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A COMPARISON OF THE EGG SURFACE STRUCTURE OF SIX ANTHROPOPHILIC PHLEBOTOMINE SAND FLIES (*LUTZOMYIA*) WITH THE SCANNING ELECTRON MICROSCOPE (DIPTERA: PSYCHODIDAE)[1]

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Abstract: A scanning electron microscope was used to study the fine surface structures on the eggs of 6 species of anthropophilic phlebotomine sand flies: *Lutzomyia panamensis, L. pessoana, L. sanguinaria, L. trapidoi, L. ylephiletor,* and *L. gomezi.* These structures are illustrated and discussed.

The scanning electron microscope (SEM) has been used by many authors to study the fine surface structure of mosquito eggs. Hinton (1968a, b) and Hinton & Service (1969) used the SEM to characterize the function and structure of *Anopheles, Culex* and *Aedes* egg surfaces. Matsuo et al. (1972) described the egg surface structure of 5 species of *Aedes* and 1 species of *Armigeres* from Japan and Malaysia. Matsuo et al. (1974) continued their study of the egg surface structure with 13 *Aedes* species from Taiwan.

Ward & Ready (1975) examined by use of the SEM the outer chorionic sculpturing of 13 species of neotropical sand flies from Brazil. Three different species-specific sculpture patterns were found which included polygons, parallel ridges and mountain- or volcano-like structures. They discussed the role of the chorionic sculpturing in plastron respiration. The same authors reviewed the literature concerning the descriptions of eggs of Old and New World sand flies, which had been made using the light microscope. Barretto (1941) used the light microscope to obtain photographs of eggs of 12 sand fly species but found it very difficult to separate the eggs of closely related species using this technique.

The eggs of 6 Neotropical anthropophilic phlebotomine species were examined in this study with the SEM. This work supplements the study done by Ward & Ready (1975) to advance the taxonomy of Phlebotominae.

MATERIALS AND METHODS

Eggs were obtained from blood-engorged females captured from a horse near Achiote, Canal Zone, Panama. The females were placed in moistened plaster of paris-lined glass vials and allowed to oviposit. Two days after oviposition the eggs were fixed in 5% glutaraldehyde and then passed through an alcohol dehydration series and allowed to dry. The specimens were then sent from the Gorgas Memorial Laboratory to the Center for Electron Optics, Michigan State University, East Lansing, Michigan, for further processing. There the eggs were mounted on specimen stubs, sputter-coated with approximately 300 Å of gold, and examined in an AMR-900 SEM at varying beam currents, voltages, and tilt angles.

The techniques employed in this study often resulted in collapsed eggs, but the surface structure remained unchanged. The term "chorion" used in the descriptions refers to the outer layer (exochorion). The descriptions are presented according to the format of Matsuo et al. (1974). Egg measurements are in microns. The number of eggs measured (N) is followed by the range of variations in length (L), the widths at the widest point (W) and the means of each in parentheses. The descriptions follow the nomenclature of Barretto (1941).

The following species were examined: *Lutzomyia panamensis* (Shannon), *L. pessoana* (Barretto), *L. sanguinaria* (Fairchild & Hertig), *L. ylephiletor* (Fairchild & Hertig) and *L. gomezi* (Nitzulescu).

RESULTS AND DISCUSSION

Sand fly eggs are generally elongate and ellip-

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oidal in shape. The sculptured outer layer exochorion) is sticky, allowing a species like *Lutzomyia panamensis* to lay its eggs singly on the oviposition substrate. The eggs remain in place and are difficult to dislodge even with considerable jarring. The eggs are very fragile and will collapse if allowed to dry before eclosion.

Only 2 basic exochorionic sculpturing patterns described by Ward & Ready (1975) were observed in this study. The mountain- or volcano-like pattern was shown in *L. panamensis* and *L. pessoana*, and the polygonal pattern was exhibited by *L. sanguinaria*, *L. trapidoi*, *L. ylephiletor* and *L. gomezi*. The parallel ridging pattern observed by Ward & Ready (1975) was not found in any of the eggs studied. The egg morphology of each species is described in the following paragraphs.

Lutzomyia panamensis (Shannon, 1926)
FIG. 1–3

Size: N = 43, L 364–429 (398), W 102–156 (122).

Chorion: Reticulation composed of flattened irregular raised areas shaped like mesas (mountain- or volcano-like), approximately equidistant from each other; surface of mesas uneven and pitted; area between mesas very rugose at 2000× magnification.

Remarks: The chorion is unlike any in the descriptions by Barretto (1941). The egg is similar to, but longer and wider than, those of *L. pessoana* and *L. wellcomei* (Ward 1972). The *L. panamensis* micrographs closely resembled those of *L. complexus* (Mangabeira), *L. carrerai* (Barretto), *L. davisi* (Root), *L. lainsoni* (Fraiha & Ward), *L. paraensis* (Costa Lima) and *L. flaviscutellata* (Mangabeira) (Ward & Ready 1975).

Lutzomyia pessoana (Barretto, 1955)
FIG. 4–6

Size: N = 9, L 357–391 (337), W 95–129 (103).

Chorion: Similar to that of *L. panamensis* (mountain- or volcano-like) except mesa-like prominences elongated; not as rugose as that of *L. panamensis*, with irregularly shaped cells formed between lower irregular ridges at 5000× magnification.

Remarks: The egg is similar to, but usually shorter than, those of *L. wellcomei*. The eggs of those species mentioned in the *L. panamensis* remarks section resemble those of *L. pessoana* even more so than those of *L. panamensis*.

Lutzomyia sanguinaria (Fairchild & Hertig, 1957)
FIG. 7–9

Size: N = 8, L 344–388 (371), W 95–122 (108).

Chorion: Provided with conspicuous, more or less parallel longitudinal ridges; connecting cross-ridges form pentagonal, hexagonal or polygonal cells; ridges generally continuous except where damaged.

Remarks: The egg is similar to that of *L. trapidoi*. The surface polygonal and ridge structure is like that found in the eggs of the sand fly groups studied by Barretto (1941) and Ward & Ready (1975).

Lutzomyia trapidoi (Fairchild & Hertig, 1952)
FIG. 10–11

Size: N = 14, L 327–378 (350), W 92–122 (104).

Chorion: Bears conspicuous, narrow, more or less longitudinal ridges; crossridges of the same consistency form generally elongated rectangular cells; ridges seem more often discontinuous than those of *L. sanguinaria*.

Remarks: The egg is similar to that of *L. sanguinaria* but generally smaller in size.

Lutzomyia ylephiletor (Fairchild & Hertig, 1952)
FIG. 12–14

Size: N = 6, L 333–374 (350), W 92–106 (101).

Chorion: Bears conspicuous, flattened, more or less parallel longitudinal ridges formed from flattened globular mounds, discontinuous in pattern at 2000× magnification; crossridges formed the same way. Ridges may be discontinuous, cells somewhat elongate, rectangular to polygonal, no distinct pattern to cells.

Remarks: The egg is very similar in size to that of *L. trapidoi* but the ridging pattern is different. These 2 sand fly species from the *L. intermedia* group have very similar external characteristics in the adult and immature stages. The differences in the egg sculpturing should make them easier to separate. The pattern closely resembles those of *Lutzomyia antunsie* (Coutinho), *Lutzomyia yuilli* (Young & Porter) and *Lutzomyia* sp. no 260.43, all of the *L. intermedia* group (Ward & Ready 1975).

Lutzomyia gomezi (Nitzulescu, 1931)
FIG. 15–16

Size: N = 18, L 300–347 (332), W 95–122 (104).

Chorion: Very conspicuous, wide, flattened, longitudinally directed ridges; crossridges narrow and oblique to longitudinal ridges; cells elongate polygons at 1000× magnification.

Remarks: The egg is similar to that of *Lutzomyia monticola* (Costa Lima) described by Barretto (1941). Ward & Ready (1975) included a micrograph of *L. gomezi* in their study of Brazilian species. The ridging pattern of the eggs from Panama and Brazil

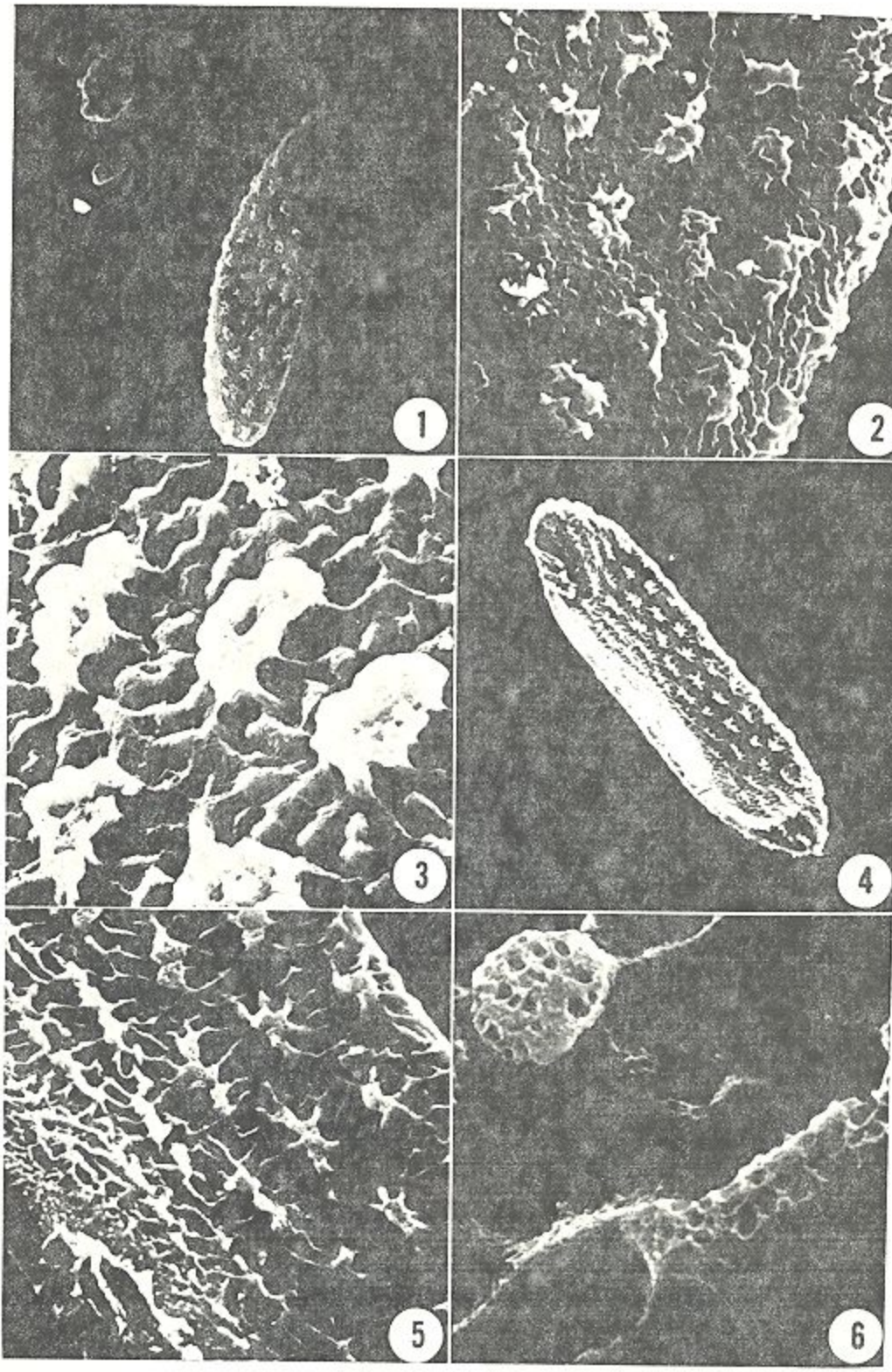

FIG. 1–6. (1) Egg of *L. panamensis*, 200 × ; (2) Egg of *L. panamensis*, 1000 × ; (3) Egg of *L. panamensis*, 2000 × ; (4) Egg of *L. pessoana*, 200 × ; (5) Egg of *L. pessoana*, 1000 × ; (6) Egg of *L. pessoana*, 5000 × .

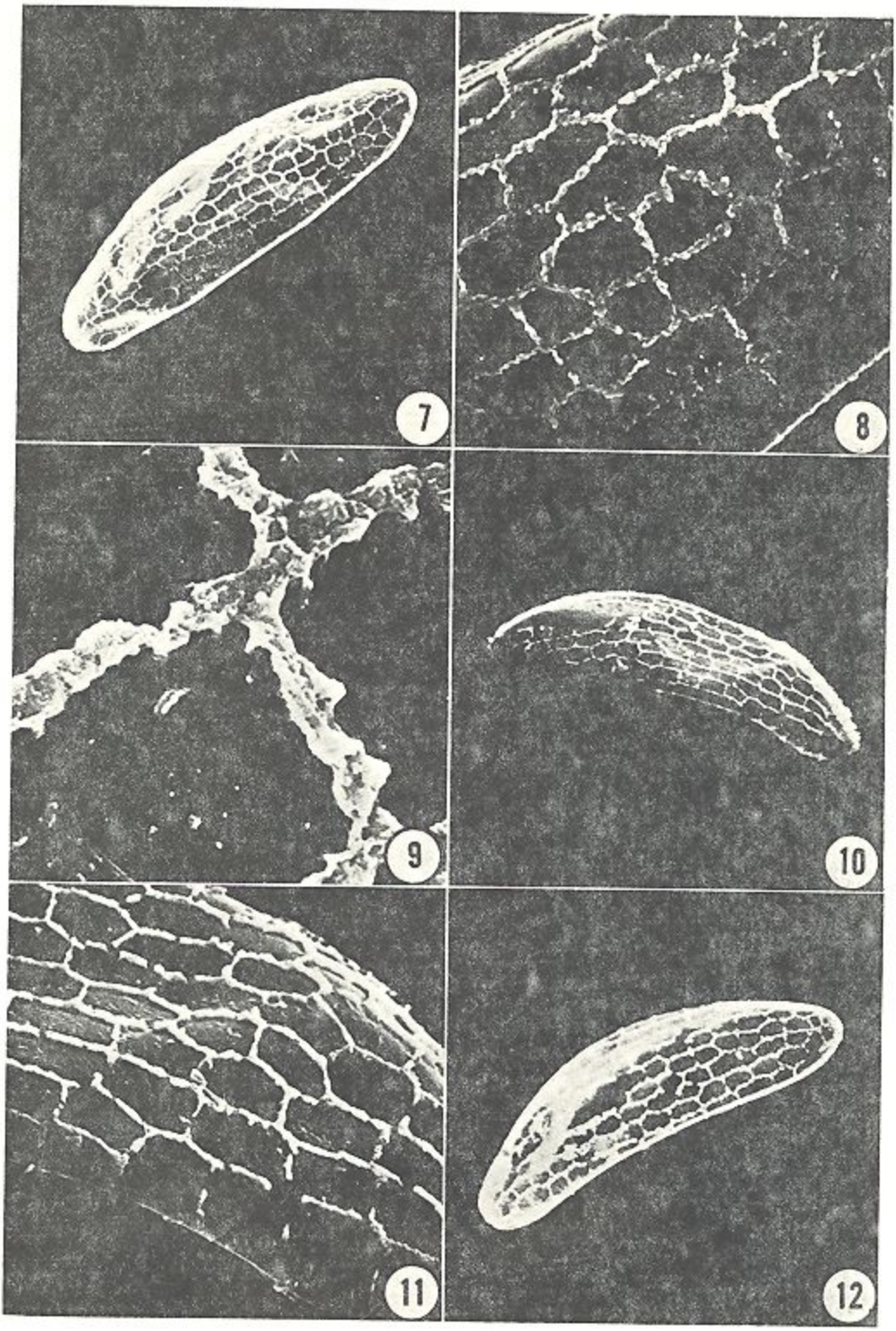

FIG. 7–12. (7) Egg of *L. sanguinaria*, 200×; (8) Egg of *L. sanguinaria*, 1000×; (9) Egg of *L. sanguinaria*, 5000×; (10) Egg of *L. trapidoi*, 200×; (11) Egg of *L. trapidoi*, 1000×; (12) Egg of *L. ylephiletor* 200×.

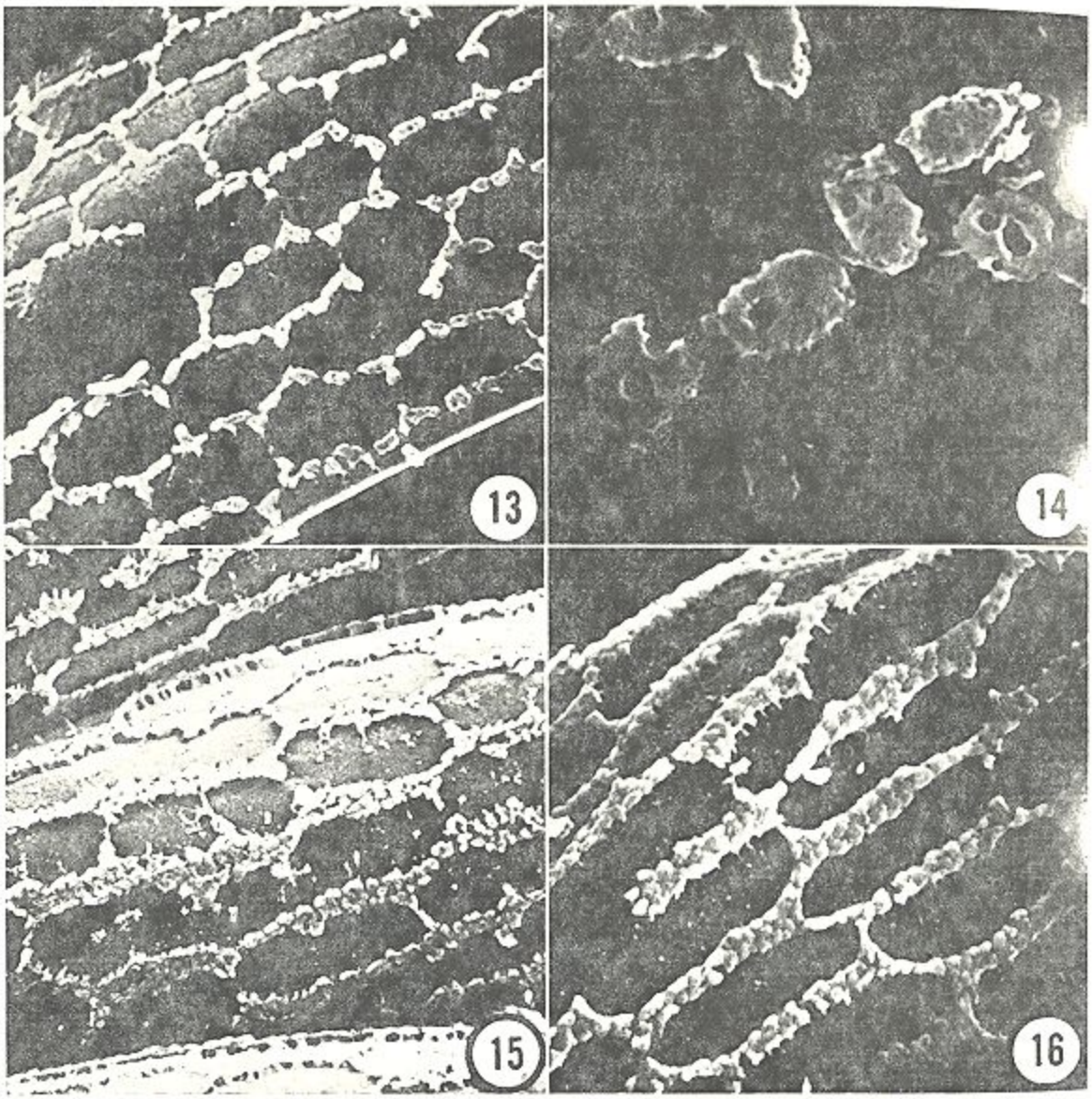

FIG. 13–16. (13) Egg of *L. ylephiletor*, 1000×; (14) Egg of *L. ylephiletor*, 5000×; (15) Egg of *L. gomezi*, 1000×; (16) Egg of *L. gomezi*, 1000×.

is distinctly different, suggesting geographic differences in egg sculpturing pattern.

CONCLUSION

The SEM showed 2 distinct patterns in the surface ultrastructure of eggs of the 6 anthropophilic sand fly species studied. They were the mountain- or volcano-like pattern of *L. panamensis* and *L. pessoana* and the polygonal pattern of *L. sanguinaria*, *L. ylephiletor*, *L. trapidoi* and *L. gomezi*. It appears that geographic differences exist between eggs of the same species, as exhibited by *L. gomezi* in this study and that of Ward & Ready (1975). Egg measurements were included in the descriptions in an effort to further distinguish the very closely related species.

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