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THE REMARKABLE EGG OF *ANOPHELES PERYASSUI* (DIPTERA: CULICIDAE)

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ABSTRACT. An ultrastructural description of the egg of *Anopheles* (*Anopheles*) *peryassui* Dyar and Knab is given from material collected in Venezuela. The egg is remarkable in several respects. Its deck and frill, on the ventral surface, have been reduced to two small, oval, crownlike areas at the anterior and posterior ends. The remainder of the ventral surface and all the dorsal surface are covered by plastron-type chorionic cells, which on the ventral side are of an unusual type. The very long floats are positioned much more toward the dorsal surface than is usual in an anopheline egg. They are extraordinarily modified anteriorly and posteriorly to form five to seven pairs of tubular, porous filaments, which are longest at the two ends of the egg. When eggs are oviposited on damp paper, the filaments lie flat along the egg's sides; immersion in water separates and spreads the filaments laterally. Almost all eggs float with the ventral side down, the reverse of the usual position for *Anopheles*.

INTRODUCTION

Anopheles peryassui Dyar and Knab is one of 22 species in the Arribalzagia Series of the subgenus *Anopheles* (Wilkerson and Peyton 1990). It is distributed from Brazil through the Guyanas, Venezuela, Colombia, and Peru into Bolivia (Knight and Stone 1977). Little is known of the biology of *An. peryassui,* although Shannon and Davis (1930), Deane et al. (1948), Zulueta (1950), and Renjifo and Zulueta (1952) provided some information on larval habitats and adult behavior and indicated that dissections of females had not revealed the presence of malaria parasites. Notwithstanding the limitations of our present knowledge of this species, it has been apparent to students for some time that, at least with respect to the egg, *An. peryassui* is quite unusual. Causey et al. (1944) illustrated the eggs of 30 anopheline species from Brazil, among which those of *An. peryassui* and *An. shannoni* Davis were shown as having long filaments arising from the floats, the filaments being longest near each end of the egg and diminishing toward the middle. From the time of these early descriptions, later followed by Lane (1953), nothing more has been learned of the structural detail of these remarkable eggs, which must have excited the curiosity of every entomologist who ever beheld them. On a recent field trip, one of us (L.P.L.) collected some females of *An. peryassui* from Amazonas State, Venezuela, where the species had not previously been recorded (Mora-Rodriguez 1988). Subsequent oviposition by these females provided eggs for the ultrastructural description presented here.

MATERIALS AND METHODS

Female *An. peryassui* collected July 21, 1993, at human bait at Sabatena, Amazonas State, Venezuela (5°20′58″N, 67°31′45″W), were allowed to oviposit on damp filter paper in small vials. Eggs were left undisturbed for 30 hr at 26°C to embryonate and were then divided into two groups. The first was washed directly off the paper with alcoholic (80% ethanol) Bouin's fixative to preserve eggs with the float filaments appressed along the sides of the egg (as they were when removed from paper). The second group was first washed off with water to cause the filaments to spread laterally, then the water was removed with a

Fig. 2. a, Entire egg, dorsal view, anterior end at top, arrows show seamlike divisions in float; b, Posterior end of egg, lateral view, showing filaments appressed along sides of float. Scale = 100 μm (a), = 50 μm (b).

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Fig. 1. a, Entire egg, ventral view, to show complete lengths of laterally expanded filaments, anterior end at right; b, Entire egg, details of ventral surface, anterior end at top; c, Entire egg, lateral view, anterior end at top, ventral surface at right. Scale = 200 μm (a), = 100 μm (b, c).

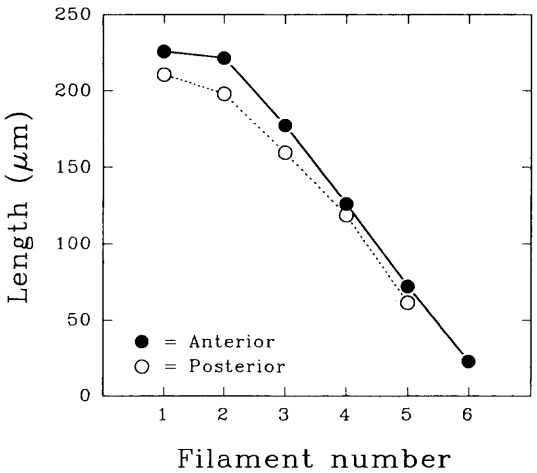

Fig. 3. Average (left and right sides) length of anterior and posterior float filaments in an egg of *An. peryassui* (Fig. 1a). Filament 1 is nearest to end of egg in each case.

pipet and the eggs were flooded with fixative. After fixation overnight, eggs were washed in two changes of 80% ethanol to remove picric acid, then completely dehydrated in ethanol by 5% concentration increments and dried finally by the critical point method. Dry eggs were positioned with a fine artist's brush on stubs coated with sticky tape, sputter-coated with gold/palladium, and examined in a Hitachi S-510 scanning electron microscope.

Measurements of egg dimensions and various structural features (means in the text given ± SE) were made with a digitizing tablet and SigmaScan software (Jandel Scientific, San Rafael, CA). An equal number of determinations was made from five separate eggs for most structures. For filament lengths, however, only those of the egg in Fig. 1a were recorded, as this was the only egg in which almost all the filaments were present, visible, and positioned so as to allow reasonably accurate measurement. The descriptive terminology follows that proposed by Harbach and Knight (1980).

RESULTS

Size: Length 585.1–616.8 μm (mean 597.7 ± 4.8, n = 7), width 144.7–157.3 μm (mean 149.4 ± 1.7), length/width ratio 3.85–4.14 (mean 4.00 ± 0.04). *Color*: Black. *Shape, overall appearance*: Egg boat-shaped in ventral view (Fig. 1a,b), widest at about 0.2 from anterior end, then gradually tapered to posterior. In lateral profile, ventral surface slightly curved, dorsal surface much more so, anterior end slightly wider and more curved than posterior (Fig. 1c). Deck reduced to two small, crownlike areas at extreme anterior and posterior ends, frill surrounding each elevated (Fig. 1c). Floats long and thin, positioned much more toward dorsal side than usual in an anopheline egg (Fig. 1c), only a short length of floats visible from ventral side, at ends of egg (Fig. 1a,b), almost all of floats visible from dorsal aspect (Fig. 2a). Float structure highly modified, at each end produced into 5–7 filaments on each side of egg (Fig. 1a,b), filament lengths greatest (210–230 μm) at ends of egg, diminishing more or less linearly toward middle (Fig. 3). Filaments spread laterally only when egg floated on water, folded along sides of egg when laid and if contact with water denied (Figs. 1c, 2b).

Ventral (upper) surface: Uniformly covered with hexagonal (occasionally pentagonal) plastron-type outer chorionic cells (Fig. 1b), length 34.5–48.7 μm (mean 41.1 ± 0.9 μm, n = 20), width 14.8–20.9 μm (mean 17.7 ± 0.3 μm), that extend well down lateral surfaces in middle of egg (Fig. 1c). Structure of cells unusual, each bounded by walls made up of raised, closely spaced, saddlelike tubercles, with numerous, tightly arrayed, disk-shaped tubercles in each cell (Fig. 4a). Centers of disk tubercles slightly cavitated, edges raised, connected by short bridges (Fig. 4b,c), surfaces rough, occasionally with tiny pores (Fig. 4c).

Float: Long, narrow, positioned well toward dorsal surface, especially from about 0.3 of egg length from anterior end to posterior end (Figs. 1c, 2a). Float surface extensively perforated, lacelike (Fig. 4d), with pores along entire length (Fig. 2a), which is divided into approximately equidistant sections, length 26.1–36.8 μm (mean 31.0 ± 0.6 μm, n = 20), bounded by shallow seams where pores are fewer (arrowheads, Fig. 2a). At anterior and posterior ends, float modified to form perforated, ribbed, pyramidal bases (Fig.

, Plastron cell detail, ventral surface; c, Detail of plastron reticulum and tubercles, ventral surface; d, Float, middle of egg, dorsal surface at bottom; e, Dorsal surface, middle of egg, showing dorsal plastron and lower margin of floats; f, Chorionic cells, plastron of dorsal surface. Scale = 50 μm (a, e), = 10 μm (b, d, f), = 5 μm (c).

Fig. 5. a, Pyramidal bases of float filaments, ventral view, anterior end at left; b, Filament bases more toward middle of egg, filaments short, fingerlike; c, Filament detail, showing tubular structure, pores; d, Distal ends of filaments as seen when folded along sides of float; e, Detail, distal extremity of filament, showing swollen, rounded end; f, Detail, anterior crown and micropylar apparatus. Scale = 10 μm (a, b, d, f), = 5 μm (c, e).

5a) extending into long, perforated filaments, spreading laterally and tending to be arched dorsally (Fig. 2a), longest at both ends of egg (Figs. 1a, 3), progressively shorter and fingerlike toward middle of egg (Fig. 5b). Filaments tubular, diameter 2.7–4.7 μm (mean 3.7 ± 0.1 μm, n = 20), perforations oval, length 0.44–1.89 μm (mean 0.97 ± 0.05 μm, n = 40), width 0.26–0.97 μm (mean 0.52 ± 0.02), fairly densely distributed over filament surfaces (Fig. 5c). Before flotation on water, filaments aligned in parallel along sides of float (Figs. 1c, 2b, 5c), ends tending to be curled ventrally in a group (Figs. 2b, 5d), pores persisting to filament ends, which are closed and usually slightly expanded, bulbous (Fig. 5e).

Dorsal, lower surface: Covered with plastron-type cells, width of covered area narrowed in middle and posterior parts of egg by extreme dorsal position of floats (Fig. 2a). Cell length 29.9–42.3 μm (mean 34.6 ± 0.7, n = 20), width 12.1–18.4 μm (mean 14.8 ± 0.3 μm), dimensions smaller than on ventral side, and structure also different. Plastron more open than on ventral surface (Fig. 4d–f), tubercles not as closely packed and smaller, diameter 1.16–3.36 μm (mean 1.99 ± 0.08 μm, n = 40), more buttonlike, edges not raised (Fig. 4f).

Anterior end, micropyle: Anterior end somewhat conical, pyramidal filament bases aligned laterally, extreme anterior ventral surface with small deck enclosed by crownlike frill, length 42.1–43.5 μm, width 29.1–35.5 μm (Fig. 6a). Frill elevated, walls often pitted (Fig. 5f), interior deck with irregularly shaped tubercles (Fig. 6c), diameter (widest) 1.00–1.86 μm (mean 1.38 ± 0.04 μm, n = 30), projecting prominently from deck surface, with smaller, lower tubercles between (Fig. 6d). Micropylar apparatus slightly separated from crown (Figs. 5f, 6b), collar 4.1–8.6 μm in diameter, surface fairly smooth (Fig. 5f), disk diameter 18.1–21.6 μm, surface slightly rough, with prominent radial rays delineating 6–8 sectors (Fig. 5f), micropylar orifice recessed in low mound.

Posterior end: Filament bases attached laterally (Fig. 6e,f), extreme end of ventral surface with small deck surrounded by elevated frill (Fig. 6e,f), length 35.3–38.6 μm, width 23.3–25.5 μm, enclosed area usually slightly smaller than at anterior end. Deck tubercles similar to those at anterior end (Fig. 6g), diameter 0.5–1.3 μm, but not as protuberant (Fig. 6h).

DISCUSSION

The terminal float filaments on the eggs of *An. peryassui* and *An. shannoni* make them unique among known anopheline eggs. To our knowledge there are no others that have comparable structures, representing a degree of modification of the float considerably beyond that in any other species. The examples of *An. peryassui* illustrated by Causey et al. (1944) in fact have more filaments at each end than the specimens we examined, diminishing in length almost to the middle of the egg. Eggs of *An. shannoni* are shown with larger (wider) floats, with well-developed filaments restricted more to each end. Comments on the eggs of *An. peryassui* are given by Deane et al. (1948), but they are not readily comprehensible. Two geographically different forms from Brazil are mentioned, one in which the floats unite in the mid-line on the "top" side and a second population where there are "clear borders around each end between extremities of the floats." The first of these comments may suggest, if the top surface referred to is the dorsal one, that some eggs have floats so shifted dorsally as to unite in the mid-dorsal line, as opposed to somewhat separated (Fig. 2a). The meaning of the reference to clear borders is obscure.

We have already mentioned that *An. peryassui* eggs in the laboratory floated on water with the dorsal side uppermost, the reverse of other anopheline eggs. Much about the structure of the egg suggests that this is the natural flotation position and not an artifact of laboratory manipulation. In their extreme dorsal position, the floats would make ventral side up orientation unstable, and the arching curvature of the filaments (Figs. 1a, 2a) suggests that they are designed to spread across the surface, presumably providing both

buoyancy and stability, when the egg's dorsal side is uppermost. In keeping with the physiological requirements imposed by its reversed orientation, the egg's immersed ventral surface is completely covered with plastron-type cells that allow exchange of respiratory gases (Hinton 1981).

From the description given by Deane et al. (1948) of the larval habitats of *An. peryassui*, in "medium or large bodies of clean water, with green algae, exposed to the sun or partially shaded," there seem to be no special conditions that one might speculate could be connected with the development of specialized floats. However, both Deane et al. (1948) in Amapa, Brazil, and Zulueta (1950) in the *llanos* of eastern Colombia recovered larvae of *An. peryassui* in association with water that collected at the bases of palms (*Mauritia flexuosa* L. f.). In fact, water surrounding the bases of "moriche" palms is regarded as the characteristic larval habitat of this species in the Colombian savannahs (Zulueta 1950), where *An. peryassui* is extremely abundant during the rainy season (Renjifo and Zulueta 1952). Conceivably, the unusual flotation method may have evolved in response to some unknown characteristic of this atypical anopheline larval habitat. Because *An. peryassui* eggs are covered overall with plastron cells that permit respiration, they are arguably adapted to submergence. The eggs float in still water in the laboratory, but not with great buoyancy. On a natural water surface, agitated by wind or rain, many eggs might sink, and the filaments might serve an anchorage function by becoming entangled with algal strands or palm roots.

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Fig. 6. a, Anterior end, ventral view; b, Anterior end, end-on view; c, Crownlike frill and enclosed deck tubercles, anterior end; d, Detail of anterior deck tubercles; e, Posterior end; f, Posterior end, end-on view; g, Crownlike frill and deck tubercles, posterior end; h, Detail of posterior deck tubercles. Scale = 50 μm (a, b, e, f), = 10 μm (c, g), = 5 μm (d, h).

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