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ULTRASTRUCTURAL DESCRIPTIONS OF THE EGGS OF *AEDES MCINTOSHI* AND *AEDES CIRCUMLUTEOLUS* (DIPTERA: CULICIDAE)

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ABSTRACT. Scanning electron micrographs are used to illustrate descriptions of the eggs of *Aedes* (*Neomelaniconion*) *mcintoshi* Huang and *Ae.* (*Neo.*) *circumluteolus* (Theobald). The eggs of both species are rhomboidal in dorsal or ventral view, particularly in *Ae. circumluteolus*, with the ventral surface in each case being substantially more curved than the dorsal. The chorionic cells are irregularly shaped in both species. Their structure is uniform over the whole egg surface in *Ae. circumluteolus*; in *Ae. mcintoshi* the anterior ventral cells have a distinct, large, central tubercle instead of the more uniformly sized tubercles present in cells over the remaining egg surface. The micropylar apparatus of both species is unusual in that the disk in most eggs may be covered with a dense mat of fine filamentous strands connected to small, sharp, irregular papillae.

INTRODUCTION

Aedes (*Neomelaniconion*) *mcintoshi* was described by Huang (1985) after a study of the very widespread mosquito *Aedes* (*Neomelaniconion*) *lineatopennnis* (Ludlow). Huang concluded that the African form was distinct from that in the Oriental and Australian regions, which retains the name *Ae. lineatopennis*. Currently, the subgenus *Neomelaniconion* is being revised (T.J. Zavortink, personal communication), and justifications have been presented (Zavortink 1993) for retaining the names *Ae. mcintoshi* and *Ae.* (*Neo.*) *circumluteolus* (Theobald), the second species considered here, which also is common and widely distributed in sub-Saharan Africa.

The subgenus *Neomelaniconion* is important as it includes a number of species implicated in the transmission of numerous arboviruses (including four Flaviviridae, four Togaviridae, and five Bunyaviridae). Specific isolations have been documented from both *Ae. mcintoshi* and *Ae. circumluteolus* (e.g., Worth et al. 1961, McIntosh et al. 1972). However, despite the interest in these mosquitoes as possible vectors of disease, little knowledge exists concerning the eggs. Mattingly (1974) cited an early description of the egg of *Ae. lineatopennis* by Banks (reference *in* Mattingly 1969), based on material collected in the Philippines, and supplemented the description with simple drawings from his own examination of four eggs collected in Negri Sembilan (southwestern Malayan peninsula). In this paper, with the aid of the scanning electron microscope, we are able to provide much more complete descriptions of the eggs of *Ae. circumluteolus* and *Ae. mcintoshi*.

MATERIALS AND METHODS

Eggs of both species were obtained from females reared from larvae hatched from eggs collected in soil samples in Kenya. The females were blood-fed, and several laid probably infertile eggs on wet filter paper. After 48 hr, the eggs were fixed in alcoholic Bouin's solution, dehydrated through a very gradual ethanol series, and dried finally by the critical point method. As a precaution against collapse of the eggs during pressure equalization in the critical point chamber, the rate of depressurization was somewhat accelerated

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(Linley 1988), which probably contributed to preserving the natural shape of the eggs throughout subsequent examination. Dried eggs were lifted with a fine artist's brush and oriented on stubs to allow scrutiny of all surfaces. After being sputter-coated with gold, the eggs were scanned immediately in a Hitachi S-510 scanning electron microscope.

At least 30 eggs of each species were examined as a basis for the descriptions. Measurements of the outer chorionic cells and tubercles were derived from five cells of five eggs of each species and were made with a digitizing tablet and SigmaScan software (Jandel Scientific, San Rafael, CA). Means cited in the text are given ± SE. The terminology is that of Harbach and Knight (1980), supplemented by "outer chorionic cell field" (Linley 1989) and "micropylar dome" (Linley et al. 1991a).

DESCRIPTIONS

Aedes (*Neomelaniconion*) *mcintoshi*
(Figs. 1–3)

Size: Length 714.0 ± 14.2 μm (range 656.1–782.8 μm), width 290.6 ± 6.3 μm (range 250.1–318.2 μm), length/width ratio 2.46 ± 0.06 (range 2.22–2.46). *Color*: Very dark brown. *Overall appearance*: Shape asymmetrical in lateral view, ventral surface more curved, dorsal surface posterior to widest point usually flat or slightly concave, anterior end conical (Fig. 1). Shape in dorsal or ventral view (Fig. 2A) somewhat rhomboidal, widest point slightly anterior to middle of egg. Boundaries of outer chorionic cells difficult to distinguish, particularly in posterior 0.5 of egg. Cells of anterior 0.4 contain a single tubercle that is distinctly larger than the remainder (Figs. 1, 2A), but tubercles in posterior cells more or less equal in size. Gradation in tubercle size less apparent on lateral and dorsal surfaces (Fig. 1). Micropylar collar fairly conspicuous (Figs. 1, 2A).

Chorion, ventral, lateral, and dorsal surfaces: Except that ventral and to some extent lateral chorionic cells have a distinctly larger central tubercle (Figs. 1, 2A), cells on all other

Fig. 1. *Aedes mcintoshi*. Entire egg, lateral view, anterior end at top, dorsal side at left. Scale = 100 μm.

surfaces similar, very irregular in outline, tending to be wider circumferentially than in the longitudinal axis of the egg (Fig. 2B), boundaries indistinct, with no apparent structural transition from ventral to dorsal surfaces (Fig. 2B). Single large tubercle in most anterior ventral cells substantially larger (Fig. 2C) (diameter (widest point) 8.1–11.3 μm (mean 9.6 ± 0.2 μm, n = 20)) than small peripheral tubercles (diameter 1.1–5.4 μm (mean 2.7 ± 0.2 μm, n = 40)). Large tubercles moderately prominent, usually widened at base (Fig. 2C), top surfaces domed, rough, small tubercle surfaces with somewhat more distinct nodular texture (Fig. 2D). Posterior cells of ventral surface with no large central tubercle, cell fields instead more or less evenly covered with flatter tubercles of more uniform size (Fig. 2E), and this structure typical also of cells of lateral (Fig. 2F) and dorsal (Fig. 2G) surfaces. Diameters of these tubercles, as represented by lateral cells, 0.8–6.1 μm (mean 3.4 ± 0.1 μm, n = 78). Cell fields on all surfaces of egg slightly rough (Fig. 2D–G), outer chorionic reticulum narrow (0.5–1.5 μm wide), usually widest in cell corners, surface occasionally perforated (Fig. 2E), edges connected to cell floors by narrow pillars (Fig. 2E–G). Irregular strips of reticulumlike material also present on interior cell surfaces, sometimes partly surrounding tubercles (Fig. 2E–G).

Anterior end, micropyle: Outer chorionic cells smaller toward micropylar collar, large tubercle distinct even in cells on dorsal side (bottom, Fig. 3A), numbers of small tubercles fewer (Fig. 3B). Micropylar collar quite distinct, height 7–12 μm, several gaps present in all of many specimens examined (Fig. 3B–D). Collar outer diameter 43–50 μm, wall width 5–8 μm, surface fairly smooth (Fig. 3C,D), inner collar diameter 28–32 μm, inner wall rather shallow, but with distinct excavations (Fig. 3C,D). Edge of micropylar disk fairly distinct, disk diameter 20–25 μm, surface (including micropylar dome) occasionally bare (Fig. 3C), but much more frequently covered with fine strands fused into closely spaced, narrow papillae, creating velcrolike appearance (Fig. 3D). Micropylar dome distinct (Fig. 3C), diameter 9–13 μm, orifice trilobed (Fig. 3D), diameter 1.9 μm.

Posterior end: Structure of chorionic cells on all surfaces very little changed close to posterior end (Fig. 3E), except that cells smaller and tubercles fewer and tending to become larger in cells very close to end of egg (Fig. 3E,F).

Aedes (*Neomelaniconion*) *circumluteolus* (Figs. 4–7)

Size: Length 697.5 ± 5.9 μm (range 642.9–733.1 μm), width 280.3 ± 2.8 μm (range 259.1–299.5 μm), length/width ratio 2.49 ± 0.03 (range 2.27–2.72). *Color*: Very dark brown. *Overall appearance*: Shape distinctly asymmetrical in lateral view (Fig. 4), ventral surface considerably more curved than dorsal, occasionally tending to be slightly humped at widest point, just anterior to middle (Fig. 4). Dorsal surface more or less straight except for some curvature at anterior and posterior ends. Profile in ventral and dorsal views rhomboidal, posterior end rather pointed (Fig. 5A). Outer chorionic cells irregular in shape, boundaries as delineated by reticulum easily visible (Fig. 4), no distinct patterns visible in arrangement of tubercles within each cell. Micropylar collar rather inconspicuous (Figs. 4, 5A).

Chorion, ventral, lateral, and dorsal surfaces: All surfaces very similar, no transition in structure apparent from ventral to dorsal surfaces (Figs. 4, 5B). Chorionic cells tending to be wider circumferentially than longitudinally, containing tubercles of fairly uniform

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Fig. 2. *Aedes mcintoshi*. A, Entire egg, ventral view, anterior end at right; B, chorionic cells, lateral surface, just anterior to middle of egg, ventral surface at top; C, chorionic cells, anterior ventral surface; D, cell detail, anterior ventral surface; E, cell detail, ventral surface, just posterior to middle of egg; F, lateral surface cell detail, middle of egg; G, dorsal surface cell detail, middle of egg. Scale = 200 μm (A), = 50 μm (B, C), = 10 μm (D–G).

Fig. 3. *Aedes mcintoshi.* A, Anterior end, lateral view; B, anterior end, chorionic cell detail; C, micropylar apparatus, disk surface rough but without fine strands or protrusions; D, micropylar apparatus, disk with filamentous strands and small, dense papillae; E, posterior end, lateral view; F, posterior end detail. Scale = 50 μm (A, E), = 20 μm (B–D, F).

Fig. 4. *Aedes circumluteolus*. Entire egg, lateral view, anterior end at top, dorsal side at left. Scale = 100 μm.

size, diameter (lateral cells) 0.9–4.9 μm (mean 2.8 ± 0.1 μm, n = 103), dispersed more or less evenly over cell fields (Fig. 5B). Tubercles of ventral cells moderately raised, consisting of a smooth base with distinctly nodular, slightly domed cap (Fig. 5C), this structure typical also in lateral (Fig. 5D) and dorsal (Fig. 5E) cells. Floors of cell fields somewhat rough (Fig. 5C,D,F), outer chorionic reticulum raised, width 1.0–2.3 μm (widest at cell corners), surface perforated by tiny pores (Fig. 5F) and supported on thin pillars (Fig. 5D). Reticulum occasionally branched into cell fields, sometimes completely crossing cell (Fig. 5D), or present as isolated pieces (Fig. 5C,E).

Anterior end, micropyle: Anterior end conical, outer chorionic cells smaller toward micropylar collar, tubercles not smaller but more closely packed (Fig. 6A), cells even immediately posterior to collar containing reticulumlike strips within cell fields (Fig. 6B). Collar of micropyle rather inconspicuous, posterior margin often ragged, poorly defined (Fig. 6B,C), height 2.5–7.0 μm and variable, outer diameter 31–42 μm, wall width 4.0–7.5 μm, surface rough and often perforated on outer margin (Fig. 6C). Internal collar diameter 20–26 μm, inner wall shallow, excavated (Fig. 6C,D). Micropylar disk clearly defined, edge slightly elevated, close to inner wall of collar (Fig. 6C,D), diameter 19–24 μm, surface with fine strands and papillae (Fig. 6C), these less developed than in *Ae. mcintoshi,* or with fewer coalesced strands (Fig. 6D). Micropylar dome distinct (Fig. 6C,D), diameter 8–10 μm, micropylar orifice 1.7 μm in diameter.

Posterior end: Chorionic cell structure very little modified close to posterior end, except that cells smaller (Fig. 6E), reticulumlike strips within cell fields more closely surrounding tubercles (Fig. 6F). In some eggs, areas of both ventral and dorsal chorion at posterior end of egg made up of cells with slightly different structure (Fig. 7A). In these areas, cells with usual complement of tubercles, but lacking distinct reticulum or reticulumlike material within cells (Fig. 7B). Reticulum instead very indistinct, visible as a line of more

Fig. 5. *Aedes circumluteolus*. A, Entire egg, ventral view, anterior end at left; B, chorion, lateral surface, just anterior to middle of egg; C, cell detail, ventral surface; D, cell detail, lateral surface, middle of egg; E, cell detail, dorsal surface; F, detail of tubercles and chorionic reticulum, ventral surface. Scale = 200 μm (A), = 50 μm (B), = 10 μm (C–F).

Fig. 6. *Aedes circumluteolus.* A, Anterior end, lateral view; B, anterior end, chorionic cell detail; C, micropylar apparatus, disk overlain with fine strands and papillae; D, micropylar apparatus, disk less occluded with strands, which appear in some places to be fused to form clumps; E, posterior end, lateral view; F, posterior end, chorionic cell detail. Scale = 50 μm (A, E), = 20 μm (B–D, F).

or less evenly spaced papillae, tubercles as in more common form (Fig. 7C).

DISCUSSION

The eggs of these two species of *Neomelaniconion* are unusual in a number of respects. They are structurally extremely uniform, particularly in *Ae. circumluteolus,* with very irregularly shaped chorionic cells and little or no differentiation between the ventral and dorsal surfaces. Surface differences may be very pronounced in species that glue their eggs to the substrate, as, for example, in the subgenera *Stegomyia* (Linley 1989), *Finlaya* (Linley et al. 1991a, 1991b), or, particularly, *Halaedes* (Linley et al. 1992). The lack of chorionic differentiation or traces of cement on the scanned eggs indicates that both these *Neomelaniconion* species almost certainly do not attach their eggs at oviposition but rather simply deposit them on wet soil.

In their individual structure, the chorionic cells of *Ae. mcintoshi* and *Ae. circumluteolus* are unusual in having strips of material structurally indistinguishable from the outer chorionic reticulum deposited within the cell fields (e.g., Figs. 2E,F; 5D,E), which makes the already irregular cell outlines difficult to distinguish, particularly in *Ae. mcintoshi.* In some *Aedes* species, there may be sheets of material extending from the reticulum to a central large tubercle, partially covering the cell, as in *Ae.* (*Gymnometopa*) *mediovittatus* (Coquillett) (Linley and Clark 1989), or completely covering cells over large areas of the chorion, as in *Ae.* (*Ochlerotatus*) *mariae* (Sergent and Sergent) (J.R.L., unpublished observations), but the branching of thin strips of reticulum into and sometimes across the cells is unique among *Aedes* eggs so far examined ultrastructurally. As far as we are aware, also, the micropylar disks in the two *Neomelaniconion* species differ from others known at this level of structural detail in that their surfaces may be covered with a velcrolike texture of fine strands associated with tiny, ridgelike papillae.

Fig. 7. *Aedes circumluteolus.* A, Posterior end, showing alternate form of chorion (right and top), lacking narrow, raised chorionic reticulum or reticulumlike material within the cells; B, boundary between alternate form of chorion (right) and more usual form (left); C, chorionic cell detail, alternate form of chorion. Scale = 50 μm (A, B), = 10 μm (C).

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