

FINE STRUCTURE OF THE EGGS OF *ANOPHELES (ANOPHELES) PUNCTIMACULA*

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ABSTRACT. The egg of *Anopheles (Anopheles) punctimacula* is described from scanning electron micrographs. Eggs of *An. punctimacula* are boat shaped, with lateral floats extending 70% of the length of the egg. Plastronlike polyhedral chorionic cells with distinctive boundaries and round tubercles in the cell field cover the dorsal, lateral, and ventral surfaces. Narrow decks enclosing a field of irregular jagged tubercles and 2-4 lobed tubercles are present at both egg poles.

KEY WORDS *Anopheles punctimacula*, Diptera, Culicidae, eggs, scanning electron microscopy

INTRODUCTION

Anopheles (Anopheles) punctimacula Dyar and Knab, along with *An. malefactor* Dyar and Knab, *An. guarao* Anduze and Capdevielle, and *An. calderoni* Wilkerson, belongs to the Punctimacula Group of the Arribalzagia Series (Reid and Knight 1961, Wilkerson and Peyton 1990, Wilkerson 1991). Synonyms of *An. punctimacula* include *An. venezuelae* Evans and *An. strigimacula* Dyar and Knab. The synonymy of *An. venezuelae* was confirmed (Wilkerson 1988); however, some features in the primary aedeagal leaflet seem to distinguish *An. strigimacula* from *An. punctimacula*. Because few specimens are available for examination, Wilkerson (1990) decided to retain this synonymy.

Anopheles punctimacula is distributed at low elevations from northern South America (Colombia and Venezuela), through Central America and the Yucatan Peninsula, to the states of Sinaloa, San Luis Potosí, and Veracruz in Mexico (Gabaldon and Cova-García 1946, Vargas and Martínez-Palacios 1955, Wilkerson 1990). No records of *An. punctimacula* infected with malaria parasites exist in Mexico, but infected specimens have been collected in Panama (Simmons 1936, Rozeboom 1938) and Colombia (Huffaker et al. 1945, Rey et al. 1945), and the coexistence of malaria and *An. punctimacula* populations led Kumm and Ruiz (1939) to suspect this mosquito as a vector in Costa Rica. However, the identity of these specimens is uncertain.

The most recent descriptions of the larva, pupa, and adults of *An. punctimacula* were provided by Wilkerson (1990), but only 2 general descriptions of the egg from light microscope photographs are available (Kumm 1941, Cova-García 1961). We

present herein a detailed description of the egg of this species by scanning electron microscopy (SEM).

MATERIALS AND METHODS

Horse-baited nylon-screened traps (Fernandez-Salas et al. 1994) were used to collect female *An. punctimacula* in Nueva Independencia (14°37'30"N, 92°16'14"W), located on the Pacific Ocean coastal plain of Chiapas State in southern Mexico. Mosquitoes were identified using the key of Wilkerson and Strickman (1990). Blood-engorged females were kept in screened cages at 26-27°C and 85% relative humidity. Eggs were collected on moist filter paper. One hundred eggs were examined under a dissecting microscope and measurements were made from the anterior to the posterior end and between the convex edges of the floats at the middle of the egg.

For electron microscope observations, individual blood-engorged females were maintained in 50-ml plastic test tubes and their eggs were collected on moist filter paper located at the bottom of the tube. Eggs were held on distilled water at 26-27°C for 45 h, and embryonated eggs were transferred to 2.5% glutaraldehyde in 0.1 M cacodylate buffer (Electron Microscopy Sciences, Washington, PA), pH 7.2. Samples were treated with increasing concentrations of ethanol, CO₂ critical-point dried, and gold-coated by ion sputtering (Ion Sputter JFC-1100; Jeol, Tokyo, Japan). Specimens were examined in a Jeol JSM-35 electron microscope (Jeol). Micrographs of 5 eggs from each of 6 females were used to determine the number of ribs in each float, the internal and external diameters of the micropyle, the number of sectors in the micropyle, and the number of lobed tubercles and their lobes at both extremes of the egg. Except for the terms "plastron cells" (Hinton 1968), "chorionic cell field" (Linley 1989), and "micropylar ray" (Linley et al. 1993a), the terminology of Harbach and Knight (1980) is used to describe the egg.

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RESULTS

Size: Mean length (\pm SE) $452.9 \pm 28.9 \mu\text{m}$ ($n = 102$, range 400–500 μm), mean width (\pm SE) $105.9 \pm 16.2 \mu\text{m}$ ($n = 102$, range 100–150 μm).

Color: Black.

Overall appearance: Boat-shaped in ventral and dorsal view (Figs. 1A, 1C), slightly broader in anterior one-third, anterior end more rounded than posterior, with narrow elongate decks at both ends, lobed tubercles at both ends within decks (Fig. 1A). Dorsal surface curved, more prominently at both ends, ventral surface almost straight on lateral view (Fig. 1B). Floats moderately long, very deep, mid-laterally positioned.

Dorsal (lower) and lateral surfaces: Outer chorion on lower and lateral surface continuous around floats, central part of ventral surface not covered by decks (Figs. 1A, 1B, 1C). Surface covered with pentagonal and few hexagonal plastron-type chorionic cells with distinct boundaries more marked on lateral surface and at ends of egg on lower surface (Fig. 1C). Cell elongate with length oriented in long axis of egg on lateral surfaces and at end of eggs on the lower surface. Cell field occupied by smooth polyhedral almost round tubercles separated by pores (Figs. 2A, 2B, 4A, 4B). Cell boundaries formed by larger round, evenly separated tubercles connected by small bridges (Fig. 2C), except at junction with dorsal margins of floats where tubercles interdigitate or fuse with ribs (Fig. 3C). Floats wider at middle, middle situated on lateral surface, with 28–38 ribs (mean \pm SE = 31.8 ± 2.04 , $n = 30$) divided into 2 ridges oriented towards middle of egg. Floats extending about 70% of egg ($n = 30$), concave at dorsal margin, ventral margin linear, ribs ending abruptly leaving a strip about 4–5 μm wide. Egg surface under floats, seen by retraction (Figs. 1A, 3C) and removal of floats (Fig. 3A) covered with polygonal irregularly jagged tubercles of variable size.

Ventral (upper) surface: Space between floats slightly wider at anterior third of egg (Fig. 1A). Entire surface, except at both ends occupied by deck, covered by hexagonal and pentagonal chorionic cells elongate with length oriented in long axis of egg, similar to those on lateral surfaces (Figs. 4A, 4B).

Anterior end, micropyle: Ventral surface occupied by ovoid deck extending to egg pole. Deck deeper on lateral sides, inner wall grooved, with fringes (Figs. 4A, 5B). Lobed tubercles (2–4, mean \pm SE = 2.8 ± 0.71 , $n = 30$) with 6–10 lobes located at extreme end of crown (Fig. 4C). Tubercles smooth with continuous rugose membranelike wall. Rest of deck surface surrounded by frill covered by tubercles of irregular shape (Fig. 5B). Micropyle located next to deck. Micropylar collar smooth with irregular outline, inner margin excavated with 5–7 (mean \pm SE = 6.56 ± 0.6) lobes from which micropylar rays extend. Disk surface slightly rugose

with a radial pattern and a central depression (Fig. 4D).

Posterior end: Slightly more pointed than anterior end. Deck elliptical, longer than anterior deck, lobed tubercles (2–4, mean \pm SE = 3.03 ± 0.49 , $n = 30$) with 5–10 lobes located at egg pole. Rest of ornamentation similar to anterior end (Fig. 5A).

DISCUSSION

Previous descriptions of eggs of *An. punctimacula* are limited to light microscopy observations. Kumm (1941) presents a lateral view photograph of 1 egg from Costa Rican specimens depicting dorsal frills that are continuous from 1 end to the other on each side of the egg. In other photographs of eggs laid by the same female, the frills are interrupted near 1 end or are confined to the poles, forming decks similar to those we observed in specimens from southern Mexico. Similar features are presented by Cova-García (1961) in 3 drawings of eggs of *An. punctimacula* from Venezuela, although no indication of the origin of the specimens is made. In our observations of 102 eggs under a dissecting microscope and 30 eggs under a scanning electron microscope, only eggs with decks confined to the poles were seen. These differences could reflect geographical variation of eggs laid by females of the same species (*An. punctimacula*), or perhaps the eggs with continuous frills described by Kumm (1941) originated from other Central American species, such as *Anopheles malefactor* Dyar and Knab, which was previously considered a synonym of *An. punctimacula* (Wilkerson 1990). Our specimens are smaller than those measured by Kumm (1941) (length 525.3 μm , width 189.4 μm), also most likely reflecting geographical variation or the nutritional condition of females. All other features described by these authors, including the polyhedral pattern of the exochorion and the position and extension of floats, are similar to ours.

The general ornamentation of *An. punctimacula* eggs follows the same design as the eggs of other *Anopheles* (*Anopheles*). Hexagonal plastron-type chorionic cells with distinctive boundaries are present on the dorsal and lateral egg surfaces of *An. vestitipennis* Dyar and Knab (Rodríguez et al. 1999), *An. atropos* Dyar and Knab (Linley 1992), *An. quadrimaculatus* Say (Linley et al. 1993a), *An. punctipennis* Say (Linley and Kaiser 1994), *An. perplexens* Ludlow (Linley and Kaiser 1994), *An. fluminensis* Root (Lounibos et al. 1997b), *An. apicimacula* Dyar and Knab (Rodríguez et al. 1996), *An. peryassui* Dyar and Knab (Linley and Lounibos 1994), and *An. shannoni* Davis (Lounibos et al. 1997b). The eggs of *An. mattogrossensis* Lutz and Neiva have the same pattern, but the boundaries of the chorionic cells are less evident (Linley and Milstrey 1995). These cells are formed by the exochorion (Sahlén 1996), the extension of which is interrupted by the position and extension of the floats,

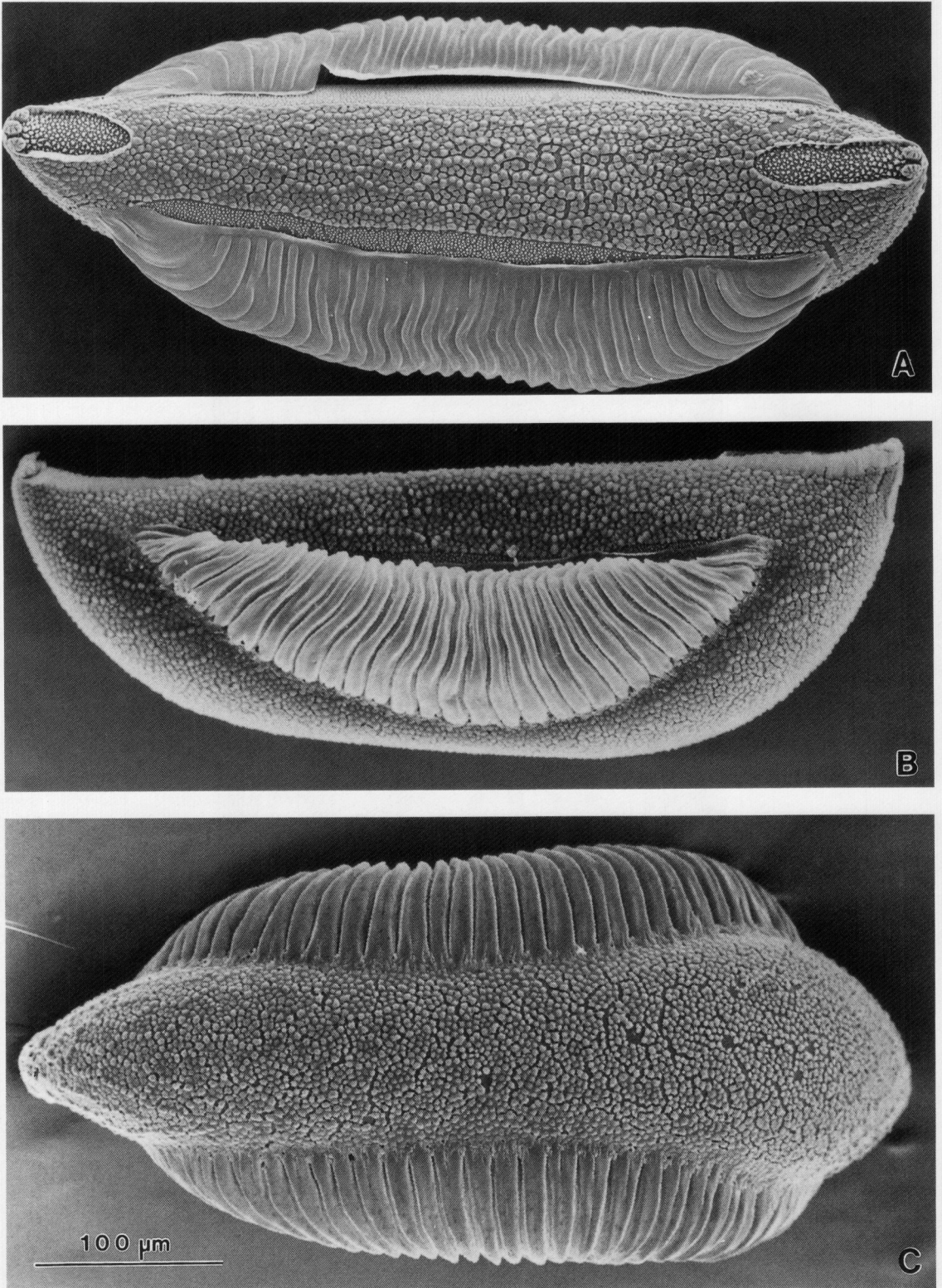
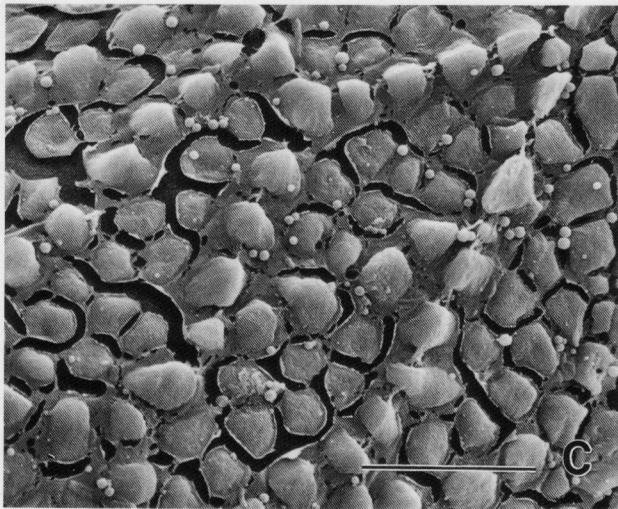
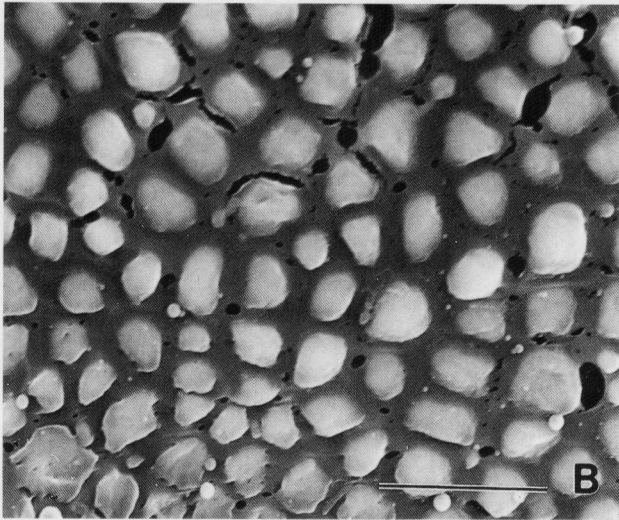
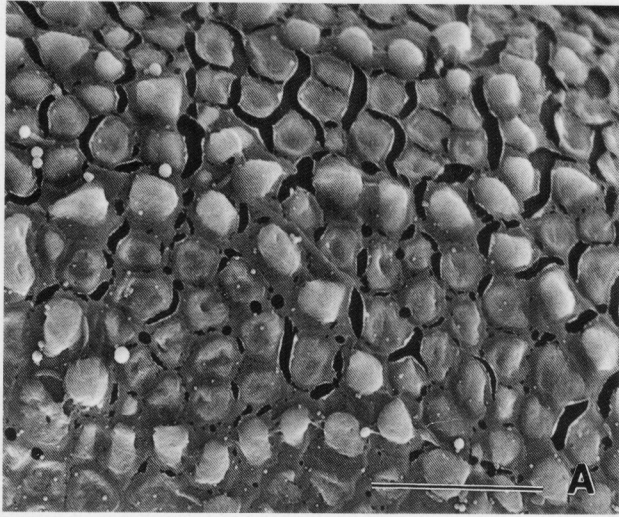


Fig. 1. *Anopheles punctimacula*. A. Entire egg ventral view, anterior end at right. B. Entire egg, lateral view ventral surface at top, anterior end at right. C. Entire egg, dorsal view, anterior end at right. Scale = 100 μm .



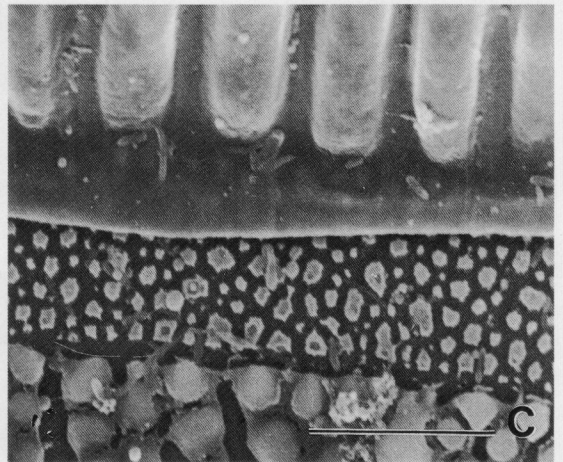
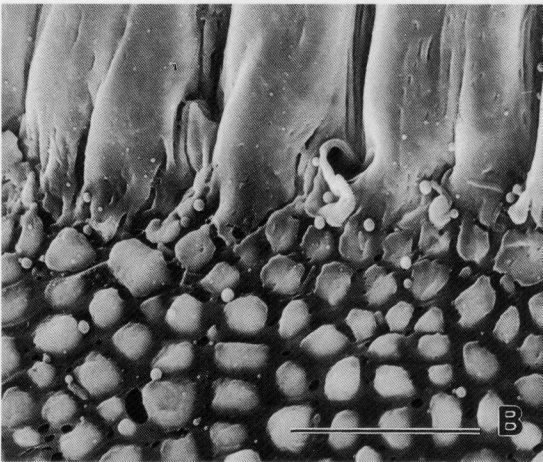
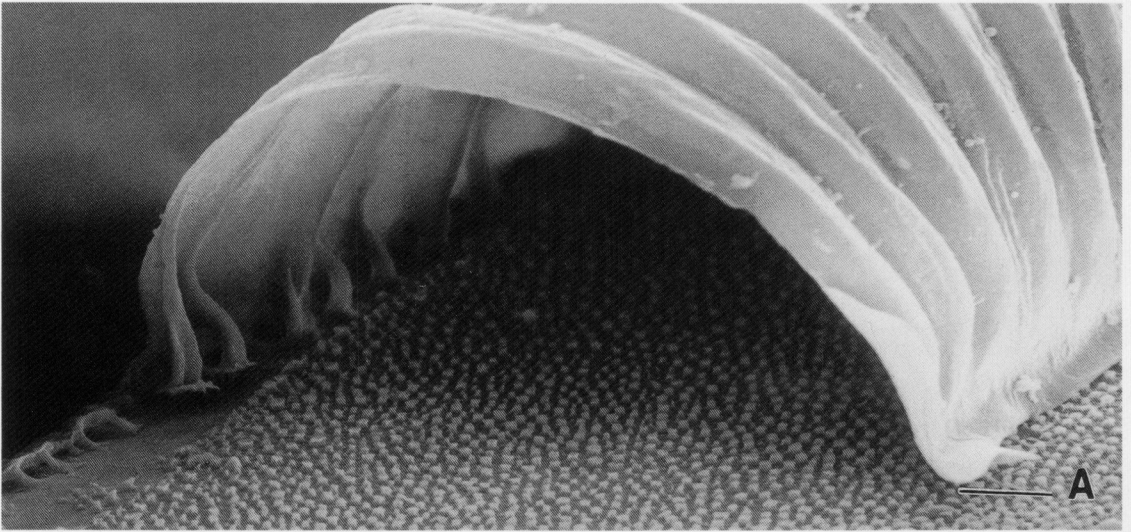


Fig. 3. *Anopheles punctimacula*. A. Outer chorionic cell detail on lateral surface under float. B. Outer chorionic cell detail on the dorsal margin of float. C. From top to bottom: upper margin of float, partial view of outer chorionic cell detail under float, plastronlike chorionic cell detail on lateral surface continuous with ventral surface. Scale = 10 μ m.

frills, and decks. Under the floats and in the spaces limited by frills and decks, the egg surface is covered by tubercles of irregular shape originating from the endochorion.

Differences among species involve features of the floats and frills and decks, which also determine the extension of the exochorion on the egg surface. In species with floats that do not extend for the entire egg length (all above except *An. vestitipennis*), hexagonal plastron-type chorionic cells extend from the dorsal surface surrounding both extremes

of the floats and cover the lateral surfaces. On the ventral surface, frills extending from pole to pole are present in *An. fluminensis*, *An. mattogrossensis*, *An. apicimacula*, *An. perplexens*, *An. punctipennis*, and *An. atropos*. In *An. fluminensis*, overlapping frills hide the deck. In *An. mattogrossensis*, frills, deeper at both ends, limit a very narrow deck, and a narrow deck is also present in *An. apicimacula*, but frills are deep in all their extension. The deck in *An. perplexens* is narrow but somewhat anteriorly and posteriorly wider, whereas in *An. puncti-*

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Fig. 2. *Anopheles punctimacula*. A. Outer chorionic cell detail on anterior end of dorsal surface. B. Outer chorionic cell field detail on middle dorsal surface. C. Outer chorionic cell detail on posterior end of dorsal surface. Scale = 10 μ m.

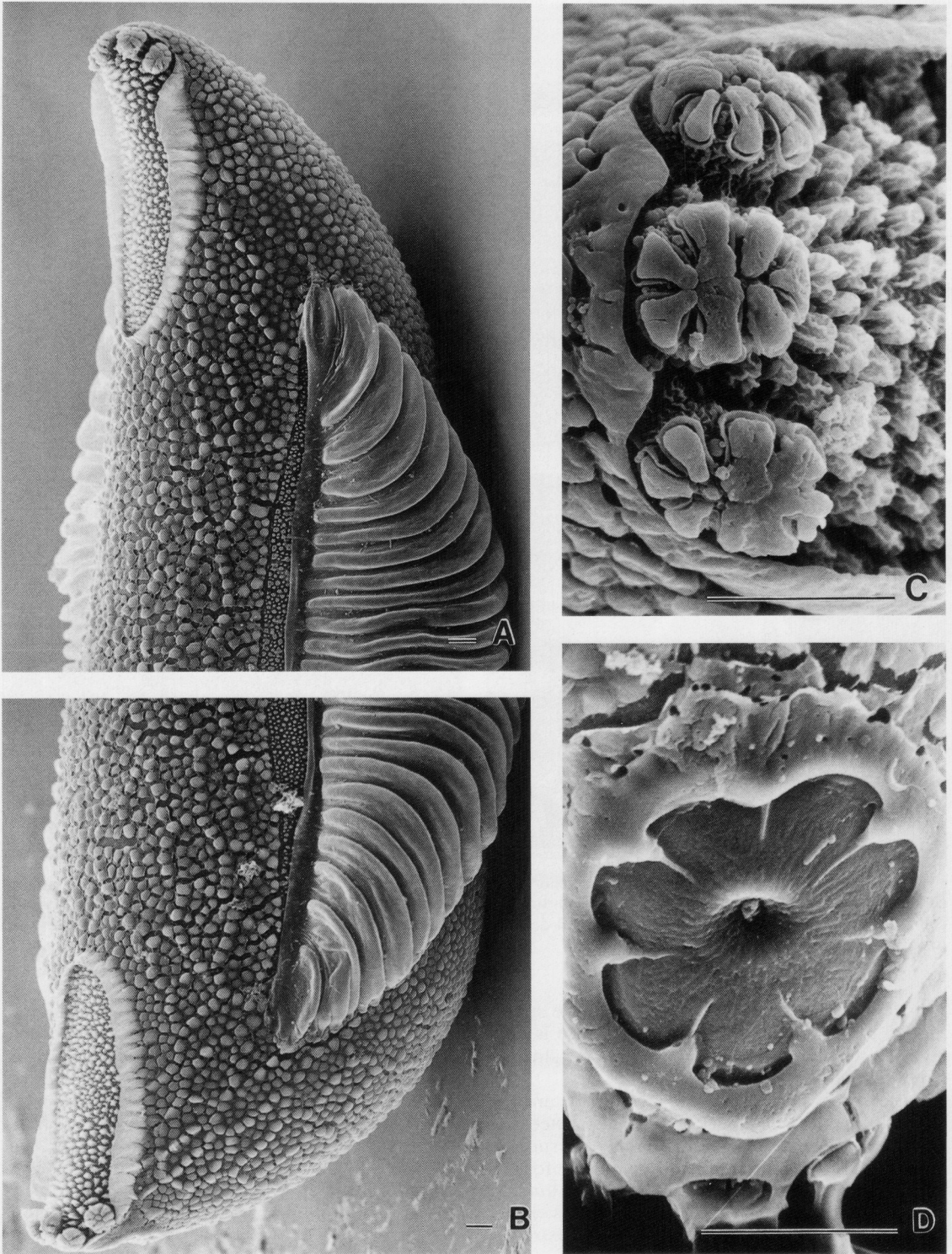


Fig. 4. *Anopheles punctimacula*. A. Lateral view of posterior end. B. Lateral view of anterior end. C. Detail of lobed tubercles at anterior ventral surface end. D. Dorsal surface, anterior end, micropyle. Scale = 10 μ m.

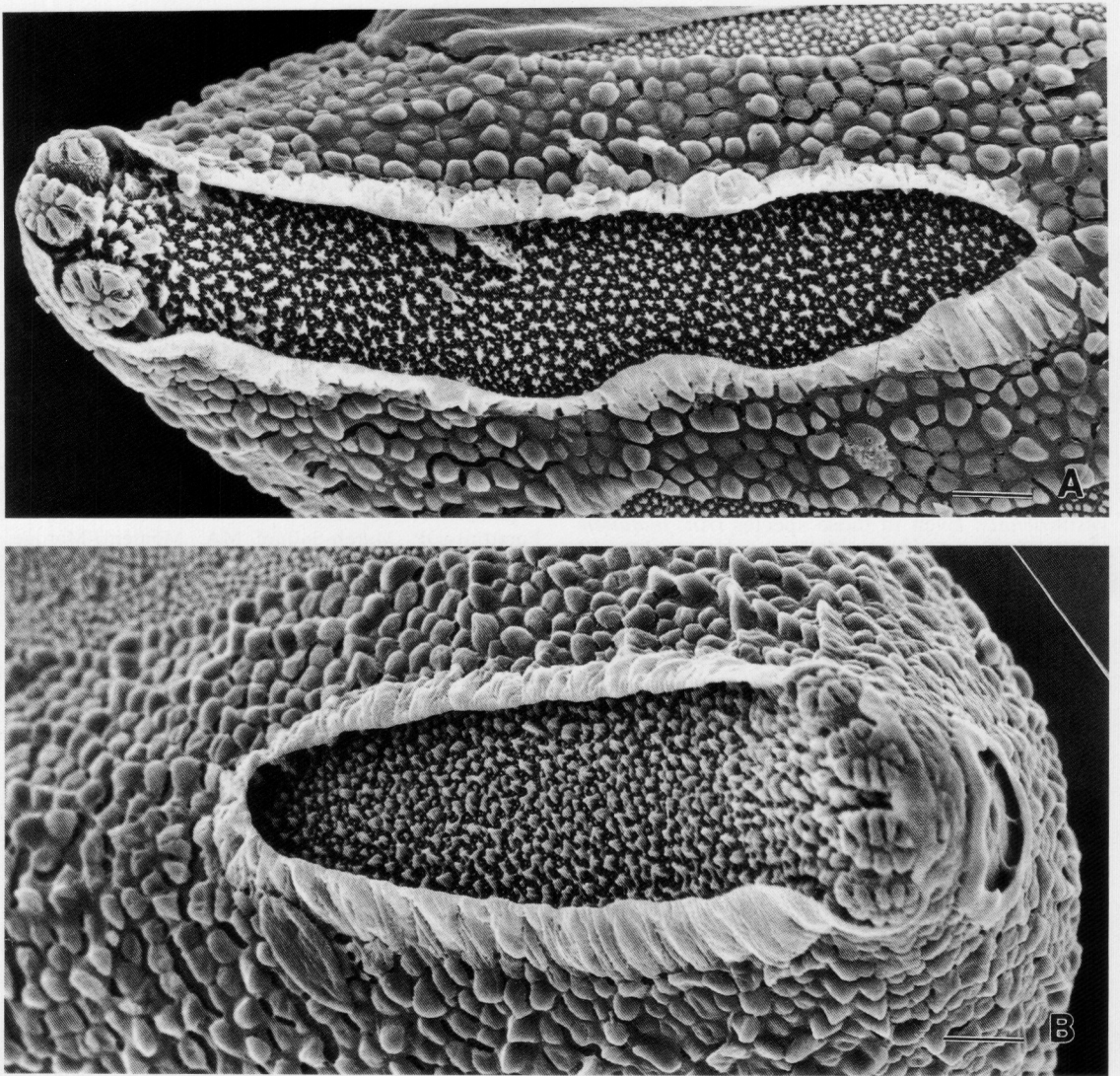


Fig. 5. *Anopheles punctimacula*. A. Ventral surface, posterior end, detail of crown. B. Ventral surface, anterior end, detail of crown. Scale = 10 μ m.

pennis frills positioned next to the upper border of floats circumscribe a wide deck. The deck is also wide in *An. atropos*, but is distinctively narrow at the middle of the egg.

Anopheles peryassui and *An. shannoni* have special features. In both species, the floats are massive and filamentous with pores perforating ribs and filaments. In these species polyhedral plastron-type chorionic cells cover the ventral surface of the egg. A similar design occurs in *An. vestitipennis*, where the floats extend the length of the egg. However, the eggs of *An. peryassui* and *An. vestitipennis* present decks, surrounded by crownlike frills, similar to those of *An. punctimacula*, whose surface is covered by irregular tubercles like those covering the decks of the other species. The lobed tubercles lo-

cated at both ends of eggs of *An. punctimacula* are also present in the other *Anopheles* (*Anopheles*) eggs, except for *An. peryassui* and *An. shannoni*.

The similarities and variations of the egg ornamentation among these mosquito species could be indicative of their genetic relationship. A comparison of the eggs examined with SEM of species belonging to the subgenus *Anopheles* with those of the subgenus *Nissorhynchus*, such as *An. rageli* Gabaldón, Cova-García, and Lopez (Linley and Lounibos 1993), *An. dunhami* Causey (Linley and Lounibos 1993), *An. aquasalis* Curry (Linley et al. 1993b), *An. nuneztovari* Gabaldón (Linley et al. 1996), *An. antunesi* Galvao and Amaral (Forattini et al. 1997), and *An. albimanus* Wiedemann (Rodriguez et al. 1992), indicates that whereas in *Anoph-*

eles, dorsal and lateral surfaces are covered by polyhedral plastron-type chorionic cells with distinctive boundaries, these are difficult to distinguish in eggs of *Nyssorhynchus*. Also, lobed tubercles are common at both ends of eggs of *Anopheles*, but are absent in *Nyssorhynchus*; and except for crownlike structures present in some species of both subgenus, *Anopheles* decks are limited by frills that separate them from floats, whereas *Nyssorhynchus* decks are limited laterally by floats that are continuous with frills at the egg poles.

Common features are less evident among eggs of species belonging to the Arribalzagia Series. Nevertheless, the main feature, in those for which SEM descriptions are available, is the absence or limited extension of the decks. Thus, no deck is present in *An. shannoni*; narrow decks are located at the egg poles in *An. punctimacula*, and decks are limited by crownlike structures in *An. peryassui* and *An. vestitipennis*. Even in those species (*An. fluminensis*, *An. mattogrossensis*, and *An. apicimacula*) with decks extending the length of egg, they form narrow strips. In contrast, available SEM photographs of eggs of other *An. (Anopheles)* mosquitoes (*An. punctipennis*, *An. perplexens*, and *An. atropos*) reveal wider decks. Likewise, in *An. quadrimaculatus*, the deck is narrow in the middle, but wide at both ends of the egg.

The status of *An. punctimacula* as a vector of malaria in Central and South America, reviewed by Wilkerson in 1990, is still uncertain. Laboratory infections of Panamanian specimens with *Plasmodium vivax* Grassi and Feletti and *P. falciparum* Welch were obtained by Simmons (1937), but the identity of these and naturally infected specimens collected in Central America (Simmons 1936, Rozboom 1938), where *An. punctimacula* coexists with *An. malefactor*, is in doubt. Further studies, including collection of infected specimens, are necessary for its definition. In South America, the malaria vector previously classified as *An. punctimacula* on the coast of the Pacific side of the Andes (Levi-Castillo 1949, Calderon et al. 1974) was proven to be *An. calderoni* (Wilkerson 1991). Other records of *An. punctimacula* at higher elevations (Itaqui, elevation 1,625 m, and Medellin, elevation over 1,538 m in Colombia; Gast-Galvis 1943), including malaria-infected specimens (Huffaker et al. 1945, Rey et al. 1945), possibly also correspond to a different species (Wilkerson 1991).

Scanning electron microscopic examination of eggs can be useful to distinguish sibling species (Damrongphol and Baimai 1989, Rodriguez et al. 1999), and to elucidate relationships among species groups (Linley et al. 1995, Lounibos et al. 1997a). In the particular case of *An. punctimacula*, comparison of its egg with topotypic material of previous synonyms (*An. malefactor* and *An. calderoni*) would be interesting to investigate how much the eggs of closely related species have in common. On the other hand, the examination of the eggs of to-

potypic specimens of *An. strigimacula*, which synonymy is unconfirmed (Wilkerson 1990), may contribute to elucidate its classification.

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