Ultrastructure of the eggs of two species of *Anopheles* (*Anopheles*) Meigen (Diptera, Culicidae)[1]

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Abstract. Egg ultrastructure of two morphologicaly similar species of the Arribalzagia Series, *Anopheles* (*A*.) *costai* Fonseca & Ramos, 1939 and *A*. (*A*.) *mediopunctatus* (Lutz, 1903) are described and illustrated using scanning electron micrographs. Although similar in the adult stage, male genitalia structures and larval and pupal stages, the eggs of these species are distinct. The eggs of *A. costai* and *A. mediopunctatus* are compared with that of *A. forattinii* Wilkerson & Sallum, 1999. Incomplete illustration of egg of *A. forattinii* is provided for comparison.

Keywords. *Anopheles*; Arribalzagia; Culicidae; egg; ultrastructure.

Resumo. Ultraestrutura dos ovos de duas espécies de *Anopheles* (*Anopheles*) Meigen, 1818 (Diptera, Culicidae). A ultraestrutura dos ovos de duas espécies morfologicamente semelhantes da Série Arribalzagia, *Anopheles* (*A*.) *costai* Fonseca & Ramos, 1939 and *A*. (*A*.) *mediopunctatus* (Lutz, 1903), é descrita e ilustrada usando-se microscopia eletrônica de varredura.. Embora sejam morfologicamente semelhantes nos estádios adulto, estruturas da genitalia masculina, larva de quarto estádio e pupa, os ovos destas duas especies são distintos. Os ovos de *A. costai* e *A. mediopunctatus* são comparados com os de *A. forattinii* Wilkerson & Sallum, 1999. Apresenta-se a ilustração incompleta do ovo de *A. forattinii* para comparação com os ovos das outras duas espécies.

Palavras-Chave. *Anopheles*; Arribalzagia; Culicidae; ovo; ultraestrutura.

Anopheles (*Anopheles*) *costai* Fonseca & Ramos, 1939 and *A*. (*A*.) *forattinii* Wilkerson & Sallum, 1999 have been largely misidentified as *A*. (*A*.) *mediopunctatus* (Lutz, 1903). The taxonomic status of these three taxa was clarified by Sallum *et al*. (1999) and Wilkerson & Sallum (1999). These authors also defined several morphological characters useful to distinguish the three species, which are members of the Arribalzagia Series as defined by Wilkerson & Peyton (1990). The Arribalzagia Series was demonstrated to be a monophyletic group within the subgenus *Anopheles* by Sallum *et al*. (2000, 2002). As far as it is known, *A. mediopunctatus* occurs on the coastal region of Brazil in the states of Rio de Janeiro and São Paulo, whereas *A. costai* is known from Argentina, Bolivia, Brazil, Colombia, Ecuador, the Guianas, Mexico, Panama, Peru, Suriname, Trinidad Tobago and Venezuela (Sallum *et al*., 1999), and *Anopheles forattinii* is known from Amazonian Brazil, Peru, Colombia and French Guiana (Wilkerson & Sallum 1999; Pecor *et al*. 2000). The only illustrations of eggs of several specimens identified as *A. mediopunctatus* from distinct localities in Brazil are those of Causey *et al*. (1944). These authors also suggested characters which could be useful to separate several species of the genus *Anopheles*. However, based on characters given in their identification key, as well as in the small illustrations, it is possible to recognize that eggs identified as being of *A. mediopunctatus* in Plate I and in drawing h in Plate II may belong to *A. costai* or *A. forattinii*, whereas drawing j in Plate II seems to be the egg of *A. mediopunctatus*. The present study describes the ultrastructure of the egg of two species of the Arribalzagia Series, *A. mediopunctatus* and *A. costai* and compare them with egg of *A. forattinii*, and with those of morphologically similar species of this series that were also described using scanning electron microscopic (Rodriguez *et al*. 2002; Lounibos *et al*. 1997; Forattini *et al*., 1997).

MATERIAL AND METHODS

Eggs were obtained from seven females of *A. costai*, three of *A. mediopunctatus* and one of *A. forattinii*. Adults of *A. costai* and *A. mediopunctatus* were collected in a Shannon trap in Icapara, Iguape municipality ($24^0$42'29"S $47^0$33'19"W), State of São Paulo, Brazil. A female of *A. forattinii* was collected in Iquitos, Peru. Eggs for SEM micrographs were obtained using the same procedure described by Forattini *et al*. (1997). Eggs were allowed 36 hours to embrionate, then 20 eggs from each oviposition were transferred to vials containing Bouin's fixative. The remaning eggs of each species were allowed to hatch and immatures were raised to adults for morphological identification of species using male genitalia structures. Length and width dimension of living eggs were measured with a stereomicroscope and digital length-measuring set. Eggs were examined in JEOL JSM P15 scanning electron microscope (Akishima, Tokyo, Japan). A full description of the egg of the egg of *A. forattinii* was not possible because we were enable

to obtain a large amount of eggs in adequate condition for the study. For this reason, the egg of *A. forattinii* was only used for comparison, and for the illustration of few characters. Voucher specimens are deposited in the entomological collection of Faculdade de Saúde Pública, Universidade de São Paulo, Brazil (FSP-USP).

RESULTS

Anopheles (*Anopheles) costai* Fonseca & Ramos, 1939 (Figs. 1, 2)

Anopheles (*Shannoniella*) *costai* Fonseca & Ramos 1939: 385.

Size: width 189 – 222 µm (mean = 204 µm + 0.01), length 485 – 522 µm (mean = 501 µm + 0.01), ratio of length to width 2.28 – 2.64 (mean = 2.46 + 0.1) (n= 82 eggs from 7 females).

Overall appearance. Black, boat-shaped in dorsal and lateral views (Fig. 1A; 2C), in lateral view contour is more or less flat dorsally and curved ventrally (Fig. 2C). Floats wide, lateral in position, long, well developed, extending most of egg length except at anterior and posterior ends (Figs. 1A; 2C,D,E,F); frill not continuous with floats, frill positioned dorsally at anterior and posterior ends, delimiting area in which lobed tubercles are placed (Fig. 1A,B,C).

Dorsal surface. Deck wide, slightly narrow at both anterior and posterior ends. Deck tubercles irregular in shape, forming well defined pattern of hexagonal outer chorionic cells with distinct boundaries, chorionic cells longer than wide, long dimension oriented in long axis of egg (Fig. 2A,B). Interior of each cell formed of round, flat tubercles, connected by narrow bridges, each chorionic cell surrounded by slightly raised outer chorionic reticulum formed of tubercles similar to those placed inside cells (Fig. 2A,C). Tubercles present on both anterior and posterior areas, which are encircled by frills, irregularly shaped with tiny tubercles intermixed with larger irregular in outline, more prominent tubercles, tubercle walls with vertical ridges and clefts (Fig. 1D,E,F).

Anterior and posterior ends. Similar in form, shape and development, however posterior end is slightly narrower than anterior end. Frill forming narrow dorsal ridge, collar-like, placed at both anterior and posterior ends; inner surface of frill irregular with tiny ridges (Fig. 1D,E,F). Area encircled by collar irregular in shape, longer than wide, somewhat oval in outline (Fig. 1D,E). Lobed tubercles well developed, 2,3 in number, somewhat oval or round in shape, tubercle wall covered with tiny ridges. Number of lobes per tubercle variable (Fig. 1D-F). Micropylar collar separated from anterior margin of frill by narrow area (Fig. 2D). Micropylar collar surface smooth, inner edge excavated, peaks between excavations tapering to form radial ridges, dividing micropylar disk into sectors (Fig. 2D).

Ventral and lateral surfaces. Plastron uniformly covered with pentagonal or hexagonal outer chorionic cells, each cell longer than wide, long dimension oriented in long axis of egg except for anterior and posterior ends (Fig. 2F). Interior of each cell formed of small, flat tubercles, irregular in shape and connected by narrow bridges, each chorionic cell surrounded by outer chorionic reticulum formed of slightly raised, irregularly shaped tubercles similar to those inside cells (Fig. 2F). Floats long, well developed, extending from anterior to posterior end of egg (Fig. 2C); ribs about 32-35 in number, slightly divided into irregular lobes (Fig. 2A,C,F).

Anopheles (*Anopheles*) *mediopunctatus* (Lutz, 1903) (Fig. 3)

Cycloleppteron mediopunctatus Lutz, 1903 *in* Theobald, 1903: 60

Size: width 164 – 209 µm (mean = 186 µm + 0.01), length 534 – 594 µm (mean = 567 µm + 0.01), ratio of length to width 2.69 – 3.48 (mean = 3.06 + 0.2) (n = 70 eggs from 3 females).

Overall appearance. Black, boat-shaped in dorsal (Fig. 3A) and lateral views (not shown). Floats long, shallow, displaced far ventrally (Fig. 3A,I). Frill well developed, forming ridges that extend along dorsal surface of egg, encircling deck and both anterior and posterior poles (Fig. 3C,H). Striation of dorsal ridge oblique and coalesced into whorls (Fig. 3A,B,G). Lobed tubercles well developed, present on both anterior and posterior poles, placed inside boundaries of frill (Fig. 3B,C,G,H).

Dorsal surface. Deck narrow, completely enclosed by dorsal ridges. Deck tubercles irregularly shaped, larger tubercles intermixed with tiny tubercles (Fig. 3F); tubercles placed at anterior and posterior ends around lobed tubercles somewhat larger than remaining deck tubercles (Fig. 3C,H). Each deck tubercle irregular in outline (Fig. 3C,F,H), tubercles at anterior and posterior ends with walls with vertical ridges and clefts, irregular at top (Fig. 3C,H). Dorsal area between frill and floats uniformly covered with hexagonal chorionic cells, each cell longer than wide, long dimension oriented in long axis of eggs (Fig. 3A,E). Interior of each chorionic cell formed of small, somewhat flat tubercles connected by narrow bridges. Outer chorionic reticulum of each chorionic cell formed of slightly raised tubercles similar to those inside chorionic cells.

Anterior and posterior ends. Frill well developed, continuous, encircling both anterior and posterior poles of egg (Fig. 3C,H), posterior end (Fig. 3G) slightly narrower than anterior end (Fig. 3B). Lobed tubercles placed dorsally on both anterior and posterior poles of egg , around 5 in number, somewhat oval in outline, walls covered with tiny ridges; number of lobes per lobed tubercle variable (Fig. 3C,H). Micropyle situated at anterior end of egg in center of low mound, micropylar collar smooth, inner boundary nearly straight between sectors and divided into sectors by short rays, which extend from collar to mycrophylar disk (Figs. 3D).

Ventral and lateral surfaces. Ventral and lateral surfaces uniformly covered with well defined, hexagonal outer chorionic cells, most cells longer than wide, long dimension oriented in long axis of egg (Fig. 3D,I). Interior of each cell covered with tubercles nearly round in outline and somewhat flat and

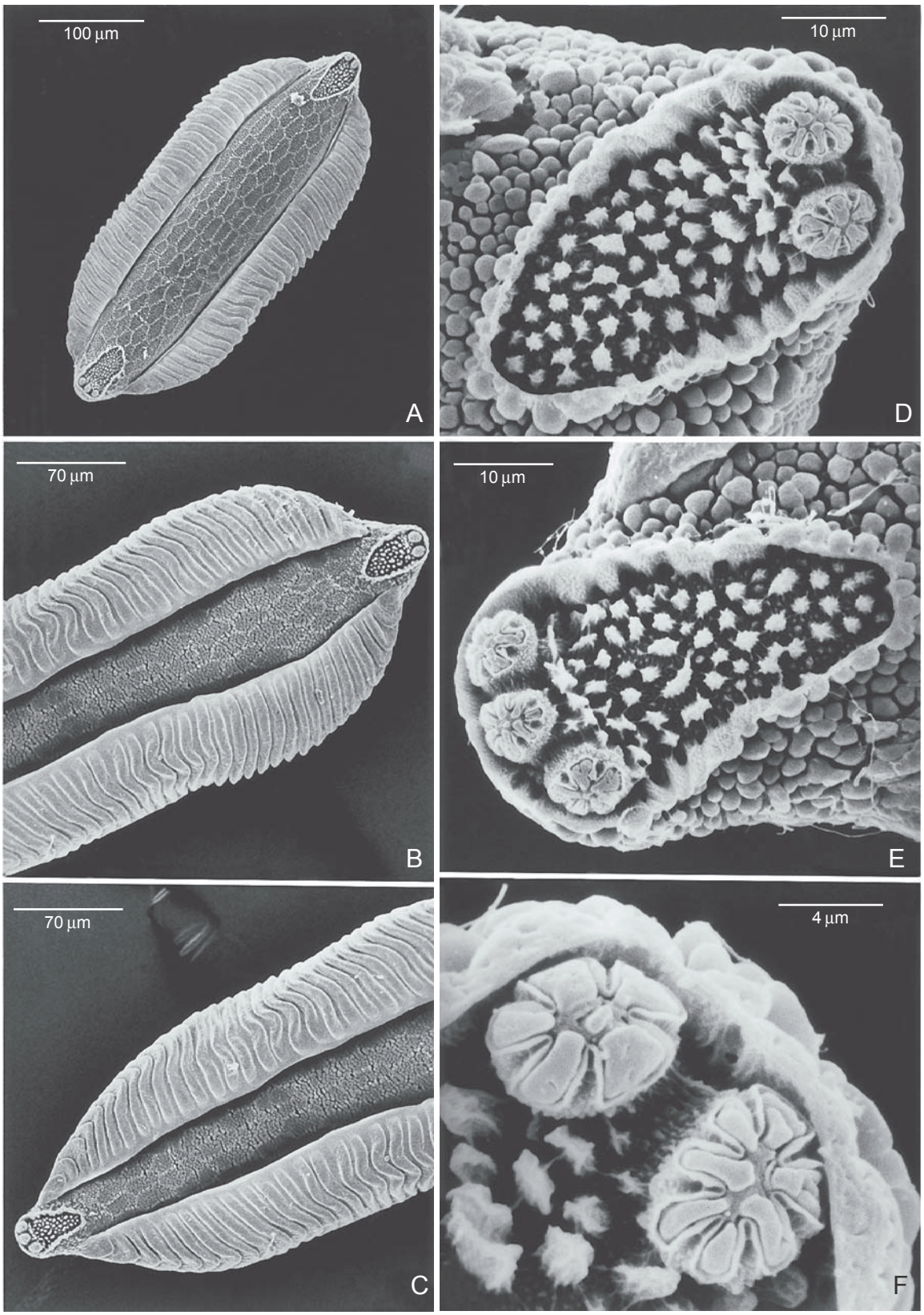

Fig. 1. Egg of *Anopheles* (*Anopheles*) *costai*. **A,** entire egg, anterior end at top, dorsal view; **B,** anterior end, dorsal view; **C,** posterior end, dorsal view; **D,** detail of anterior end, showing lobed tubercles and frill, dorsal view; **E,** detail of posterior end, showing lobed tubercles and frill, dorsal view; **F,** detail of lobed tubercles at anterior end.

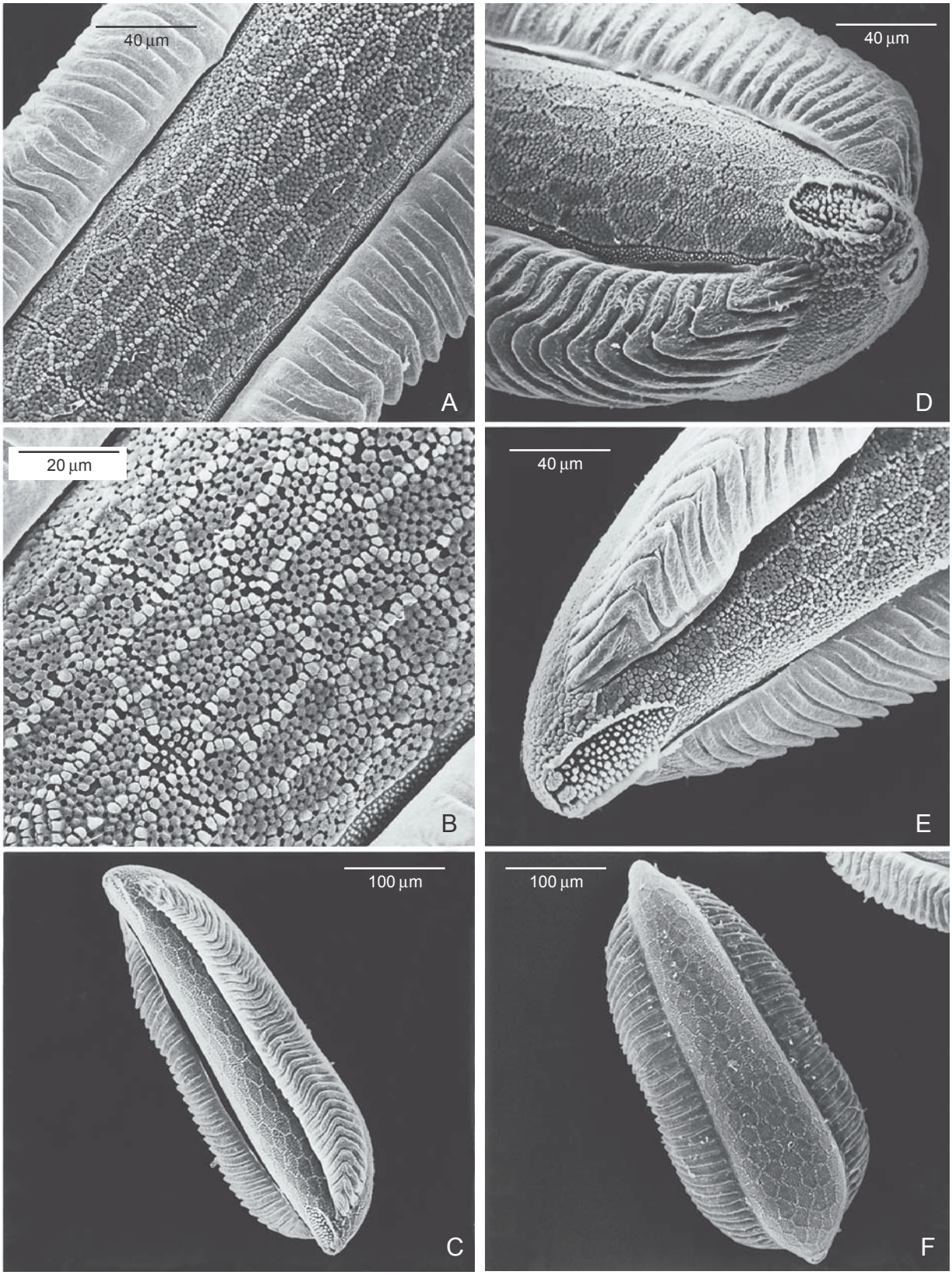

Fig. 2. Egg of *Anopheles* (*Anopheles*) *costai*. **A,** deck, middle region showing outer chorionic cells and floats; **B,** detail of deck, middle region; **C,** entire egg in dorsolateral view, anterior end at right top; **D,** anterior pole, showing microphyle; **E,** posterior end, in dorsolateral view; **F,** entire egg, anterior end at right top, ventral view.

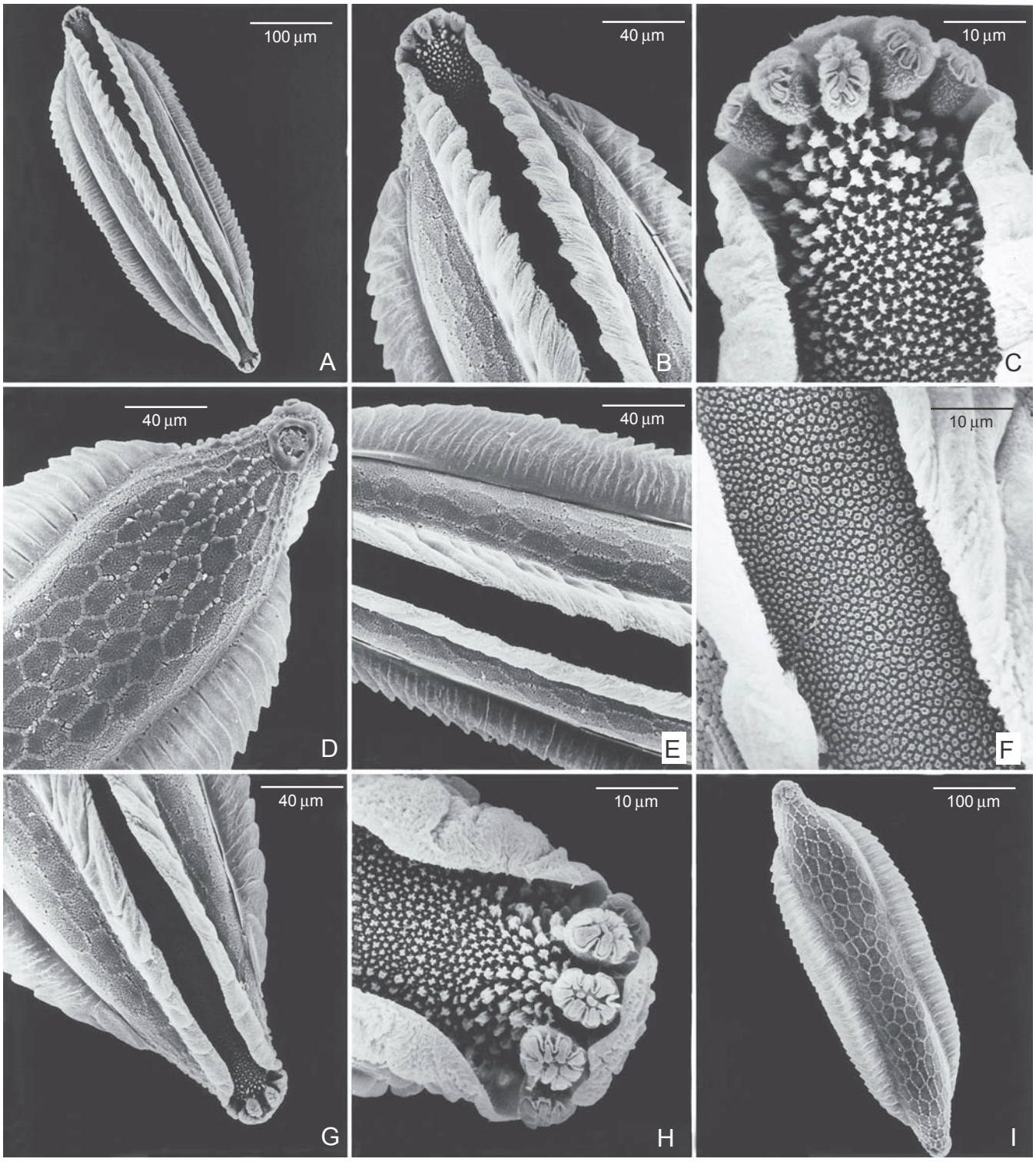

Fig. 3. Egg of *Anopheles* (*Anopheles*) *mediopunctatus*. **A,** entire egg, anterior end at top, dorsal view; **B,** anterior end, showing frill, dorsal view; **C,** anterior end, showing detail of deck tubercles and lobed tubercles, dorsal view; **D,** anterior end, ventral view, showing microphyle and outer chorionic cells; **E,** middle region of egg, showing frill and floats, dorsal view; **F,** deck tubercles, middle region, dorsal view; **G,** posterior end, showing detail of frill, dorsal view; **H,** posterior end, showing detail of deck tubercles and lobed tubercles; **I,** entire egg, anterior end at top, ventral view.

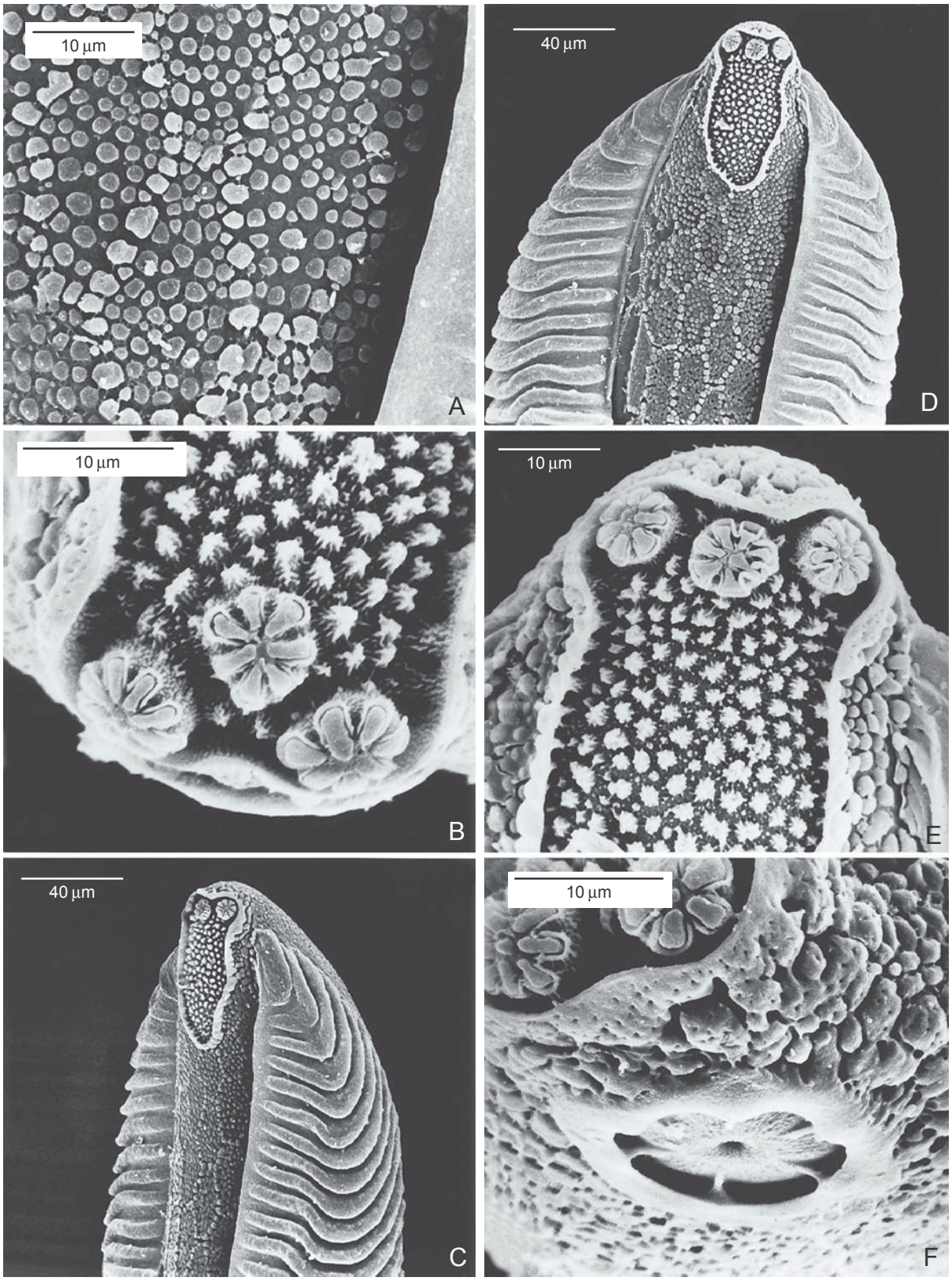

Fig. 4. Egg of *Anopheles* (*Anopheles*) *forattinii*. **A,** deck tubercles, dorsal view; **B,** anterior end, showing detail of lobed tubercles and deck tubercles, dorsal view; **C,** posterior end, showing floats, dorsolateral view; **D,** posterior end, showing floats and deck; **E,** posterior end, showing detail of lobed tubercles and deck tubercles; **F,** anterior pole, showing micropyle, anteroventral view.

smooth at top (Fig. 3D); tubercles connected by narrow bridges. Outer chorionic reticulum distinct, formed of slightly raised tubercles, larger than those found inside cells (Fig. 3D,I). Floats well displaced ventrally, shallow, long, extending along egg except at anterior and posterior ends, ribs about 34-37 in number, slightly divided into irregular lobes (Fig. 3A,E,I).

DISCUSSION

Causey *et al*. (1944) pointed out that the eggs of specimens identified as *A. mediopunctatus* from several localities in Brazil had distinct morphological features depending on the locality where the adults were collected. Although the limited resolution of the light microscopic drawings of each distinct type of egg, it seems that the drawing of the "typical" egg of *A. mediopunctatus* shown in Plate I (Causey *et al*. 1944) is not, in fact, of *A. mediopunctatus*, but from either *A. costai* or *A. forattinii*. The egg of *A. costai* (Figs. 1,2) seems to be indistinguishable from that of *A. forattinii* from Peru (Fig. 4). One of the drawings of eggs considered "atypical" by Causey *et al*. (1944) (Plate II, j) seems to be of *A. mediopunctatus* because it has a continuous ridge extending along dorsal surface of egg, enclosing the deck. The other two "atypical" eggs (Plate II, h, i) may be of either *A. costai* or *A. forattinii*.

In comparison with the eggs of related species of the Arribalzagia Series examined under scanning electron microscope, the egg of *A. mediopunctatus* is more similar to those of *A. fluminensis* Root, 1927 and *A. intermedius* (Peryassu, 1908) in having shallow floats, well displaced ventrally, and frill modified into prominent dorsal ridges, which extend along egg length encircling deck region. Lounibos *et al*. (1997) pointed out that the egg of *A. mediopunctatus* is distinct from that of *A. fluminensis* in having the central deck more exposed, and a dorsal ridge coalesced into whorls, whereas in *A. fluminensis* the frill overlaps dorsally, almost hiding the deck region, and the dorsal ridge does not coalesce into whorls. Additionally, in *A. mediopunctatus* the dorsal ridges are continuous along egg surface enclosing both anterior and posterior poles, whereas in *A. fluminensis* the dorsal ridges extend along the egg except for the anterior and posterior poles. The egg of *A. intermedius* is easily distinguished from that of *A. mediopunctatus* because the deck region is well exposed, not hidden by overlapping frills, the dorsal ridges are oblique to the long axis of the egg, and similar to the egg of *A. fluminensis* do not encircle both anterior and posterior poles of the egg.

Although morphologically similar to *A. costai* and *A. forattinii* in the adult stage, male genitalia structures and fourth-instar larva and pupa, the egg of *A. mediopunctatus* is distinct from those of *A. costai* and *A. forattinii*. The eggs of these two species do not have the frill developed in a dorsal ridge enclosing the deck region, and the floats are swollen and positioned laterally. In fact, the eggs of *A. costai* and *A. forattinii* most resembles those of *A. punctimacula* Dyar & Knab, 1906 (Rodriguez *et al*. 2002) in having the frill forming a narrow, dorsal collar at both the anterior and posterior ends of the egg, the areas encircled by the collar somewhat oval in outline, and with the deck region and ventral surface covered with hexagonal chorionic cells. However, the eggs of *A. costai* and *A. forattinii* can be distinguished from that of *A. punctimacula* in having the floats long, extending almost the entire length of the egg except for anterior and posterior ends, whereas they are short in *A. punctimacula*. Additionally, in *A. costai* and *A. forattinii* the frill encircles both the anterior and posterior poles of the egg (Fig. 1D,E; 4C,D,E), while in *A. punctimacula* frill is interrupted at the level of the lobed tubercles (see Fig. 4A,B in Rodriguez *et al*. 2002).

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