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FINE STRUCTURE OF THE EGGS OF *ANOPHELES (ANOPHELES) APICIMACULA* (DIPTERA: CULICIDAE)

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ABSTRACT. The eggs of *Anopheles (Anopheles) apicimacula* Dyar and Knab are described from scanning electron micrographs. The eggs are boat-shaped, with frills that extend ventrally along the length of the egg and surround the deck region. The ornamentation on the dorsal and lateral surfaces is formed by groups of smooth, round tubercles. The ventral surface is covered by irregularly jagged tubercles. Prominent lobed tubercles are present at the anterior and posterior ends of the deck.

INTRODUCTION

Anopheles (Anopheles) apicimacula Dyar and Knab, a member of Arribalzagia Series (Reid and Knight 1961), is distributed from central Mexico to northern South America, including several countries in the West Indies and Central America (OSP 1949, Vargas and Martínez-Palacios 1955, Cova-García 1961, Forattini 1962). Despite its wide distribution and frequency in malarious areas, this species has not been conclusively incriminated as a malaria vector. However, *An. apicimacula* was suspected to be responsible for persistent malaria transmission in the state of Puebla, Mexico (Martínez-Palacios 1960), and it has been successfully infected with *Plasmodium falciparum* in the laboratory (Simmons 1937).

Several descriptions of larval, pupal, and adult stages of this species are available (Vargas and Martínez-Palacios 1955, Cova-García 1961, Wilkerson and Peyton 1990), but descriptions of its eggs are scanty (Kumm 1941, Vargas and Martínez-Palacios 1955, Cova-García 1961) and limited to light microscopy. We present herein a detailed description of the eggs of this species by scanning electron microscopy.

MATERIALS AND METHODS

Anopheles apicimacula females were collected using horse-baited nylon-screened traps (Fernandez-Salas et al. 1994) during routine entomological surveys in the Soconusco area of the state of Chiapas in southern Mexico. Specimens were identified with the key by Wilkerson and Strickman (1990). Blood-engorged mosquitoes were maintained at 26–27°C and 85% RH and allowed to oviposit on moist paper. One hundred and fifty eggs were examined under a dissecting microscope and measured from the anterior to the posterior end and at the widest point between the outer convex edges of the floats.

For scanning electron microscopy, eggs were allowed to embryonate on distilled water for 48 h. Using a fine paint brush, embryonated eggs were placed in a 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer. Samples were dehydrated in increasing concentrations of ethanol, CO_2 critical-point dried, and coated with gold by ion sputtering. Specimens were examined in a Jeol JSM-35 electron microscope. The terminology proposed by Harbach and Knight (1980) was followed for egg description.

RESULTS

Overall appearance: Boat-shaped, slightly broader in anterior one third. *Color:* Black. *Size:* Mean length (±SE) 509.2 ± 18.5 μm (n = 150, range 447.3–530.4 μm), mean width (±SE) 107.7 ± 5.2 μm (n = 150, range 105.2–118.4 μm). Anterior end more rounded than posterior (Fig. 1). Frills extend length of egg on ventral surface, well separated from lateral floats (Figs. 1A, 1C). Ventral surface concave between edge of floats and frills, narrow area uncovered by frills flat (Fig. 1C). Dorsal surface curved (Figs. 1A, 1B).

Dorsal (lower) and lateral surfaces: Outer chorion on lower and lateral surfaces not covered by floats, chorionic cell boundaries indistinct, plastron (Hinton 1968) formed of smooth, almost round tubercles (n = 20, mean diameter ± SE 3.24 ± 0.99 μm) (Figs. 1B and 2B). Floats with 30–32 long, narrow ribs, extending about 83% length of egg (n = 10) (Fig. 1A). At dorsal margin, floats concave and ribs divided into 2 or 3 ridges (Fig. 1B). Ventral margin of floats almost linear, ribs ending abruptly, terminating flat on a strip about 1 μm wide along edge of float (Figs. 1A and 2C). Outer chorion, on surface covered by floats, with same ornamentation as deck, as seen by removing floats (Fig. 2A).

Ventral (upper) surface: Deck narrow and

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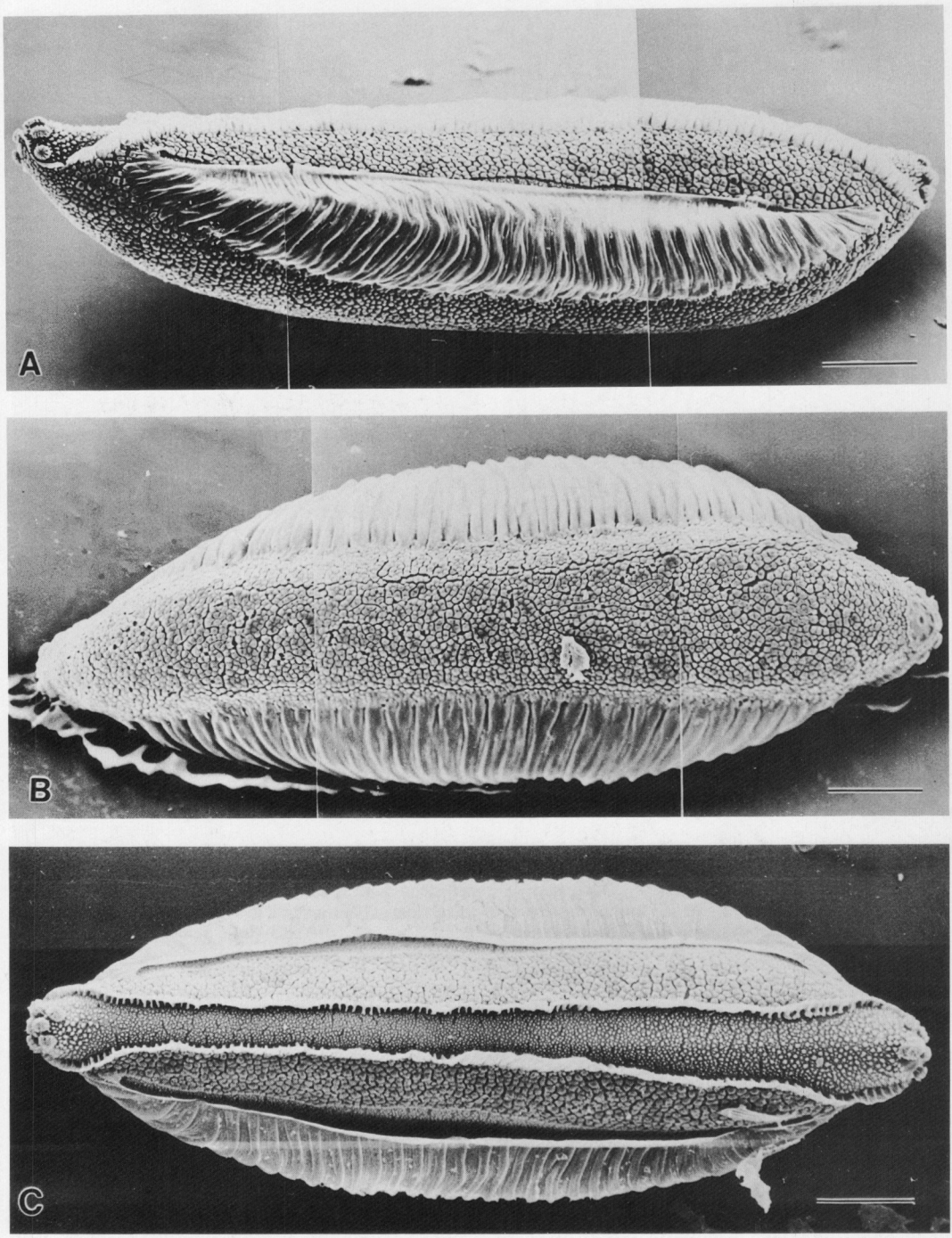

Fig. 1. *Anopheles apicimacula.* A. Entire egg, lateral view, ventral surface at top, anterior end at right. B. Entire egg, dorsal view, anterior end at right. C. Entire egg, ventral view, anterior end at right. Scale = 50 μm.

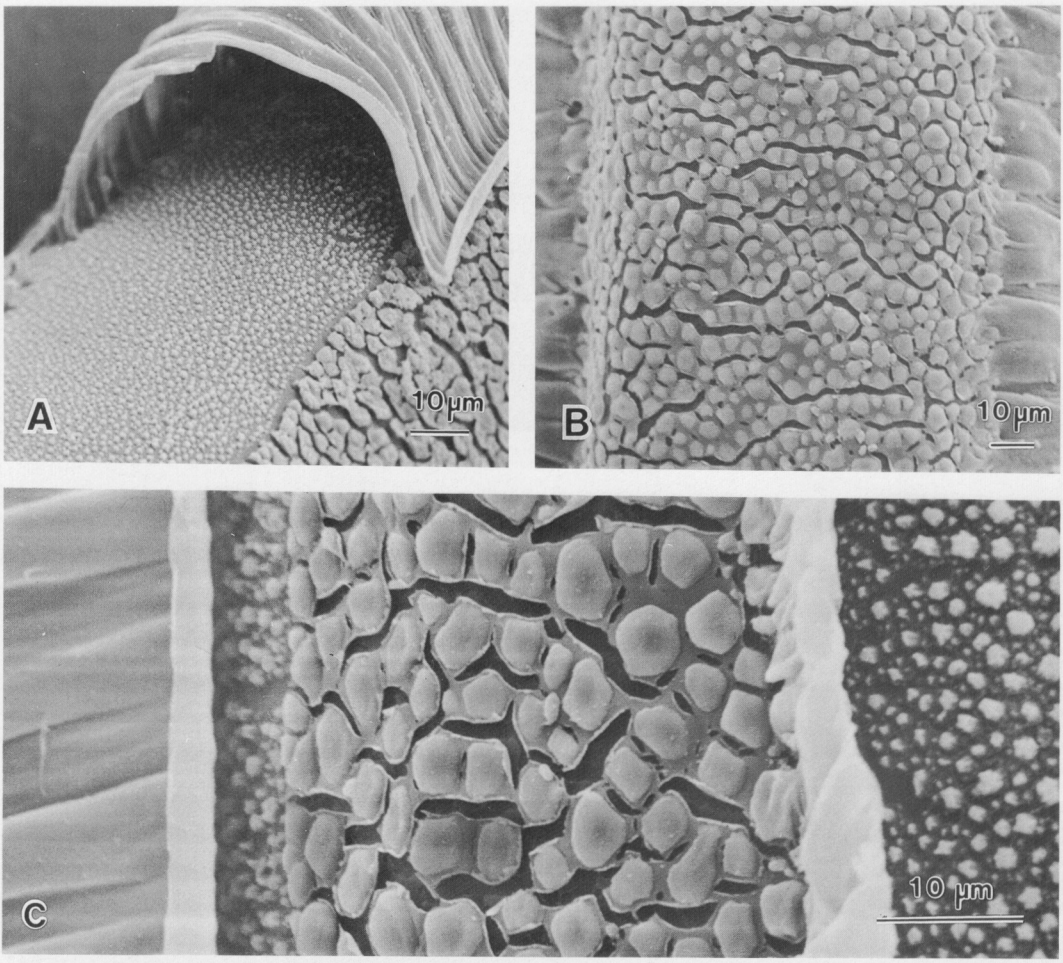

Fig. 2. *Anopheles apicimacula.* A. Outer chorionic cell detail on lateral surface under float. B. Outer chorionic cell detail on dorsal surface limited by emergence of float. C. From left to right: float, partial view of outer chorionic cell detail under float, space between edge of float and frill, and deck. Scale = 10 µm.

completely surrounded by a continuous deep frill (Fig. 1C) that narrows at both ends, its inner surface with ridged columns (Figs. 4A, 4C). Edge of frill undulated with outer surface slightly grooved (Fig. 3D). Entire deck covered with polygonal irregularly jagged tubercles of variable size (Figs. 3A, 3B, 3C) (n = 20; mean diameter ± SE of tubercles at anterior pole 2.04 ± 0.21 µm, at the middle 1.01 ± 0.37 µm, at posterior pole 1.98 ± 0.37 µm). Six to 8 prominent lobed tubercles at anterior end of deck (n = 10, mean diameter 9.33 ± 0.53 µm) (Fig. 4C). Seven or 8 similar formations at posterior end (n = 10, mean diameter 9.66 ± 0.59 µm), separated by invagination of the frill, forming 2 rows that extend to pole of egg (Fig. 4A). Tubercles with continuous outer membranelike wall that surrounds and separates 8–10 elongated smooth-surfaced structures that form lobes. Center of tubercle occupied by irregular invagination of outer wall (Fig. 4B).

Anterior end, micropyle: Micropyle separated from lobed tubercles by frill and thin plastron of tubercles similar to those on dorsal surface (Fig. 4D). The micropylar collar (n = 3, mean diameter ± SE 29.25 ± 0.75 µm) somewhat hexagonal with rugose surface. Inner margin excavated, presenting 8–9 protrusions with thornlike ridges extending toward micropyle. Disc with rugose surface, orifice covered by a plug, located at bottom of circular depression (Fig. 4D).

Posterior end: Shape pointed, ornamentation of ventral and lateral surfaces similar to deck-chorion cells. Most prominent features, the lobed tubercles, described above (Figs. 1C and 4A).

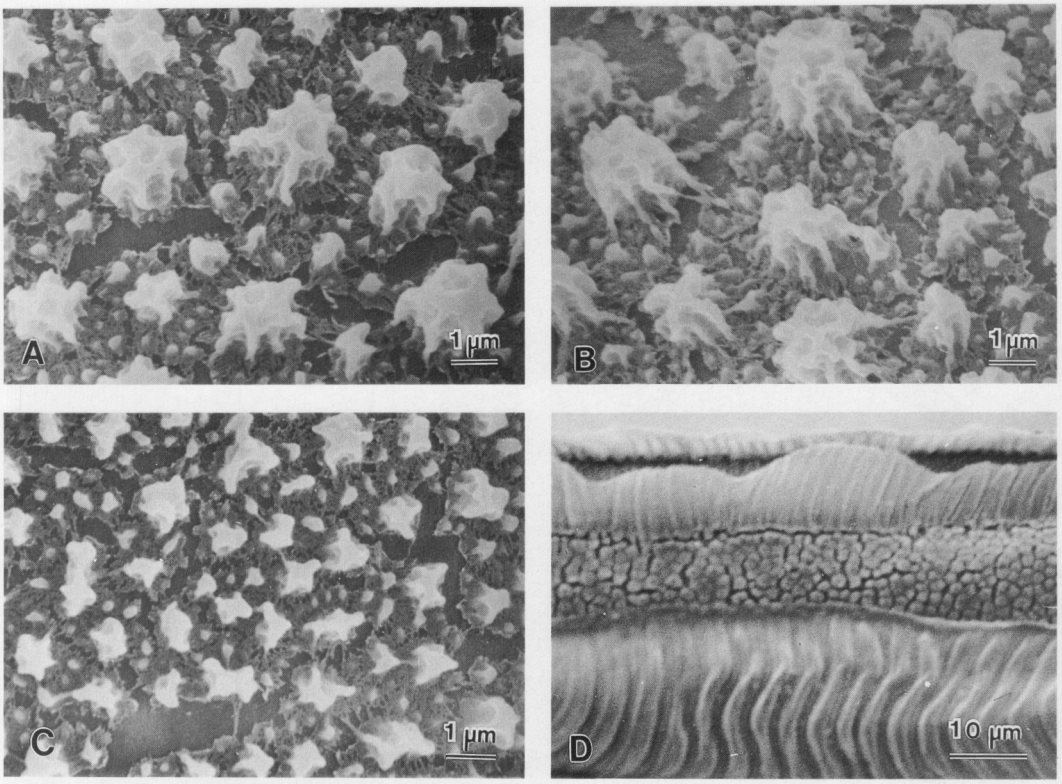

Fig. 3. *Anopheles apicimacula.* A. Outer chorionic cell detail on anterior ventral surface. B. Outer chorionic cell detail on posterior ventral surface. C. Outer chorionic cell detail on middle part of ventral surface. D. Lateral view of central region of egg. Scale = 5 µm.

DISCUSSION

Previous descriptions of eggs of *An. apicimacula* based on light microscopy are fragmentary and incomplete. Kumm (1941) presented 2 dorsal-view photographs and indicated that the frills are fused down the midline. In our observations under a dissecting microscope of 2,355 eggs, obtained from 25 *An. apicimacula* females, 0.6% of the specimens presented frills that were apparently fused. However, scanning electron microscope examination of these eggs revealed structures similar to those presented in Fig. 1. The description by Vargas and Martínez-Palacios (1955) is presented in a classification key. The main features include: size of about 550 µm but less than 600 µm, frills separated from the floats, and exochorion with a hexagonal reticular pattern. This ornamentation pattern is also depicted in a dorsal view of the egg presented by Cova-García (1961). Our measurements of the egg size are somewhat below those indicated by Vargas and Martínez-Palacios (1955) and Kumm (1941) (length 556.2 µm, width 178.6 µm), but this could reflect geographic variation, or nutritional condition of the females. The main difference from the previous descriptions is that a hexagonal pattern on the dorsal outer chorion plastron was not seen by us. Instead, groups of 5–16 tubercules separated by irregular gaps, forming polyhedrical patterns (Figs. 1B and 2C), were visible, but these gaps are most probably preservation artifacts. Few fine-structure descriptions of the eggs of other *Anopheles* (*Anopheles*) are available, but the eggs of *Anopheles atropos* Dyar and Knab (Linley 1992), *Anopheles quadrimaculatus* Say (Linley et al. 1993), *Anopheles punctipennis* Say (Linley and Kaiser 1994), and *Anopheles mattogrossensis* Lutz and Neiva (Linley and Milstrey 1995) all present hexagonal plastron-type chorionic cells with distinct boundaries on their dorsal and lateral surfaces, and this pattern also occurs on the extremely modified egg of *Anopheles peryassui* Dyar and Knab (Linley and Lounibos 1994). Except for *An. peryassui,* the eggs of all these species present lobed tubercles at the ventral surface of the anterior and posterior poles, similar to those seen on *An. apicimacula* eggs.

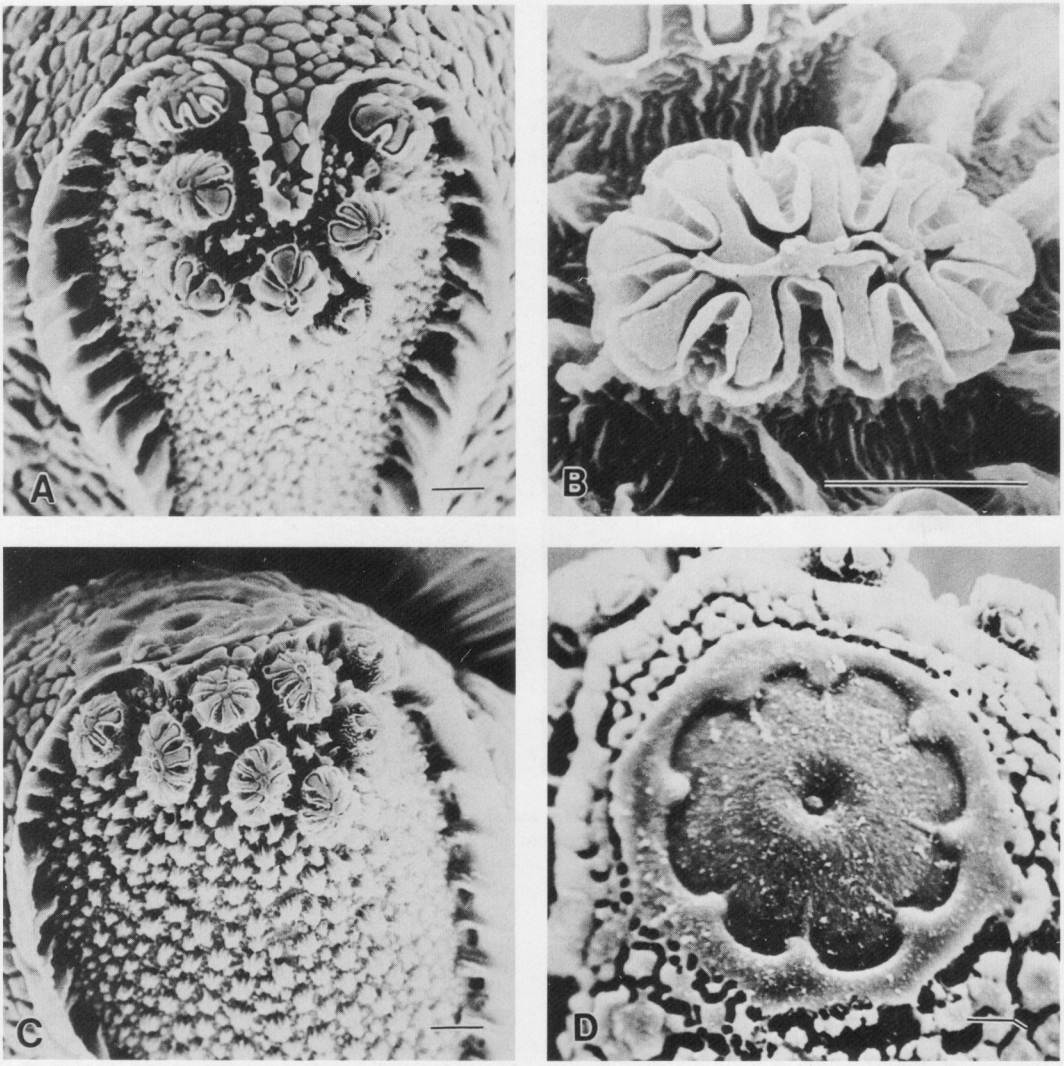

Fig. 4. *Anopheles apicimacula.* A. Ventral surface, posterior end. B. Detail of lobed tubercle at anterior ventral surface end. C. Ventral surface, anterior end. D. Dorsal surface, anterior end; micropyle. Scale = 5 μm.

The general aspect and ornamentation of the eggs of *An. apicimacula* were remarkably constant among the specimens examined. The ultrastructural examination confirms many of the features observed by light microscopy, but adds considerable detail on the surface ornamentation that could only be evidenced by scanning electron microscopy.

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