Mosquito Systematics, 25(3):157–169, 1993

THE EGGS OF *ANOPHELES* (*NYSSORHYNCHUS*) *RANGELI* AND *ANOPHELES* (*NYSSORHYNCHUS*) *DUNHAMI* (DIPTERA: CULICIDAE)

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ABSTRACT. The eggs of *Anopheles rangeli* from Ecuador and Bolivia and *An. dunhami* (formerly *An. trinkae*) are described, the latter for the first time, from scanning electron micrographs. The most conspicuous feature in both species is the prominent anterior crown, which tends to be more elevated and widened anteriorly in *An. rangeli,* whereas in *An. dunhami* it is usually tapered anteriorly (sometimes straight), with the anterior end of the egg easily visible and slightly protruding. The egg of *An. dunhami* is longer in relation to width than that of *An. rangeli,* its floats are longer and almost invariably touch the posterior margin of the crown, while the *An. rangeli* floats are shorter, wider, and not contiguous with the crown. Eggs of the two species are readily distinguishable with a stereomicroscope, providing an alternative to unreliable morphological keys for separation of adult females.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *rangeli* Gabaldon, Cova Garcia and Lopez and *An.* (*Nys.*) *dunhami* Causey are sister species in the Oswaldoi Complex of the Albimanus Section of subgenus *Nyssorhynchus* of *Anopheles* (Faran 1980). *Anopheles dunhami* has recently been shown to be the senior synonym of *Anopheles trinkae* Faran (Peyton 1993). *Anopheles rangeli* ranges from the upper Amazon and Orinoco basins, through Colombia, Venezuela, and Ecuador, and south through Peru and into Bolivia. The known distribution of *An. dunhami* is along the eastern slope of the Andes from central Colombia to central Bolivia (Faran 1980; J. Conn, unpublished data), extending east to Tefe, Brasil, the type locality (Causey 1945). Recent evidence, based on the presence of sporozoites in the salivary glands, has incriminated both these anophelines as probable vectors of malaria in eastern Peru (Hayes et al. 1987). *Anopheles rangeli* is a suspected malaria vector in Ecuador (Forattini 1962) and a confirmed transmitter of *Plasmodium vivax* in Colombia (M. Suarez, unpublished data).

Although Faran (1980) has provided descriptions of the adult, pupal, and larval stages of these species, the egg of *An. rangeli* is known only at the light microscopic level, which is adequate for provision of a simple drawing (Causey et al. 1944), whereas the egg of *An. dunhami* is undescribed. Recent research in South America afforded the opportunity to collect gravid females of both species, whose eggs served as the bases for the present ultrastructural descriptions.

MATERIALS AND METHODS

Eggs of *An. rangeli* were obtained from four females collected August 5, 1992, from cattle 10 km north of Coca, Napo Province, Ecuador (0° 22′ S 76° 54′ W) and three from Bolivia obtained from human bait at San Ramon, Riberalta, Beni Province (11° 5′ S 66° 5′ W), December 4, 1991; Puerto Villaroel, Cochabamba Province (16° 47′ S 64° 45′ W), November 30, 1991; and Ibuelo, Villa Tunari, Cochabamba Province (16° 56′ S 65° 25′ W), November 29, 1991. Eggs of Ecuadorian females were used for the formal description, but comparative measurements also were made of Bolivian *An. rangeli* (see below). The *An. dunhami* eggs were from four females collected August 6, 1992, from cattle at Sar-

Table 1. Attributes of eggs of *An. rangeli* and *An. dunhami*. Distances measured in μm, areas in square μm.

Attribute	Mean (±SE)[1]		
	An. rangeli (E)[2] (n = 12)	*An. rangeli* (B)[2] (n = 9)	*An. dunhami* (E) (n = 12)
Egg length	455.8 ± 6.4a	461.3 ± 6.2a	462.3 ± 6.8a
Egg width	183.3 ± 2.1b	186.8 ± 2.0b	167.7 ± 2.4a
L/W ratio[3]	2.48 ± 0.04a	2.47 ± 0.05a	2.76 ± 0.04b
Float length	355.5 ± 4.9a	347.3 ± 3.8a	375.1 ± 6.1b
Float length %[4]	0.781 ± 0.006a	0.753 ± 0.009a	0.812 ± 0.005b
Ribs in float	34.71 ± 0.23a	35.22 ± 0.53ab	36.67 ± 0.59b
Rib width[5]	10.19 ± 0.17a	9.88 ± 0.19a	10.23 ± 0.08a
Float area %[6]	0.713 ± 0.007a	0.746 ± 0.012a	0.826 ± 0.013b
Deck area %[7]	0.211 ± 0.006b	0.173 ± 0.016b	0.115 ± 0.017a
Float to crown[8]	19.41 ± 2.24b	14.20 ± 2.25b	1.13 ± 0.81a
Crown width[9]	67.52 ± 1.55a	79.15 ± 1.83b	77.16 ± 2.36b

[1] Means followed by same letter do not differ significantly.
[2] E, material from Ecuador; B, material from Bolivia.
[3] Egg length/egg width.
[4] Mean float length as % of egg length.
[5] Float length/mean number of ribs (of the two floats).
[6] Area of both floats as % of whole egg area.
[7] Area of deck as % of whole egg area.
[8] Distance from anterior end of float to posterior edge of crown.
[9] Width of crown at base.

dina Yacu, Napo Province, Ecuador (0° 10′ S 77° 5 ′ W). Specific determinations were confirmed on reared progeny from the same mothers, or on conspecific individuals from the same collections, by examination of male genitalia or endonuclease profiles of mitochondrial DNA. Voucher specimens have been deposited in collections in the National Museum of Natural History, Smithsonian Institution, and the Florida Medical Entomology Laboratory.

The eggs were preserved 24 hr after oviposition in alcoholic Bouin's fixative, washed in two changes of 80% ethanol to remove picric acid, then completely dehydrated and mounted for electron microscopy as described elsewhere (Linley 1992). Examination of the specimens was carried out in a Hitachi S-510 scanning electron microscope.

For the descriptions, micrographs to be taken were selected during a systematic examination of the mounted eggs of all the females in each case. Illustrations were assembled using micrographs from several different females. Means (±SE) cited in the text were derived from an equal number of measurements from eggs of each female. Several attributes were measured from low-power (200×) micrographs of three individual eggs from each female. Six representative low-power ventral views have been assembled to allow better interspecies comparison and appreciation of size and structural variation. All measurements were done from micrographs laid over a digitizing tablet used in conjunction with Sigmascan software (Jandel Scientific, San Rafael, CA). Statistical analysis was performed using Statgraphics software (Statistical Graphics Corporation, Rockville, MD). The terminology used is that proposed by Harbach and Knight (1980).

DESCRIPTIONS

Anopheles rangeli (Figs. 1–4)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Broadly boat-shaped in ventral (Figs. 1, 2a) and dorsal views, anterior end with conspicuous, flared crown, posterior end just protruding beyond floats and rather

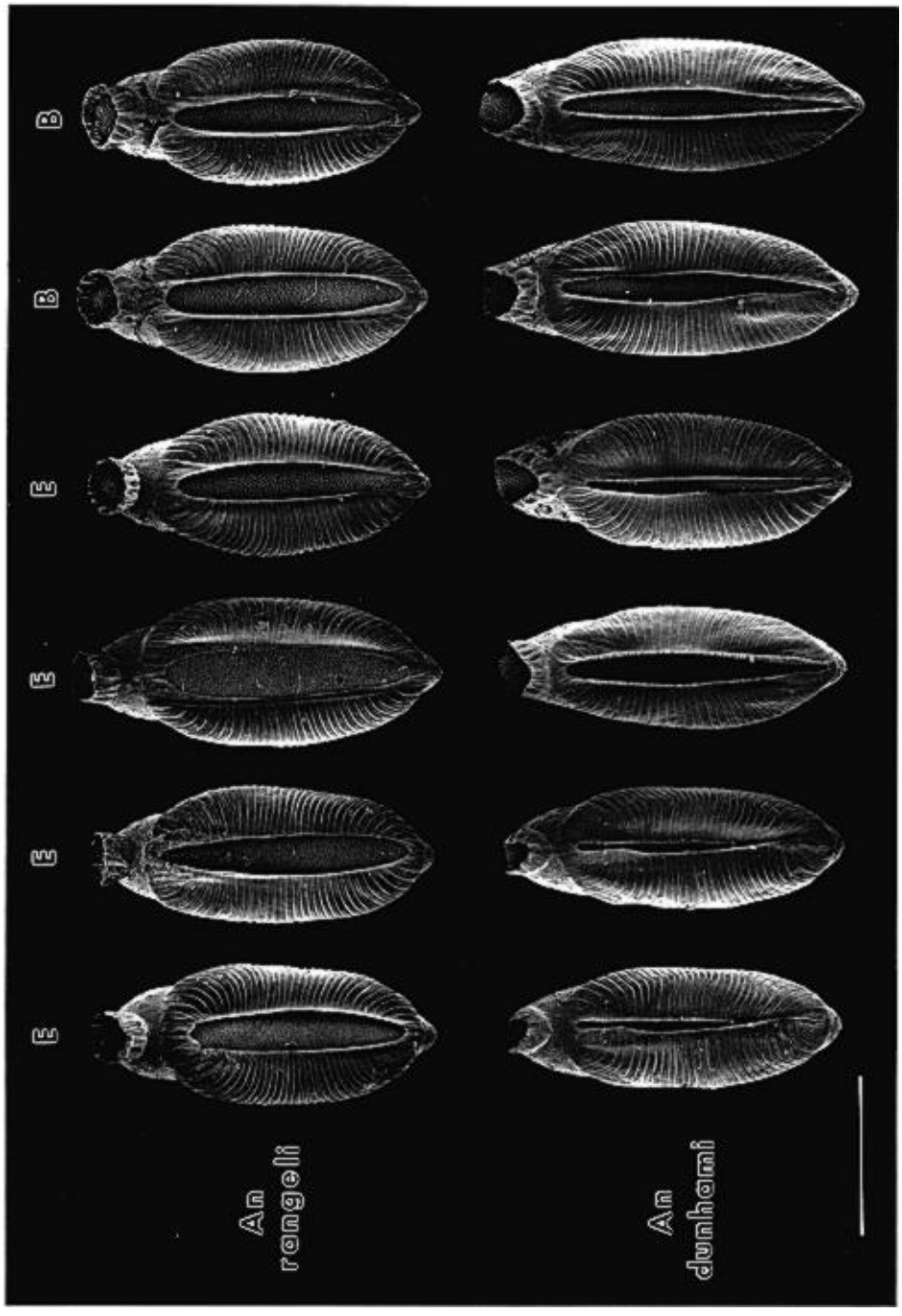

Fig. 1. Representative eggs (ventral view) of four Ecuadorian (E) and two Bolivian (B) *An. rangeli* females (egg on left from Puerto Villaroel, Cochabamba Province; egg on right from San Ramon, Beni Province) and of four Ecuadorian *An. dunhami* females. The two adjacent eggs on the left and right sides of the *An. dunhami* group are from the same female in each case. Scale = 200 μm.

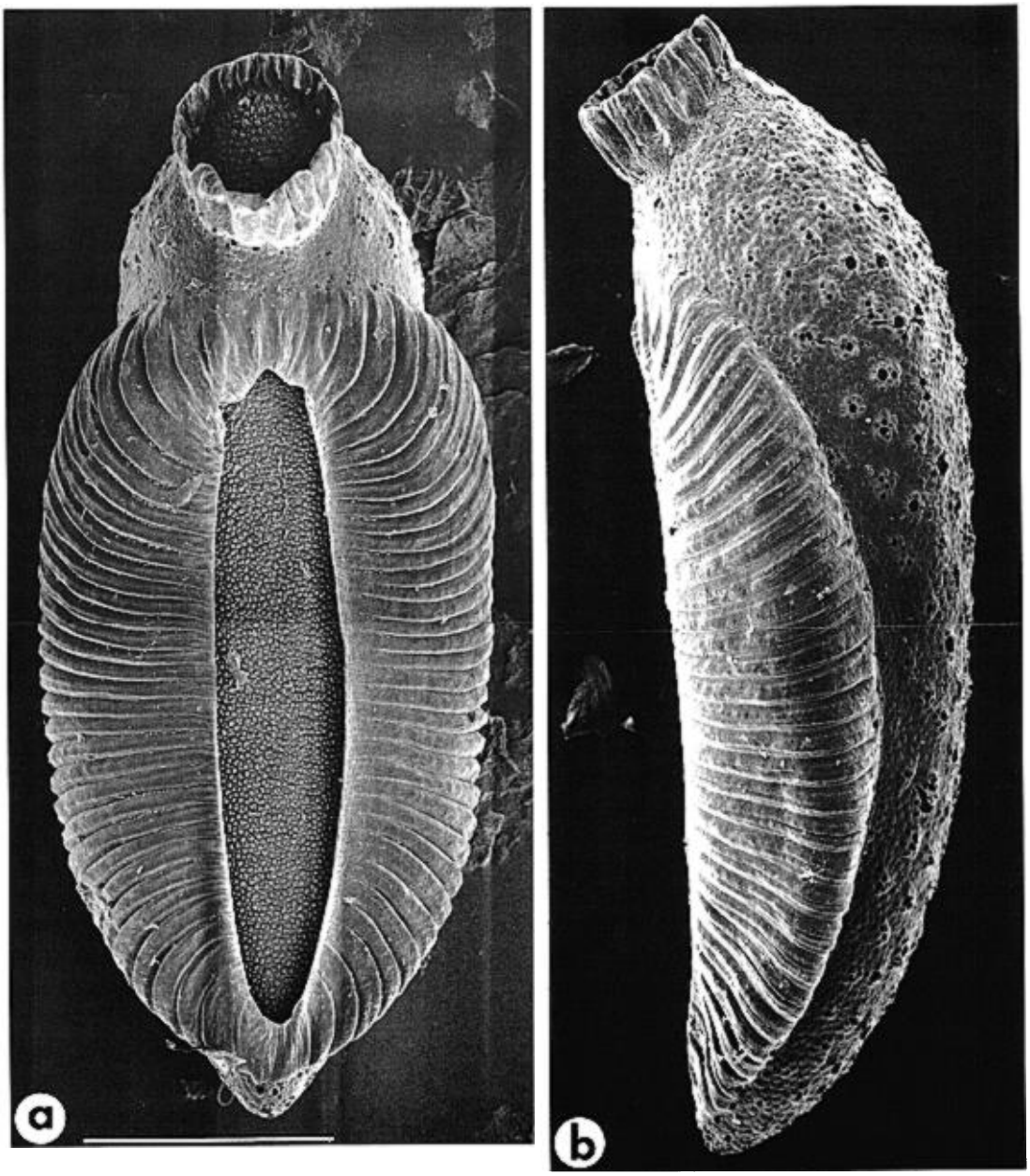

Fig. 2. *Anopheles rangeli.* a, Entire egg, ventral (top) view, anterior end at top; b, entire egg, lateral view, ventral surface at left, anterior end at top. Scale = 100 μm.

pointed (Figs. 1, 2b). Floats long and wide, creating distinct widening in outline of egg. Profile of egg in lateral view distinctly deeper anteriorly, tapered to posterior end, dorsal surface strongly curved, ventral surface almost straight (Fig. 2b).

Dorsal (lower) and lateral surfaces: Chorionic cell boundaries difficult to distinguish, cell surfaces covered with flat nodules (Fig. 3d,e) raised centrally to form a blister-like mound. Each mound in dorsal mid-line perforated by 4–14 pores, largest ones usually

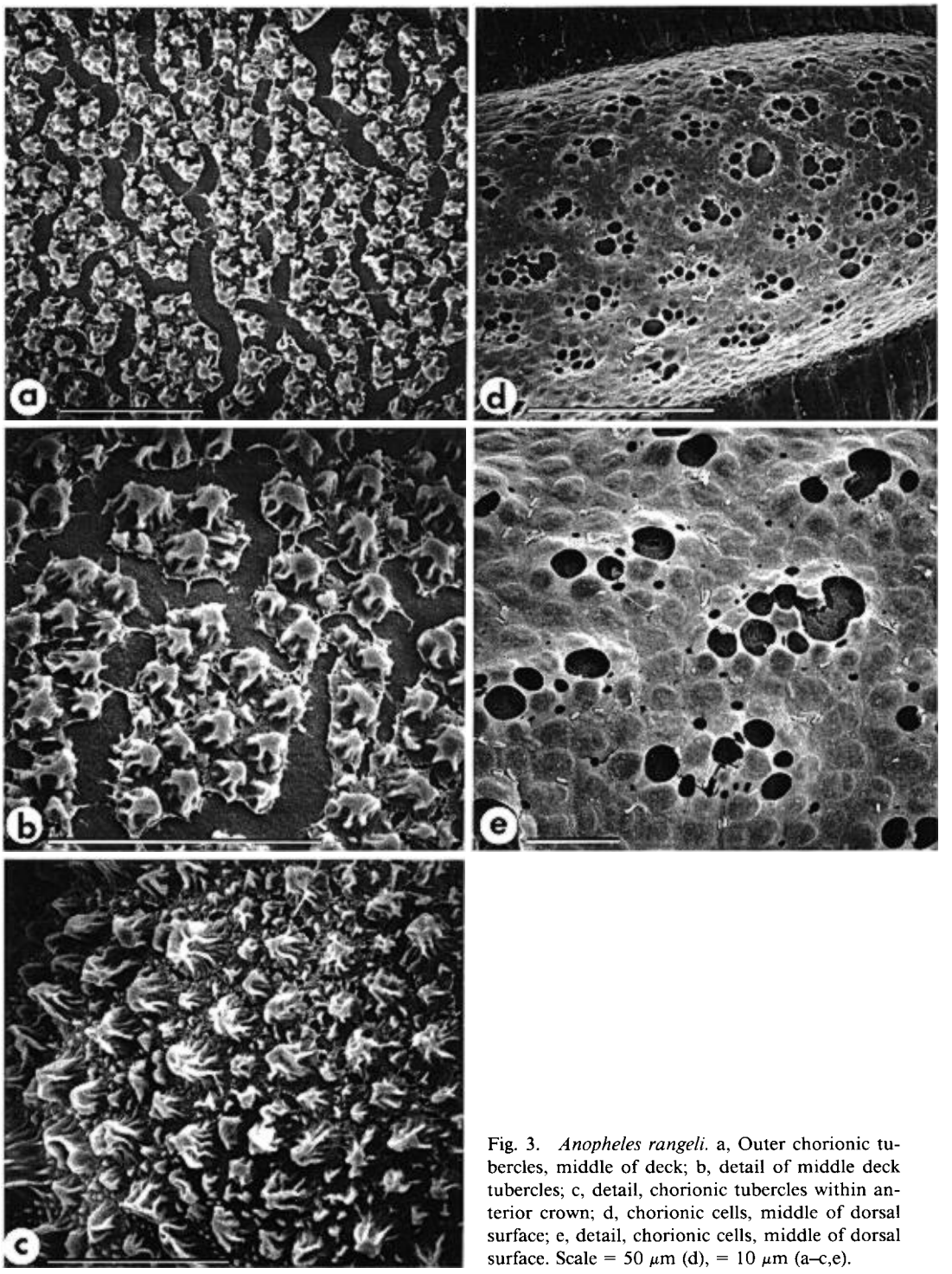

Fig. 3. *Anopheles rangeli.* a, Outer chorionic tubercles, middle of deck; b, detail of middle deck tubercles; c, detail, chorionic tubercles within anterior crown; d, chorionic cells, middle of dorsal surface; e, detail, chorionic cells, middle of dorsal surface. Scale = 50 μm (d), = 10 μm (a–c,e).

more irregular in shape, formed from several coalesced smaller ones. Number and size of pores smaller in more laterally positioned cells, particularly at anterior and posterior ends (Figs. 2b; 4b,e). Floats large (quantitative attributes as in Table 1), extending from about anterior 0.2 almost to posterior end and quite widely joined both anteriorly and posteriorly on ventral surface to enclose a fairly narrow deck (Figs. 1, 2a). Anterior float margin clearly separated from rear edge of crown (Table 1; Figs. 1, 2a). Junction of dorsal float margin and lateral plastron with occasional pores, float ribs extending and clearly defined almost to dorsal margin (Fig. 4c).

Ventral (upper) surface: In ventral view, floats conspicuously wide, invariably enclosing deck completely (Fig. 1). Deck relatively narrow, occupying a fairly small proportion of the total egg area (Table 1). Chorionic cell boundaries on deck not visible (Fig. 2a), surface covered with tubercles lacking any pattern related to size, arrayed in irregular groups separated by gaps (Fig. 3a). Tubercle diameter (widest point) 0.61–1.92 μm (mean 1.24 ± 0.04 μm, n = 40), each consisting structurally of a domed top with deeply grooved walls, smaller tubercles progressively less elevated than large ones (Fig. 3b).

Anterior end, micropyle: Anterior end bearing prominent crown, width at base and distance of its posterior edge from anterior float margin as in Table 1. Crown more or less round (Fig. 4b), its walls deeply grooved, higher ventrally than dorsally (Figs. 2a,b; 4a), and usually slightly flared (Fig. 1). Deck area within crown domed (Fig. 4b), with tubercles structurally similar to those on main deck (Fig. 3b,c), but largest ones bigger than on main deck (Fig. 4a) and with more small tubercles present (Fig. 3c). Micropylar collar irregular in outline, only slightly separated from edge of crown, surface smooth (Fig. 4f). Perimeter of disc with shallow excavations and thin radial ridges, forming 6–8 (mean 6.8 ± 0.2, n = 20) sectors. Disc surface slightly rough, micropyle 1.3 μm in diameter, set within a low mound (Fig. 4f).

Posterior end: Pointed, only slightly projecting beyond rear margin of floats (Figs. 2a; 4d,e), plastron covering end with pores, but very few pores present slightly more anteriorly, beneath floats (Fig. 4e).

Anopheles dunhami (Figs. 1, 5–7)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Boat-shaped in ventral (Figs. 1, 5a) and dorsal views, anterior end with conspicuous crown, which is usually tapered anteriorly, or straight, not flared (Figs. 1, 5a). Rounded anterior end of egg easily visible in crown, almost always protruding slightly beyond anterior crown margin. Posterior end fairly pointed, barely extending beyond floats (Figs. 1, 5a). Floats long, only slightly widening outline of egg. Lateral profile of egg deepest at about anterior 0.25, gradually tapered posteriorly till posterior 0.25, then more rapidly so, ventral surface flat (Fig. 5b).

Dorsal (lower) and lateral surfaces: Chorionic cell boundaries difficult to recognize (Fig. 6d), surfaces composed of flat nodules and raised centrally to form mounds perforated by several pores, which are often fused (both within and between cells) to form much larger elongated or irregularly shaped openings (Fig. 6d,e). Pores fewer in more lateral cells and at anterior end (Fig. 5b). Floats large and long (attributes as in Table 1), extending from anterior 0.15 almost to posterior end of egg, but not conspicuously wide (Figs. 1, 5a). Floats invariably fused anteriorly and posteriorly, to enclose very narrow deck, anterior float margin almost always contiguous with posterior edge of crown or, if not so, only slightly separated from it (Table 1, Fig. 1). Lateral plastron with few pores at dorsal float margin, float ribs clearly defined almost to dorsal margin, contiguous lateral plastron with few pores (Figs. 5b, 7c).

Ventral (upper) surface: Deck area narrow, occupying only a small proportion of the whole egg area (Table 1). Cell boundaries in deck invisible, tubercles arranged in irregular groups separated by gaps (Fig. 6a), size range considerable, diameter 0.5–2.1 (mean 1.26 ± 0.06 μm, n = 40), larger tubercles tending to be surrounded by smaller ones (Fig. 6a). Large tubercles structurally dome-shaped, with

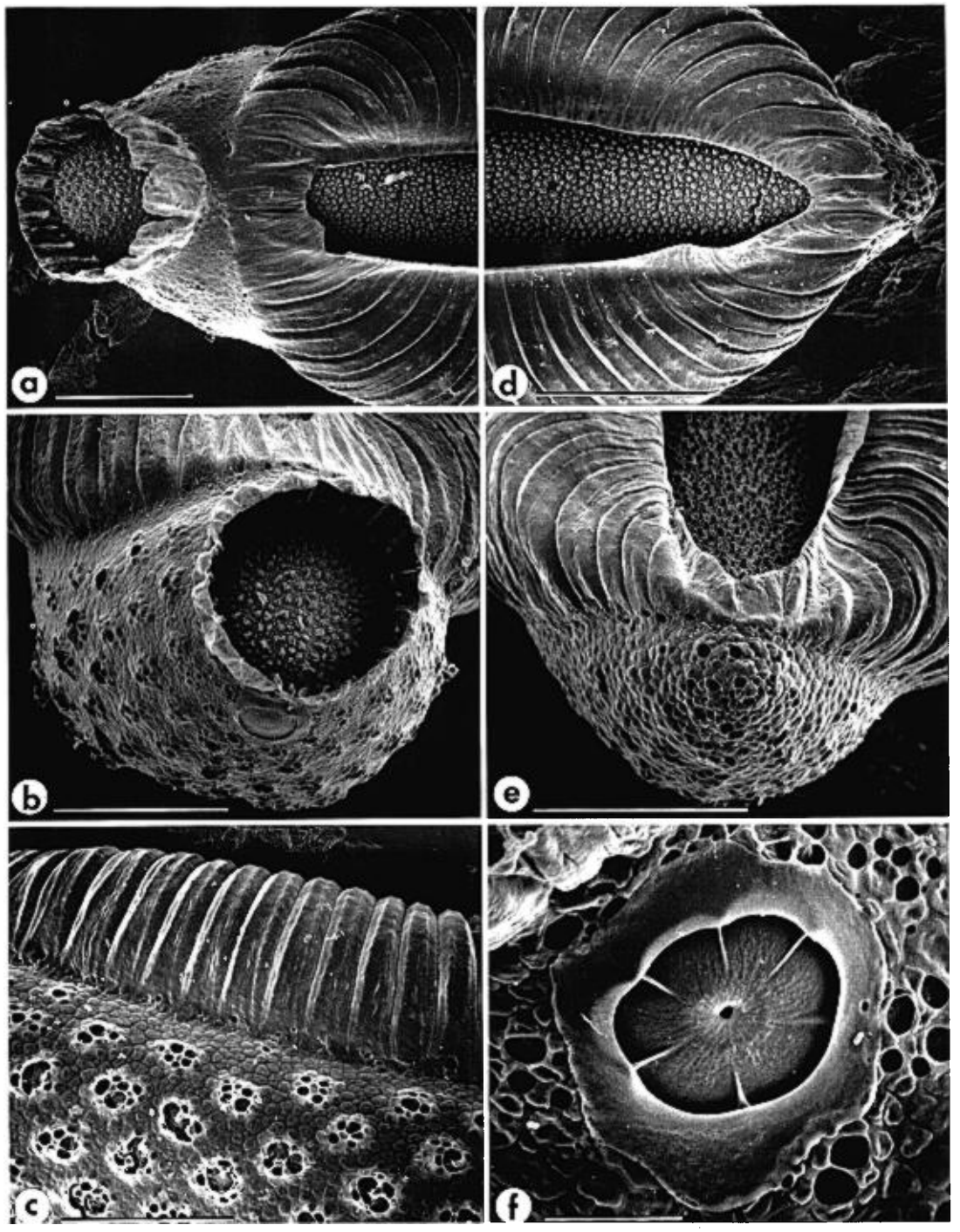

Fig. 4. *Anopheles rangeli.* a, Anterior end, ventral (top) surface; b, anterior end, end-on view; c, lateral surface, dorsal margin of float; d, posterior end, ventral surface; e, posterior end, end-on view; f, micropylar apparatus. Scale = 50 μm (a–e), = 10 μm (f).

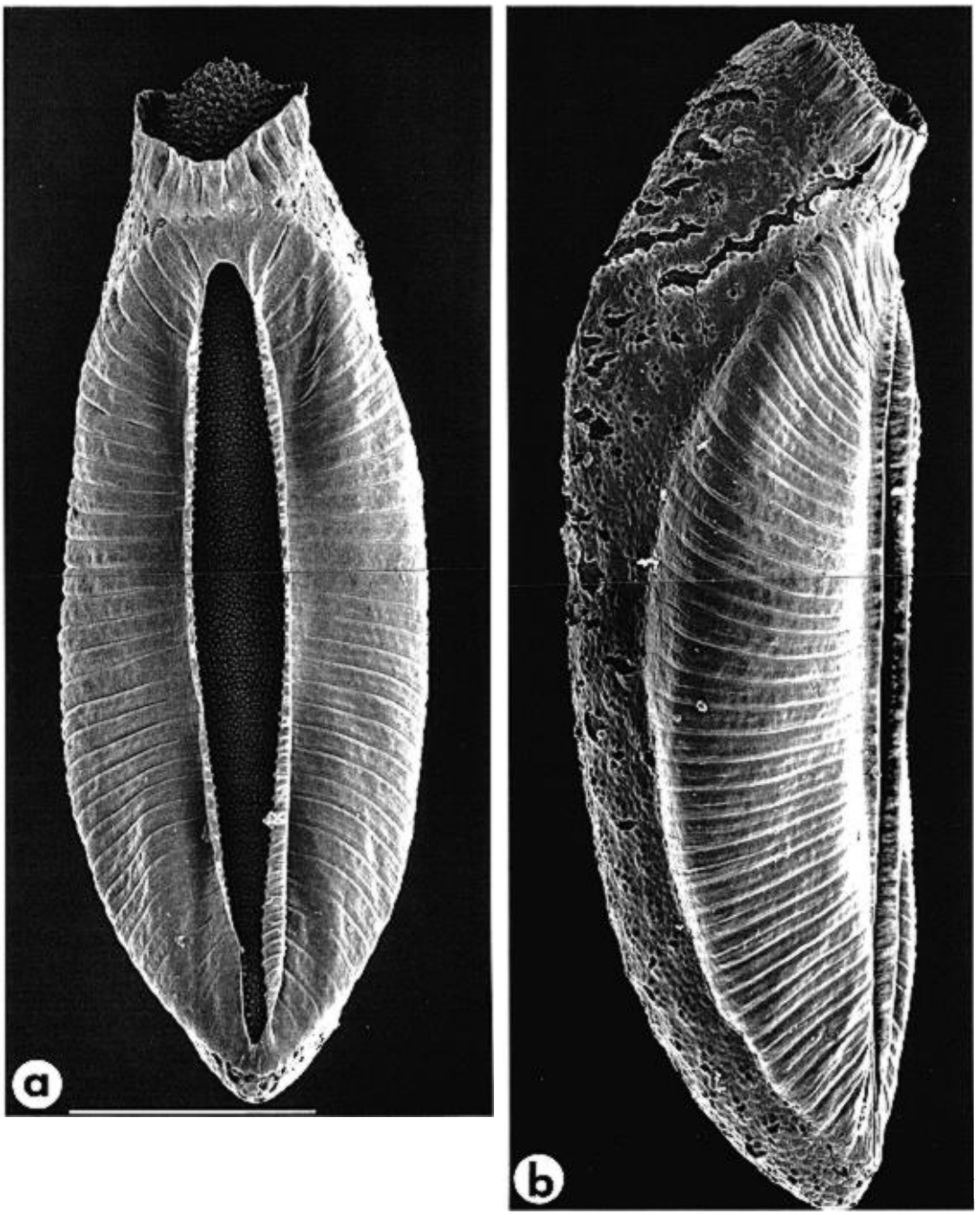

Fig. 5. *Anopheles dunhami*. a, Entire egg, ventral (top) view, anterior end at top; b, entire egg, lateral view, ventral surface at right, anterior end at top. Scale = 100 μm.

deeply buttressed walls, small tubercles simpler, much less elevated (Fig. 6b).

Anterior end, micropyle: Anterior crown large, tending to be tapered anteriorly (Fig. 1), basal width and separation from anterior float margin as in Table 1. Shape of crown more or less circular (Fig. 7b), walls grooved (Fig. 7a,b), ventral walls considerably deeper

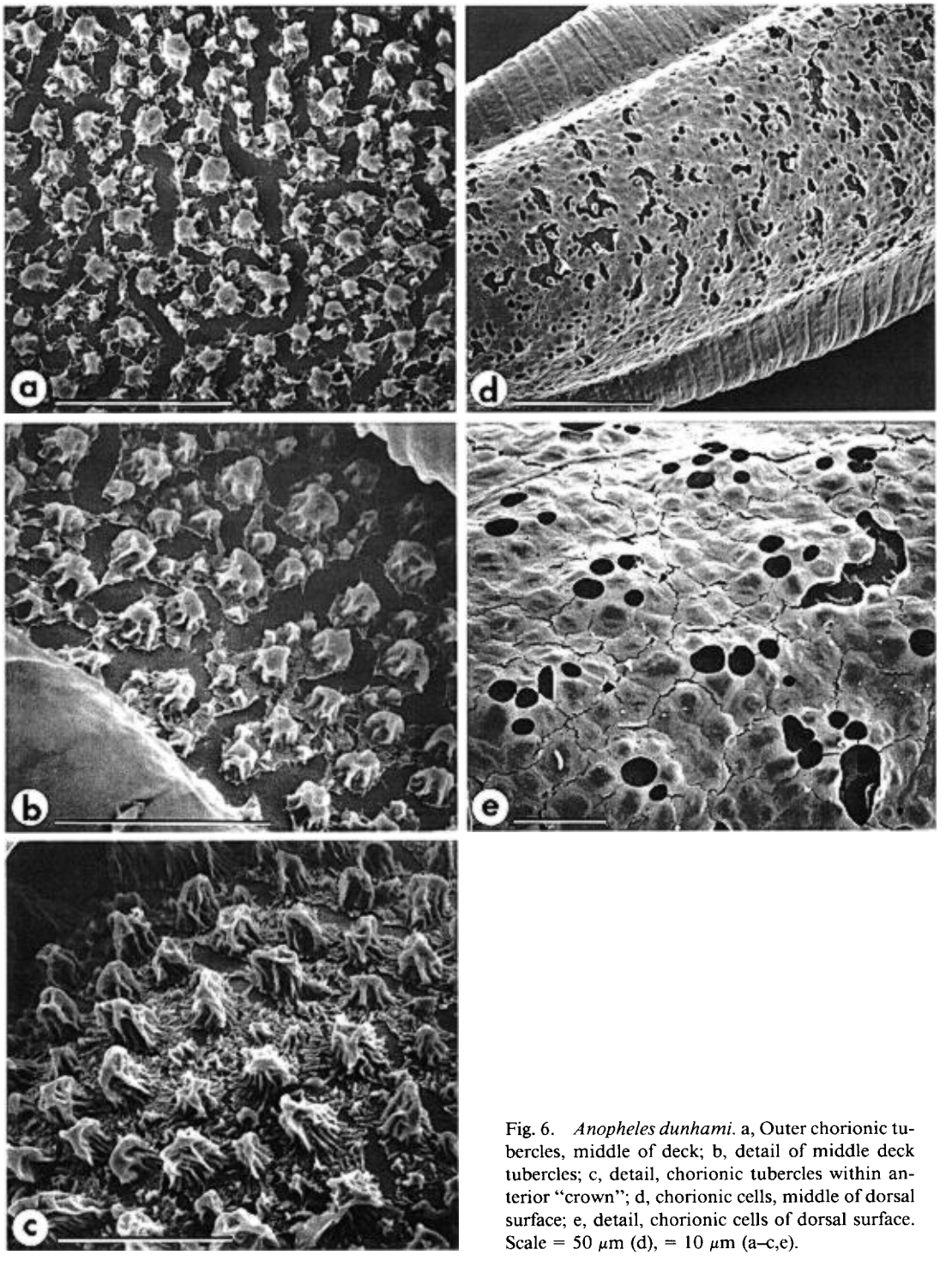

Fig. 6. *Anopheles dunhami*. a, Outer chorionic tubercles, middle of deck; b, detail of middle deck tubercles; c, detail, chorionic tubercles within anterior "crown"; d, chorionic cells, middle of dorsal surface; e, detail, chorionic cells of dorsal surface. Scale = 50 μm (d), = 10 μm (a–c,e).

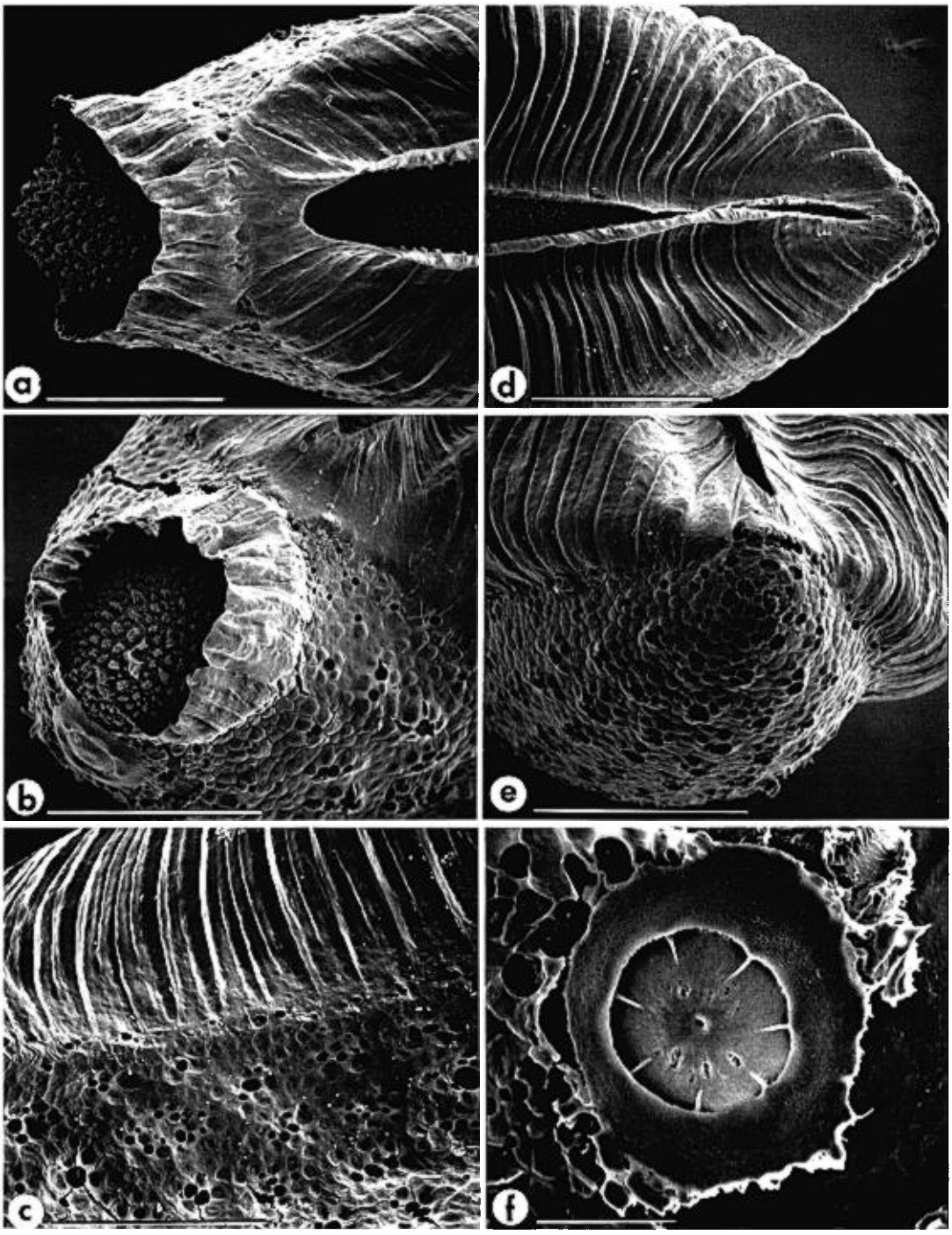

Fig. 7. *Anopheles dunhami.* a, Anterior end, ventral (top) surface; b, anterior end, end-on view; c, lateral surface, dorsal margin of float; d, posterior end, ventral surface; e, posterior end, end-on view; f, micropylar apparatus. Scale = 50 μm (a–e), = 10 μm (f).

than dorsal (Fig. 5b). Small deck within crown rounded anteriorly, jutting slightly beyond crown (Figs. 1, 7a), tubercles larger than on main deck (Fig. 7a), structurally higher and more peaked (Fig. 6c). Micropylar collar touching or extremely close to dorsal crown margin (Fig. 7b), collar surface smooth, with occasional pits, inner walls with shallow excavations and radial ridges (Fig. 7f). Sectors 6–8 in number (mean 7.1 ± 0.1, n = 20), disc surface slightly rough, micropylar orifice 0.8 μm in diameter, surrounding disc slightly domed (Fig. 7f).

Posterior end: Pointed and barely visible beyond posterior margin of floats (Figs. 1, 7d). Plastron close to and covering end perforated by pores, but these much fewer somewhat more anteriorly, just beneath float margins (Fig. 7e).

Comparison of Ecuadorian and Bolivian *An. rangeli*

Eggs of the two populations of *An. rangeli* were statistically inseparable in every measured attribute except the basal width of the anterior crown (Table 1). In this, Bolivian eggs were significantly ($P < 0.001$) larger, a difference discernible in the two Bolivian eggs shown (Fig. 1). In fact, only one of the Ecuadorian eggs exceeded the minimum diameter of the Bolivian group. Otherwise, the eggs of the two populations were obviously very similar (Fig. 1, Table 1).

Comparison of *An. rangeli* and *An. dunhami*

Although superficially rather similar (Fig. 1), the eggs of these two species differ substantially in several respects, and the differences are easily visible. Most obviously, the floats in *An. rangeli* are shorter and occupy a smaller proportion of the length of the egg (Table 1). Egg width across the floats also is greater, hence a significantly lower value is found in *An. rangeli* for the length/width ratio (Table 1). This difference, however, is related to the greater deck width in *An. rangeli,* as the mean float width (in ventral view, measured at approximately half the float length) is significantly ($P = 0.033$) narrower (mean 64.71 ± 0.91 μm, n = 24) in *An. rangeli* compared with *An. dunhami* (69.11 ± 1.78 μm, n = 24). In terms of the proportional contribution of total float area to total egg area, the *An. dunhami* floats are significantly larger, whereas the deck area is smaller (Table 1).

At the anterior end of the egg, the shorter floats in *An. rangeli* do not reach the posterior edge of the crown, and a clear gap separates the two structures (Fig. 1). In *An. dunhami,* on the other hand, the margins are almost invariably abutted, or the gap is extremely small (in the 12 eggs measured, two had gaps, of 4.4 and 9.1 μm, respectively). In the crown itself, the distinct anterior widening in *An. rangeli* contrasts with the straight or tapered appearance in *An. dunhami* (Fig. 1), and, also in the latter, the rounded anterior of the egg protrudes beyond the crown's rim.

DISCUSSION

The eggs of the two species are easily separable under a stereomicroscope, where the laterally swollen floats in *An. rangeli* create an appearance quite different from the narrower profile of *An. dunhami.* Confirmatory differences are the distinctly flared crown in *An. rangeli,* clearly separated at the posterior edge from the anterior margin of the floats, which themselves enclose a distinctly larger deck area.

Based on its abundance in zones of endemic malaria, we suspect that *An. rangeli* may be a vector of malarial parasites in both Napo Province, Ecuador, and Cochabamba Province, Bolivia. In Ecuador, one of us (L.P.L.) used egg characters described in this paper to separate *An. rangeli* from *An. dunhami,* the females of which are not reliably discriminated by existing morphological keys. Further, these two species are readily distinguished in the egg stage from their "sister" *An. nuneztovari* Gabaldon, whose ova have no crown (Linley, unpublished observation). Because egg structure may vary geographically among populations ascribed to the same

species, such as *An. nuneztovari* (Linley et al., in preparation), future work should endeavor to compare morphological features with topotypic specimens of *An. rangeli* from Puerto Cabillo, Venezuela (Gabaldon et al. 1940), and *An. dunhami* from Tefe, Brasil (Causey 1945).

Along with these two species, there are 12 others in the Albimanus Section of subgenus *Nyssorhynchus* (Faran 1980). For two of these (*An. ininii* Senevet and Abonnenc, *An. rondoni* (Neiva and Pinto)), the eggs are unknown, but in the remaining 10 the eggs are structurally distinct from the two species studied here. All except *An. strodei* Root have long floats (Causey et al. 1944), but the anterior part of the deck, although it may be rounded or oval, is either confluent with the main, open deck area or bounded posteriorly by the inner anterior parts of each float (Causey et al. 1944). In none of these species is the anterior patch of deck completely isolated at the extreme anterior end within a deep crown formed of a small circle of highly developed frill. The species in the Albimanus Section that most closely approaches this condition is, apparently, *An. benarrochi* Gabaldon, Cova Garcia and Lopez, where the very small anterior deck, although surrounded only by a shallow frill, has become separated from the floats and main deck (Causey et al. 1944). Nonetheless, the elevated walls of the crown in *An. rangeli* and *An. dunhami,* and its extreme anterior position, completely differentiate these species from the remainder. Eggs of *An. strodei* occasionally display separation of the anterior deck (Causey et al. 1944), but the floats in this species are substantially shorter than in the two we describe here.

Within the subgenus *Nyssorhynchus,* the closest and quite considerable resemblance is to *An. darlingi* Root, whose eggs do have a deep anterior crown, which is often though not invariably separated from the main deck (Causey et al. 1944, Linley 1992). Again, however, the floats in *An. darlingi* are unmistakably shorter than in the species presented here. The co-occurrence of a micropylar crown in *An. darlingi* of the Argyritarsis Section and *An. rangeli* and *An. dunhami* of the Albimanus Section suggests independent evolution of this structure in these distinctive lineages of *Nyssorhynchus.*

ACKNOWLEDGMENTS

For assistance in the field we thank J. Conn and G. Fritz (University of Florida, U.S.A.), H. Bermudez and R. Rodriguez (Universidad de San Simon, Bolivia), J. Cespedes, J. Cuba, A. Melgar, S. Villroel, and F. Yabeta Pedraza (Unidad Sanitaria, Bolivia), and J. Alarcon, E. Castro, G. Shiguango, and J. Yepez (Servicio Nacional de la Erradicacion de la Malaria, Ecuador). J. Conn and L. Hribar (University of Florida) corroborated identifications through DNA haplotypes and male genitalia. D. Duzak assisted in the electron microscopy laboratory and printed the micrographs. We appreciate reviews of the manuscript by J. Conn and L. Hribar. The research was supported by NIH grant 31034. This paper is Institute of Food and Agricultural Sciences, University of Florida Experiment Station Journal Series No. R-03001.

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