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FINE STRUCTURE OF THE EGGS OF *AEDES DURBANENSIS, AEDES WOODI,* AND *ERETMAPODITES QUINQUEVITTATUS* (DIPTERA: CULICIDAE)

J. R. LINLEY[1] AND M. W. SERVICE[2]

ABSTRACT. Ultrastructural descriptions are given of the eggs of *Aedes* (*Aedimorphus*) *durbanensis* (Theobald), *Aedes* (*Stegomyia*) *woodi* Edwards, and *Eretmapodites quinquevittatus* Theobald). In *Ae. durbanensis* the egg is broadly cigar-shaped and all surfaces are uniform in structure. Chorionic cells are considerably longer (in the egg's longitudinal axis) than wide; each contains several relatively large, central tubercles with smaller peripheral ones closer to the chorionic reticulum. The egg of *Ae. woodi* is structurally typical of the subgenus *Stegomyia,* except that it is extraordinarily long in relation to width. Cells on the ventral (upper) surface are slightly longer than wide, and each contains a single very large, central tubercle surrounded by many much smaller ones. Pronounced structural change occurs transitionally down the lateral to the dorsal (lower) surface, where cells contain only small, low tubercles scattered over the cell floor. *Eretmapodites quinquevittatus* eggs also are long relative to width and structurally different on the ventral and dorsal surfaces. They are remarkable, however, in that the transitional change, with four structurally distinct longitudinal zones, is the most complex yet documented in a mosquito egg. Ventral surface cells contain a single, very large, round central tubercle with several much smaller ones abutted against the reticulum. Progressively toward the dorsal surface, these are replaced (laterally) by cells with several low, smooth, medium-sized tubercles, then (dorsolaterally) by cells with a few larger, domed, centrally clumped tubercles, and finally (in a middorsal band) by cells in which several small tubercles are scattered randomly.

INTRODUCTION

The mosquitoes considered in this paper are all African species, none of which, as far as is known, is connected with the transmission of disease, although all are anthropophilic (Trpis and Gerberg 1974, Sharp et al. 1987, 1988). The larval and adult stages all have been described (for references see Knight and Stone 1977), but very little is known of the eggs. The egg stage is unknown in any descriptive sense in both *Aedes* (*Aedimorphus*) *durbanensis* (Theobald) and *Aedes* (*Stegomyia*) *woodi* Edwards, and only a brief verbal account, supplemented by two simple line drawings, has been provided for the egg of *Er. quinquevittatus* Theobald (Mattingly 1970). In this contribution we have used the scanning electron microscope to examine and document egg fine structure in the three species.

MATERIALS AND METHODS

The eggs of *Ae. durbanensis* and *Ae. woodi,* collected in 1969 in Tanzania and Kenya, respectively, were among those of several African species that had been preserved (by M.W.S.) in 70% ethanol for 24 years before being removed and prepared for electron microscopy. Dehydration in ethanol was continued by 5% concentration increments, followed by final critical point drying prior to mounting on stubs coated with sticky tape. Eggs were placed on stubs in the required attitudes by manipulation with a very fine artist's brush.

Eretmapodites quinquevittatus eggs were from a small laboratory colony originated

[1] Florida Medical Entomology Laboratory, IFAS, University of Florida, 200 9th St. S.E., Vero Beach, FL 32962.
[2] Vector Biology and Control, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, U.K.

from material collected in Kenya (W.A. Hawley, personal communication). Live, embryonated eggs were removed from oviposition papers and oriented on stubs, which were then kept briefly (five min) in a calcium chloride desiccator to ensure surface dryness. The mounted eggs of all three species were then sputter-coated with gold/palladium and examined immediately in a Hitachi S-510 scanning electron microscope.

All measurements were made from the micrographs with a digitizing tablet used in conjunction with SigmaScan software (Jandel Scientific, San Rafael, CA). Chorionic cell length was taken as the measurement in the longitudinal axis of the egg, width as the circumferential measurement. Data obtained for attributes of the chorionic cells were derived from an equal number of measurements from five eggs of each species. Means (±SE) are cited in the text for normally distributed attributes; median values are given for those whose distributions were not normal.

The terminology follows that proposed by Harbach and Knight (1980), supplemented by "outer chorionic cell field" and "anterior ring" (Linley 1989) and "micropylar dome" (Linley et al. 1991).

DESCRIPTIONS

Aedes (Aedimorphus) durbanensis
(Figs. 1–3)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Broadly cigar-shaped in ventral (Fig. 1) and dorsal view, lateral profile slightly more curved ventrally than dorsally (Fig. 2A), anterior ring present, micropylar collar erect, fairly conspicuous (Fig. 1). Cells of outer chorion pentagonal or hexagonal, considerably longer than wide, each cell with several tubercles, larger ones more centrally positioned (Fig. 1).

Chorion, ventral, lateral, and dorsal surfaces: All surfaces similar and uniform in structure. Outer chorionic cells longer than wide, length (measured on ventral surface) 27.4–47.4 μm (mean 36.9 ± 0.9 μm, n = 25), width 9.2–13.3 μm (mean 11.2 ± 0.2 μm),

Fig. 1. *Aedes durbanensis*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

Fig. 2. *Aedes durbanensis.* A, Entire egg, lateral view, ventral surface at top, anterior end at right; B, anterior end, ventral view; C, anterior end, chorionic cell detail; D, posterior end, lateral view; E, posterior end, chorionic cell detail; F, anterior end and micropylar apparatus; G, detail of micropylar apparatus. Scale = 200 μm (A), = 50 μm (B–D,F), = 20 μm (E,G).

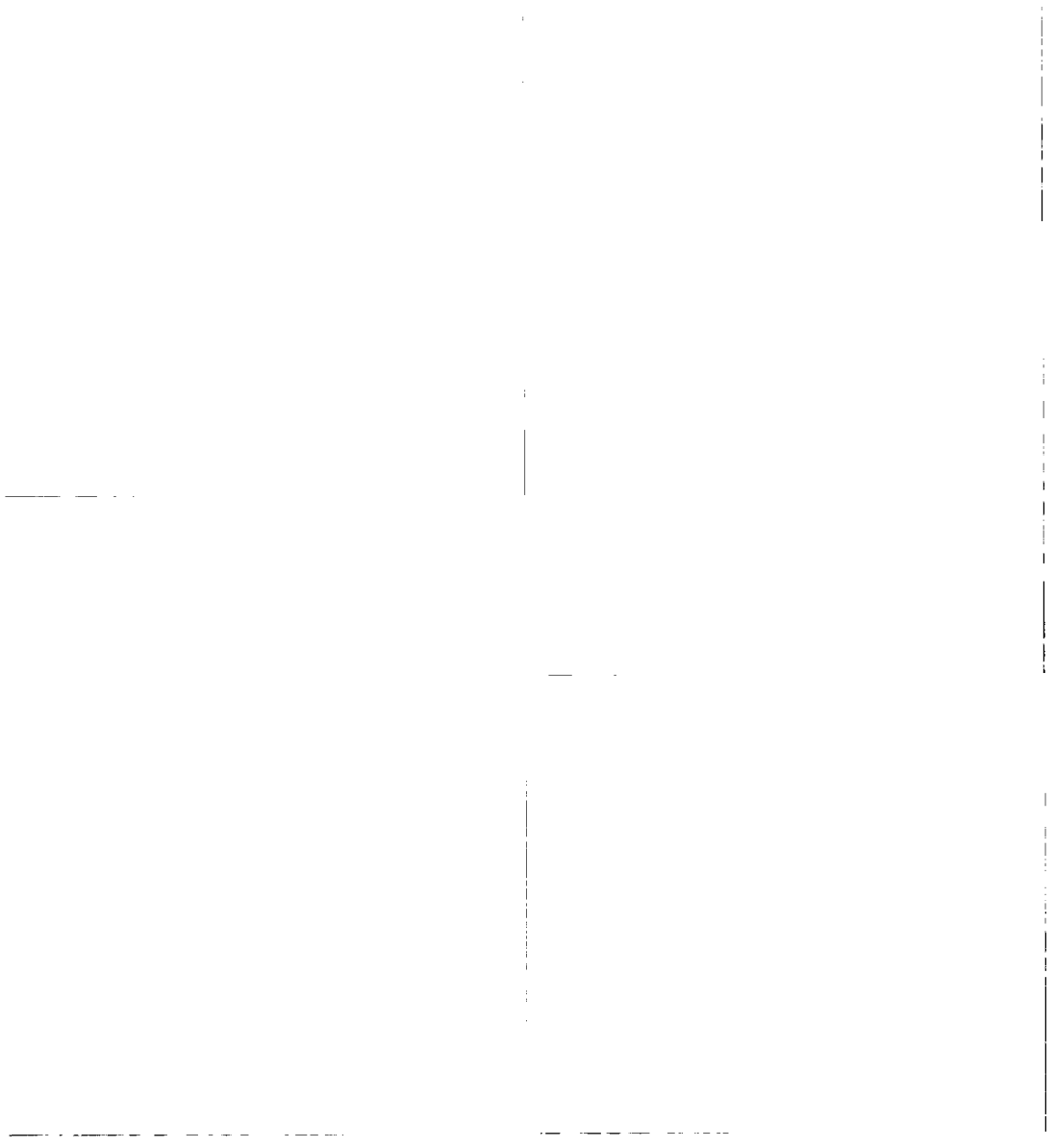

Fig. 3. *Aedes durbanensis.* A, Outer chorionic cells, ventral (upper) surface; B, chorionic cells, ventral surface, detail of tubercles and chorionic reticulum; C, lateral view, middle of egg, showing ventral–dorsal transition, ventral side at top; D, chorionic cells, lateral surface; E, chorionic cell detail, dorsolateral surface; F, unusual type of cell, dorsolateral surface, showing filamentous connections between tubercles. Scale = 50 μm (C,D), = 20 μm (A,E,F), = 10 μm (B).

anterior and posterior corners usually pointed (Figs. 1; 3A,C), floors smooth. Number of tubercles in each cell 14–28 (mean 20.8 ± 0.7, n = 25), diameter (widest point) 0.5–4.8 μm (median 1.9 μm, n = 120), shapes irregular, largest tubercles more centrally positioned, smaller ones peripheral, but not usually touching outer chorionic reticulum (Fig. 3A,B). Large tubercles more elevated than small ones (Fig. 3B), tubercle walls smooth, tops slightly domed, fairly smooth, often with ragged edges (Fig. 3A,B). Chorionic reticulum 0.8–1.7 μm wide, raised, faintly striated, and tubular except where edges incompletely folded together (Fig. 3B), edges supported on irregularly spaced fine pillars rising from cell floors (Fig. 3B).

Cell structure essentially the same down lateral surfaces of egg (Fig. 3C), except that central tubercles not relatively so large (Fig. 3D,E), reticulum tending to be more open, less completely folded. Dorsolateral and dorsal cells occasionally with filamentous material connecting tops of tubercles (Fig. 3F).

Anterior end, micropyle: Chorionic cells becoming smaller toward anterior end, with fewer tubercles (Fig. 2B), but detailed structure otherwise unchanged to posterior margin of anterior ring (Fig. 2C). Ring conspicuous, diameter 43–50 μm, formed of a palisade of tubercles clearly separated from micropylar collar (Fig. 2F,G). Collar almost invariably continuous (no gaps), erect, often flared outwardly at top (Fig. 2B,C,F,G), height 6.5–10.0 μm, outer wall indented, outer rim irregular (Fig. 2F,G). Width of collar wall 28–36 μm, surface slightly rough, peaked at outer rim and sloped on inner surface (Fig. 2F,G), inner diameter 18.0–22.5 μm, inner margin with only slight indentations. Edges of micropylar disc very indistinct, disc surface somewhat rough, micropylar dome not distinguishable, orifice diameter 2.4 μm.

Posterior end: Cells smaller toward posterior end, tubercles fewer, but structure otherwise unchanged (Fig. 2D). Shape of cells tending to become rounder very close to end of egg, reticulum flat, ribbonlike (Fig. 2E).

Table 1. Dimensions of the eggs of *Ae. durbanensis* (n = 6), *Ae. woodi* (n = 14), and *Er. quinquevittatus* (n = 15).

Species	Length (μm)		Width (μm)		L/W ratio	
	x̄ ± SE	Range	x̄ ± SE	Range	x̄ ± SE	Range
Ae. durbanensis	774.2 ± 11.0	755.6–827.6	237.2 ± 3.3	227.4–246.1	3.27 ± 0.04	3.16–3.36
Ae. woodi	932.2 ± 9.1	853.4–968.4	136.6 ± 3.9	114.7–163.8	6.88 ± 0.15	5.83–7.55
Er. quinquevittatus	691.4 ± 9.4	620.7–744.8	147.6 ± 2.7	126.7–157.1	4.70 ± 0.09	4.17–5.49

Aedes (*Stegomyia*) *woodi*
(Figs. 4–6)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Egg very long and narrow, widest at anterior 0.17, then tapering very gradually to rounded posterior end (Fig. 4). Anterior end slightly tipped upward (Fig. 5A). Outer chorionic cells prominent, regular in outline, boundaries clearly defined, each with a single large, central tubercle and several much smaller peripheral ones (Fig. 4). Micropylar collar fairly distinct.

Chorion, ventral (upper) surface: Cells mostly pentagonal, occasionally quadrilateral or hexagonal, slightly longer than wide (Fig. 6A), length 16.7–25.5 μm (mean 20.6 ± 0.5 μm, n = 25), width 12.4–21.6 μm (mean 16.3 ± 0.5 μm). Each cell with a very large, low, flat central tubercle 8.2–12.5 μm (mean 10.1 ± 0.2 μm, n = 25) long, 5.4–7.1 μm (mean 7.1 ± 0.1 μm) wide. Tubercle walls rough or nodular, tops slightly domed or flat, surfaces pitted between small, flat nodules (Fig. 6B). A narrow, mostly clear area surrounding each tubercle (Fig. 6A,B), remainder of cell floor with scattered, much smaller, peaked tubercles (Fig. 6B), 28–50 in number (mean 38.0 ± 1.1, n = 20), diameter 0.32–1.55 μm (mean 0.84 ± 0.03 μm), outer ones often touching chorionic reticulum (Fig. 6B). Reticulum 2.5–4.5 μm wide, trough-shaped, edges raised, surface with complex meshwork (Fig. 6B).

Chorion, lateral surface (ventral–dorsal transition): Outer chorionic cell structure changes substantially down lateral surface (Fig. 6C). Cells first become wider than long in ventrolateral region, large tubercles somewhat smaller (Fig. 6C,D), then substantially so progressively down the lateral region (Fig. 6E), with small tubercles much less clearly defined. Ultimately, on dorsal surface, cells contain only small, low tubercles scattered over cell floors, most tending to be peripherally distributed (Fig. 6F). Dorsal surface re-

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Fig. 4. *Aedes woodi*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

Fig. 5. *Aedes woodi*. A, Entire egg, lateral view, ventral side at top, anterior end at right; B, anterior end, lateral view; C, posterior end, ventral view; D, anterior end and micropylar apparatus; E, detail of micropylar apparatus, collar continuous; F, detail of micropylar apparatus, collar discontinuous. Scale = 200 μm (A), = 50 μm (B–D), = 10 μm (E,F).

Fig. 6. *Aedes woodi*. A, Outer chorionic cells, ventral (upper) surface, anterior end at left; B, chorionic cell detail, ventral surface; C, lateral surface, middle of egg, showing ventral–dorsal transition, ventral surface at top; D, cell detail, ventrolateral surface; E, cell detail, lateral surface; F, cell detail, extreme dorsolateral surface. Scale = 20 μm (A,C–F), = 10 μm (B).

ticulum much less conspicuous, with very faint striations and a thin, raised central line (Fig. 6F).

Anterior end, micropyle: Anterior end conically tapered, in lateral view appearing slightly tipped upward from dorsal (lower) side, ventral (upper) rim of micropylar collar protruding beyond dorsal rim (Fig. 5B). Cells becoming smaller toward margin of collar, but structure little changed until immediately at collar margin, where large tubercle may occupy almost entire cell and become fused to reticulum (Fig. 5B). Micropylar collar conspicuous, almost always with no gaps (Fig. 5D,E), a single division sometimes present (Fig. 5F), outer rim rounded, surface rough (Fig. 5E). Collar height 6.0–12.0 μm, outer diameter 29.5–36.0 μm, wall width 4.3–9.2 μm, internal diameter 17.0–20.4 μm, inner wall surface quite deep, excavated (Fig. 5E,F). Edges of micropylar disc difficult to discern, diameter 16.0–17.5 μm, surface rough (Fig. 5F). Micropylar dome also vaguely demarcated, diameter about 10 μm, orifice about 2.4 μm in diameter.

Posterior end: Approaching posterior end chorionic cells become smaller, large tubercle progressively filling more of cell, ultimately fusing with reticulum in most posterior cells (Fig. 5C). Large tubercles prominent even at very end of egg.

Eretmapodites quinquevittatus
(Figs. 7–9)

Size: As in Table 1. *Color*: Dark brown. *Overall appearance*: Cigar-shaped and long in relation to width, which is very uniform along most of length, anterior and posterior ends rounded (Fig. 7). Boundaries of chorionic cells difficult to distinguish, but their individual identity and regularity easily discerned from the single large tubercle present in each. Collar of micropyle very inconspicuous, not raised, conforming to rounded anterior shape of egg (Fig. 7).

Chorion, ventral (upper) surface: Chorionic cells almost invariably hexagonal, extremely uniform in shape (Fig. 8E), length 12.9–20.5

Fig. 7. *Eretmapodites quinquevittatus*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

Fig. 9. *Eretmapodites quinquevittatus.* A, Anterior end, lateral view; B, posterior end, lateral view; C, anterior end and micropylar apparatus; D, detail, micropylar apparatus. Scale = 50 μm (A,B), = 20 μm (C,D).

μm (mean 15.8 ± 0.3 μm, n = 25), slightly greater than width 10.8–16.4 μm (mean 13.1 ± 0.3 μm). Each cell almost entirely filled with a large, domed, smooth-surfaced, central tubercle (Fig. 8F), length 9.2–12.4 μm (mean 10.7 ± 0.2 μm, n = 25), width 6.8–11.2 μm (mean 8.8 ± 0.2 μm). Around central tubercle, abutted against reticulum, are 5–16 (mean 10.2 ± 0.7, n = 20) small, nodular tubercles 0.3–1.4 μm (mean 0.84 ± 0.03 μm, n = 60) in diameter (Fig. 8F). Reticulum itself 1.8–3.6 μm wide, consisting of short, irregular ridges overlain by an intricate meshwork (Fig. 8F,G).

Chorion, lateral and dorsal surface (ventral–dorsal transition): Change in structure from ventral to dorsal surfaces very striking, with abrupt transition through three completely distinct cell types (Fig. 8A–D). Ventrolaterally, cells become wider than long,

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Fig. 8. *Eretmapodites quinquevittatus.* A, Outer chorionic cells, ventrolateral surface, middle of egg; B, chorionic cells, dorsolateral surface; C, chorionic cells, extreme dorsolateral surface; D, low power dorsal view, middle of egg, middorsal line traversing approximate center of micrograph; E, chorionic cells, ventral (upper) surface; F, chorionic cell detail, ventral surface; G, extreme detail of tubercles and chorionic reticulum, ventral surface; H, chorionic cell detail, ventrolateral surface. Scale = 50 μm (D), = 20 μm (A–C,E), = 5 μm (F–H).

large central tubercles in each cell replaced by several low, smooth tubercles with rounded or polygonal edges (Fig. 8A,H), diameter (widest) 0.6–6.4 μm (median 2.4, n = 75), reticulum narrowed to form a thin ridge with transverse striations (Fig. 8H). In extreme dorsolateral region, cells again change abruptly (Fig. 8B) so that reticulum (cell boundaries) no longer visible, tubercles fewer in number, larger (diameter 1.7–5.5 μm, mean 3.7 ± 0.1 μm, n = 75), and clumped into fused groups (Fig. 8B,C). Finally, in a middorsal band, cells change to a form (Fig. 8C) in which scattered tubercles are more numerous and smaller (diameter 0.8–3.5 μm, mean 1.92 ± 0.05 μm, n = 75). Dorsal aspect of egg shows distinct bands of cell types from lateral to middorsal surfaces (Fig. 8D).

Anterior end, micropyle: Egg profile rounded anteriorly in lateral as well as ventral view (Fig. 9A), cell structure hardly modified close to micropylar collar. Collar wide (42.0–59.9 μm), very inconspicuous, conforming to rounded shape of egg's anterior (Fig. 9A), wall width 8.1–18.0 μm, very variable, outer margin very irregular (Fig. 9C,D), collar surface rough (Fig. 9C). Inner collar margin rounded, diameter 26.9–33.8 μm, edge with very shallow excavations, micropylar disk well defined, edge slightly raised (Fig. 9C,D), diameter 23.2–27.0 μm, surface quite rough (Fig. 8D). Orifice very slightly trilobed (Fig. 9D), diameter 2.5 μm.

Posterior end: Lateral profile rounded (Fig. 9B), chorionic cell structure near and at end of egg very similar to remainder of egg surface.

DISCUSSION

The egg of *Ae. durbanensis* has much in common with those of other species in the subgenus *Aedimorphus,* of which three species have been described ultrastructurally, *Ae. vexans* (Meigen) (Matsuo et al. 1972, Moriya et al. 1973, Linley 1990) and *Ae. dentatus* (Theobald) and *Ae. fowleri* (Charmoy) (Linley and Turell 1993). All these eggs are basically similar in shape and show little or no differentiation between the ventral and dorsal surfaces; chorionic cells are considerably longer than wide over the whole egg and uniform in structure. Exceptions to this pattern may occur in some species, however, as shown by Reinert's (1972) line drawing of the egg of *Ae. domesticus* (Theobald), where cells in a zone in the middle of the egg are wider than long, as opposed to the reverse at the anterior and posterior ends. Of the three African species described ultrastructurally, *Ae. durbanensis* is very similar to *Ae. dentatus* (see Linley and Turell 1993), but the egg of the former is significantly ($P < 0.006$) longer (mean 819.2 ± 8.4 μm *cf.* 774.2 ± 11.0 μm) and differences in the cell tubercles and structure at the anterior of the egg should make stereomicroscopic differentiation relatively simple. There are fewer tubercles in the cells of *Ae. dentatus,* they are larger, and none is as small as in *Ae. durbanensis* or distributed around the periphery of the cell close to the reticulum. Also, the anterior ring and erect micropylar collar of *Ae. durbanensis* are not found in *Ae. dentatus,* where the collar is very inconspicuous (Linley and Turell 1993). The anterior ring and micropylar collar of the *Ae. fowleri* egg are very like those of *Ae. durbanensis,* but the reticulum and tubercle structure are quite different and this would be obvious under a stereomicroscope.

Except for its remarkable length and thinness, structural features in the egg of *Ae. woodi* are typical of the subgenus *Stegomyia* (e.g., Matsuo et al. 1974, Linley 1989). Cells on the ventral (upper) surface have a single, large central tubercle surrounded by smaller ones, and there is a pronounced lateral transition leading to dorsal surface cells that contain only a few small, low, scattered tubercles. *Aedes woodi* females are known to lay their eggs only in the axils of leaves of certain species of sedge, such as *Cyperus grandis* (Trpis and Gerberg 1974), where the egg's extraordinarily thin profile would enable it to be inserted into very narrow crevices between the leaves. This presumably would offer protection from predation but might also be essential in preventing desiccation, especially as these axils contain only a few millimeters of water and, also, because the eggs of *Ae. woodi* are un-

usual among *Stegomyia* in having little resistance to drying (Trpis and Gerberg 1974).

In terms of complexity of the ventral–dorsal transition, *Er. quinquevittatus* eggs display the most elaborate structural sequence so far recorded in a mosquito egg, with remarkably abrupt and substantial changes in cell form at each successive boundary. They share the general arrangement of *Stegomyia,* however, in that larger tubercles and a more intricate reticulum are found on the ventral surface, whereas the dorsal surface, cemented in some degree to the substrate, has only small, less prominent tubercles. Eggs of *Er. quinquevittatus* are laid usually in empty snail shells [*Achatina fulica* (Ferussac)] but also in banana and pineapple axils and, rarely, in tin cans and bottles. Eggs exhibit a certain degree of drought resistance, as apparently do the eggs of some other species of *Eretmapodites* (Lounibos 1980, Service 1990).

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