles, deck tubercles that are uniformly shaped, a micropylar collar that touches the dorsal crown margin, a deck that is equally wide throughout the length, float ribs that are straight, and floats that are shorter and narrower. Based on Causey et al. (1944), the egg of *An. rondoni* also resembles those of *An. argyritarsis* and *An. nuneztovari*. However, which characters might separate *An. rondoni* from *An. argyritarsis* and *An. nuneztovari* is still unknown.

The egg of *An. triannulatus* was found to have 4 distinct types (Causey et al. 1944). The type that most closely resembles *An. rondoni* is type "4", which has frills at both ends, and floats that are widely separated throughout the length. Nascimento (1995) also observed 3 types of egg of *An. triannulatus*, which can be distinguished by features of

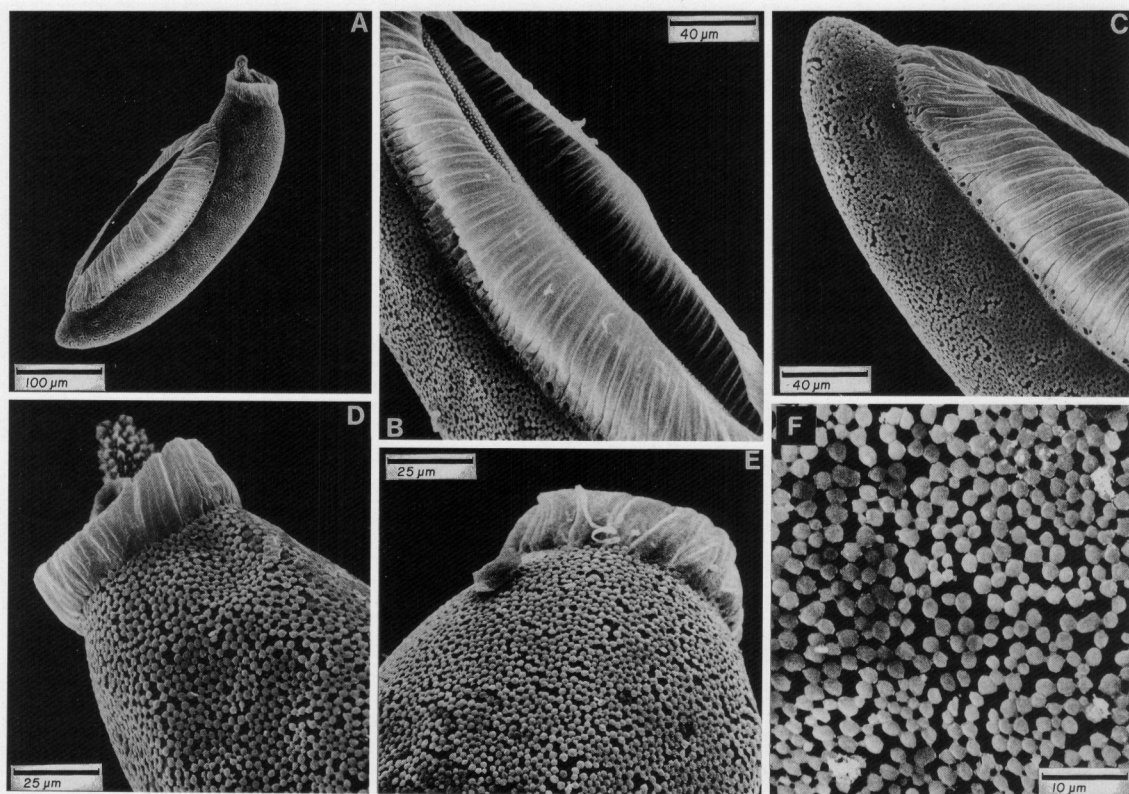


Fig. 9. Egg of *Anopheles* (*Nyssorhynchus*) *parvus*. A. Entire egg, anterior end at top, on the right, lateral view. B. Floats, lateral view. C. Posterior end, lateral view. D. Anterior pole, dorsal view. E. Outer chorion, anterior end, dorsal view. F. Outer chorion, dorsal surface.

the frill and the floats. The egg of *An. rondoni* differs from type "4" of Causey et al. (1944) and type "Salobra 1" of Nascimento (1995) in having the outer chorionic cells with more conspicuous boundaries.

The eggs of *Anopheles antunesi* Galvão and Amaral and *An. lutzii* are unusual in having floats widely joined anteriorly on the ventral side, the anterior end barely visible beyond the anterior margin of the floats, the frill absent (*An. antunesi*), and a frill that is present on the posterior end (*An. lutzii* Dourado form) or that is absent (*An. lutzii* Ribeira Valley form).

Forattini et al. (1997) described the ultrastructure of the egg of *An. antunesi* and compared it with that of *An. lutzii* of Galvão (1941). However, the description and illustration of the egg of *An. lutzii* provided by Galvão (1941) were inadequate for a critical comparison of both species. In fact, *An. lutzii* from Dourado has a shallow crown surrounding the small posterior deck area on the ventral side, not anteriorly as mentioned by Forattini et al. (1997). In conclusion, as the egg of *An. lutzii* from Ribeira Valley does not have a posterior frill, the egg of *An. lutzii* can be separated from that of *An. antunesi* by the absence of the small, posterior deck area that is barely isolated from the main deck and

supports larger tubercles than those found on the remainder of the deck.

Within the subgenus *Nyssorhynchus*, the egg of *An. parvus* is similar to those of *An. darlingi* (Linley 1992) and *An. rangeli* and *An. dunhami* (Linley and Lounibos (1993) in having a deep anterior crownlike frill. These authors hypothesized that the co-occurrence of this structure in species of distinctive lineages of the subgenus *Nyssorhynchus* suggests independent evolution of the crown. Furthermore, the presence of a crown in *An. parvus* of the *Myzorrhynchella* Section supports the hypothesis of Linley and Lounibos (1993).

Although similar, the egg of *An. parvus* differs substantially from those of *An. darlingi*, *An. rangeli*, and *An. dunhami*. Both the terminal fingerlike structure and the micropyle situated within the anterior crown make the egg of *An. parvus* easily separable from those of *An. darlingi*, *An. rangeli*, and *An. dunhami*, in which the micropyle is dorsal to the crownlike frill and the fingerlike structure is absent. Other distinctive features are the unusual presence of several lobed tubercles on the anterior fingerlike structure, narrow floats that ventrally placed and widely joined anteriorly and posteriorly on the ventral side, and the anterior end of the float that is well separated from the anterior crown.

The egg of *An. parvus*, in the laboratory, floated on water with the anterior end uppermost, so the remainder of the egg stayed submerged in the water inside a bubble. This seems to be the natural flotation position, especially because eggs from 3 females floated with the anterior pole up. When the water surface was agitated the eggs became entangled in the walls of the plastic vials. This suggests that in natural larval habitats the anterior crown and the terminal fingerlike structure with its several lobed tubercles might serve an anchorage function by becoming entangled in water debris or plants, thus preventing eggs from sinking.

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