Eggs of Floodwater Mosquitoes. XIII. Chorionic Sculpturing[1]

WILLIAM R. HORSFALL, FRANK RAY VOORHEES,[2] AND EDDIE W. CUPP[3]
Department of Entomology, University of Illinois, Urbana 61801

ABSTRACT

This paper is one of a series which seeks to aid researchers in identifying eggs and in clarifying the associations of species in higher taxa. Micrographs of whole eggs and segments of the chorion made with a scanning electron microscope are included.

Preceding papers in this series contain descriptions and illustrations of eggs of numerous floodwater mosquitoes (Horsfall et al. 1952, Horsfall and Craig 1956, Craig and Horsfall 1960, Ross and Horsfall 1965). At the time they were written no adequate means were available for illustrating the contour and sculpturing. Even with black backgrounds, micrography with visible light would not depict the eggs with sharp margins or free of excess highlights under the best of conditions. Kalpage and Brust (1968) present some of the better figures of whole eggs made with light microscopy. The scanning electron microscope permits photography showing detail of whole eggs as it appears to the human eye with the optics of a dissecting microscope at 100×. The scanning electron microscope also permits portrayal of fine aspects of chorionic sculpturing even more sharply than may be seen by phase-contrast microscopy as was shown in earlier papers.

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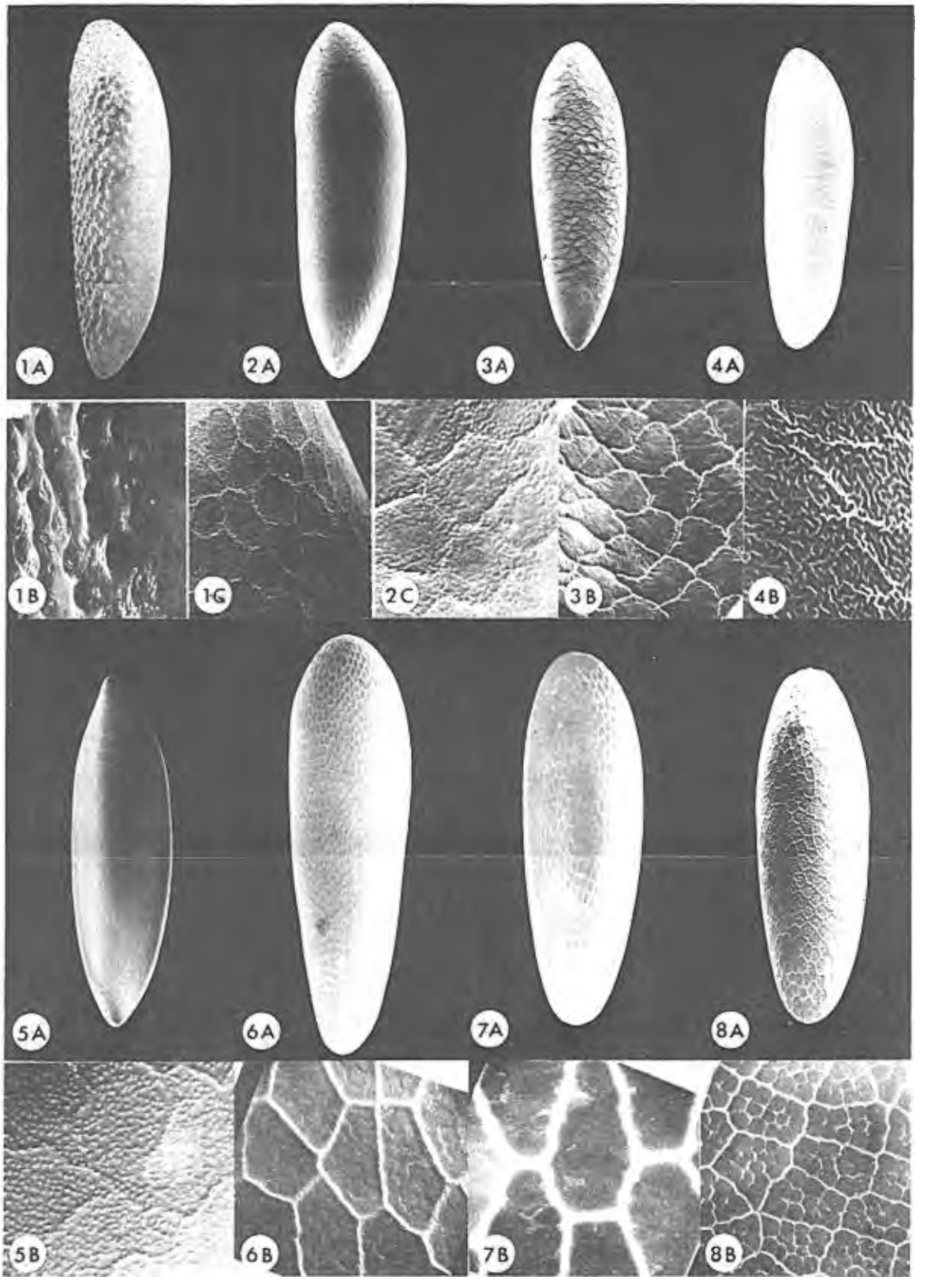
[2] Present address: Department of Biology, Knox College, Galesburg, Ill. 61401.

[3] Present address: Department of Parasitology, Tulane School of Medicine, New Orleans, La. 70100.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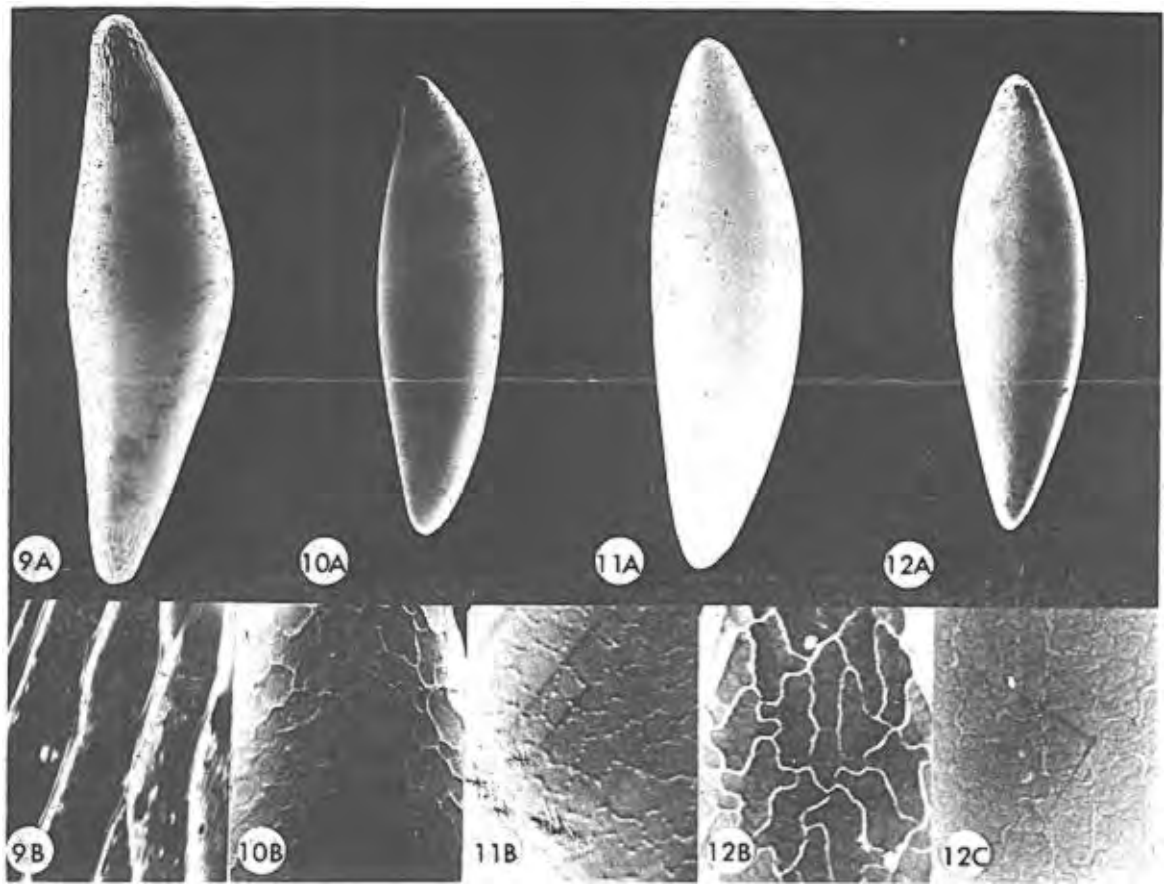


FIG. 9. *A. atlanticus* Dyar & Knab, northern Alabama. A, Whole egg ×100; B, chorionic sculpturing ×1000.
FIG. 10. *A. dupreei* (Coquillett), Champaign Co. A, Whole egg ×100; B, chorionic sculpturing ×500.
FIG. 11. *A. trivittatus* (Coquillett), Champaign Co. A, Whole egg ×100; B, chorionic sculpturing, anterior third ×500.
FIG. 12.—*A. trivittatus* variant, Champaign Co. A, Whole egg ×100; B, chorionic sculpturing near anterior end ×500; C, chorionic sculpturing near middle ×500.

METHODS

Sources.—Eggs were obtained from wild-caught females obtained in Alabama, Illinois, Michigan, Wisconsin, and northern Manitoba. Eggs were deposited on moist substrata, incubated in the laboratory, and stored on dry surfaces in moist chambers at 4°C prior to preparation for examination.

Preparation.—The exochorion and adherent debris were removed by rolling the eggs on the sticky surface of cellulose-acetate tape while observing them with the aid of a stereo-dissecting microscope. To the flat face of a "stub" (Stereoscan viewing platform) was added a strip of bifacially adhesive cellulose-acetate tape. Clean eggs were placed on the exposed adhesive face so that the dorsal and ventral sides of the eggs were in profile. The stub bearing eggs was immersed in a bath of acetone-dry ice at ca. −50°C for 10 min for setting the tissue by rapid freezing. While the eggs remained frozen the stub was transferred to a refrigerated vacuum (−60°C)

FIG. 1–33.—Eggs of floodwater mosquitoes. (Dorsal side to left and anterior end uppermost.)
1. *A. triseriatus* (Say), Champaign Co., Ill. A, whole egg ×100; B, chorionic sculpturing along dorsoventral line ×500; C, chorionic sculpturing on ventral side ×500.
2. *A. atropalpus* (Coquillett), from colony. A, Whole egg ×100; B, chorionic sculpturing on anterior venter ×500.
3. *A. sierrensis* (Ludlow), from California (Judson colony). A, Whole egg ×100; B, chorionic sculpturing on anterior third ×500.
4. *A. aegypti* (L.), (Larsen colony). A, Whole egg ×100; B, chorionic sculpturing ×500.
5. *A. sollicitans* (Walker), southern Illinois. A, Whole egg ×100; B, chorionic sculpturing anterior third ×500.
6. *A. stimulans* (Walker), Champaign Co. A, Whole egg ×100; B, chorionic sculpturing ×500.
7. *A. grossbecki* Dyar & Knab, Olney, Ill. A, Whole egg ×100; B, Chorionic sculpturing ×500.
8. *A. canadensis* (Theobald), central Kentucky. A, Whole egg ×100; B, chorionic sculpturing ×500.

for an hour for desiccation. The desiccated eggs were kept dry at room temperature in dry air over calcium chloride at atmospheric pressure not longer than 24 hr before photography.

Surfaces of the eggs were made more adaptable to electron microscopy by coating the chorions with metallic film. The film of choice was of gold-palladium applied in a vacuum evaporator. Filaments of gold and palladium were crushed together to form a compact nodule in a volatilizing basket heated between 2 electrodes. The eggs were exposed in the evacuated chamber at a uniform distance from the evaporation site of the metals. Since exposure of eggs to this 2nd depressurization predisposes them to collapse, metal coating should be completed within 20 min.

Exposure of eggs to the scanning electron beam and viewing and photographing them are procedural matters established by manual and require a trained operator. The stub bearing the egg may be manipulated to the proper angle while viewing the object on a fluorescent screen. Focusing for minute detail requires care and precision. Photography is accomplished by transferring the image to a fluorescent screen against a film. All pictures were made on 4×5 Polaroid film, and prints were made on bromide paper.

Culicine eggs may collapse in the vacuum of the microscope. Why this should be so is not clear. Nevertheless an egg cannot be expected to maintain its integrity in a vacuum for longer than 2 hr of total exposure. Its life expectancy in the microscope will be the difference between this theoretical 2-hr maximum and the length of time spent under vacuum during preparation. To avoid loss by collapse, stubs should bear only as many eggs as can be examined and photographed conveniently in 1 hr.

Two features inherent in the design of any electron microscope—the electron beam and the vacuum essential for its propagation—make the environment of the specimen chamber hostile to culicine eggs no matter how carefully prepared. As the beam strikes the black egg, some of the beam's radiant energy is converted into heat. After a few seconds or minutes in the beam, burn marks may appear in the chorion. The higher the magnification, the more rapidly these marks will form. Thus it is essential that focusing and photography be carried out as rapidly as possible. Burning can be delayed by operating the microscope at the lowest voltage consistent with good resolution. For photographs ca. 100×, 3kv is adequate; for magnifications of 500–2500×, 10kv is necessary.

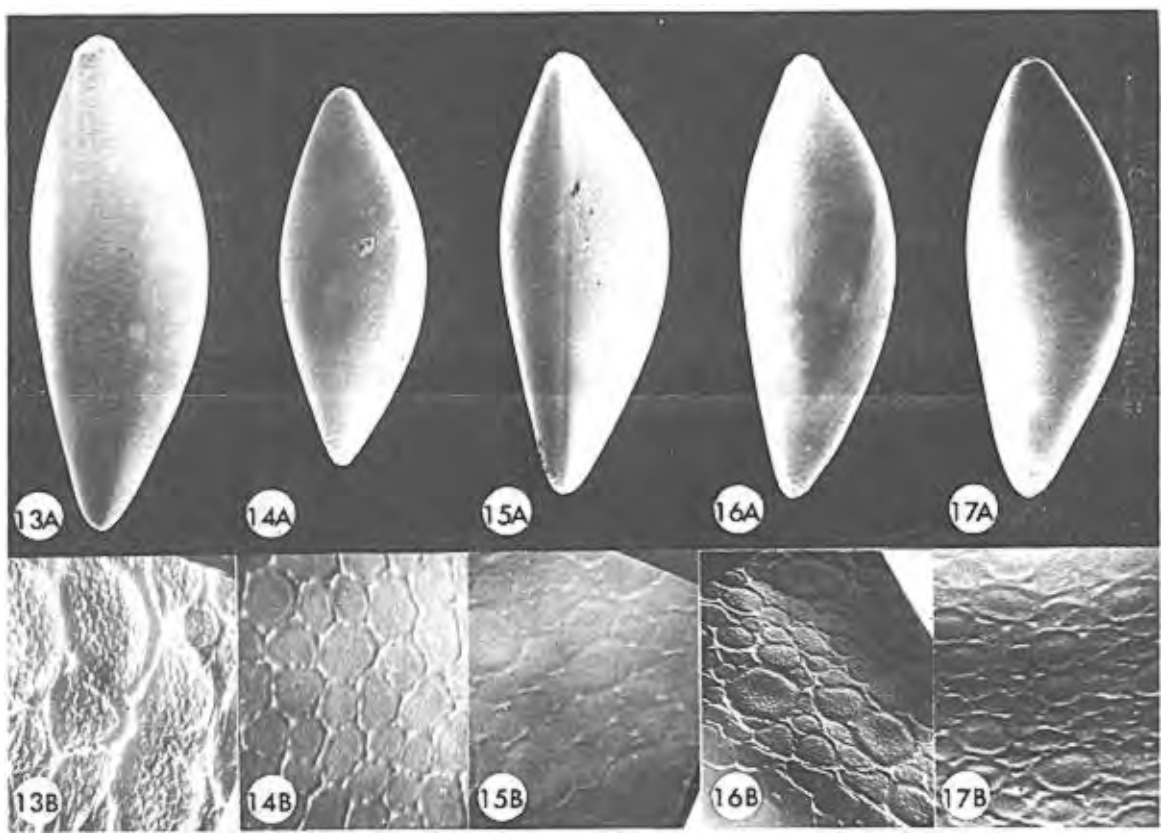


FIG. 13–17.—*A. sticticus* (Meigen), variants. 13. From northern Wisconsin. A, Whole egg ×100; B, chorionic sculpturing ×2500. 14. From northern Alabama. A, Whole egg ×100; B, chorionic sculpturing ×1000. 15. From northern Alabama. A, Whole egg ×100; B, chorionic sculpturing ×1000. 16. From Champaign Co. A, Whole egg ×100; B, chorionic sculpturing ×1000. 17. From southern Illinois. A, Whole egg ×100; B, chorionic sculpturing ×1000.

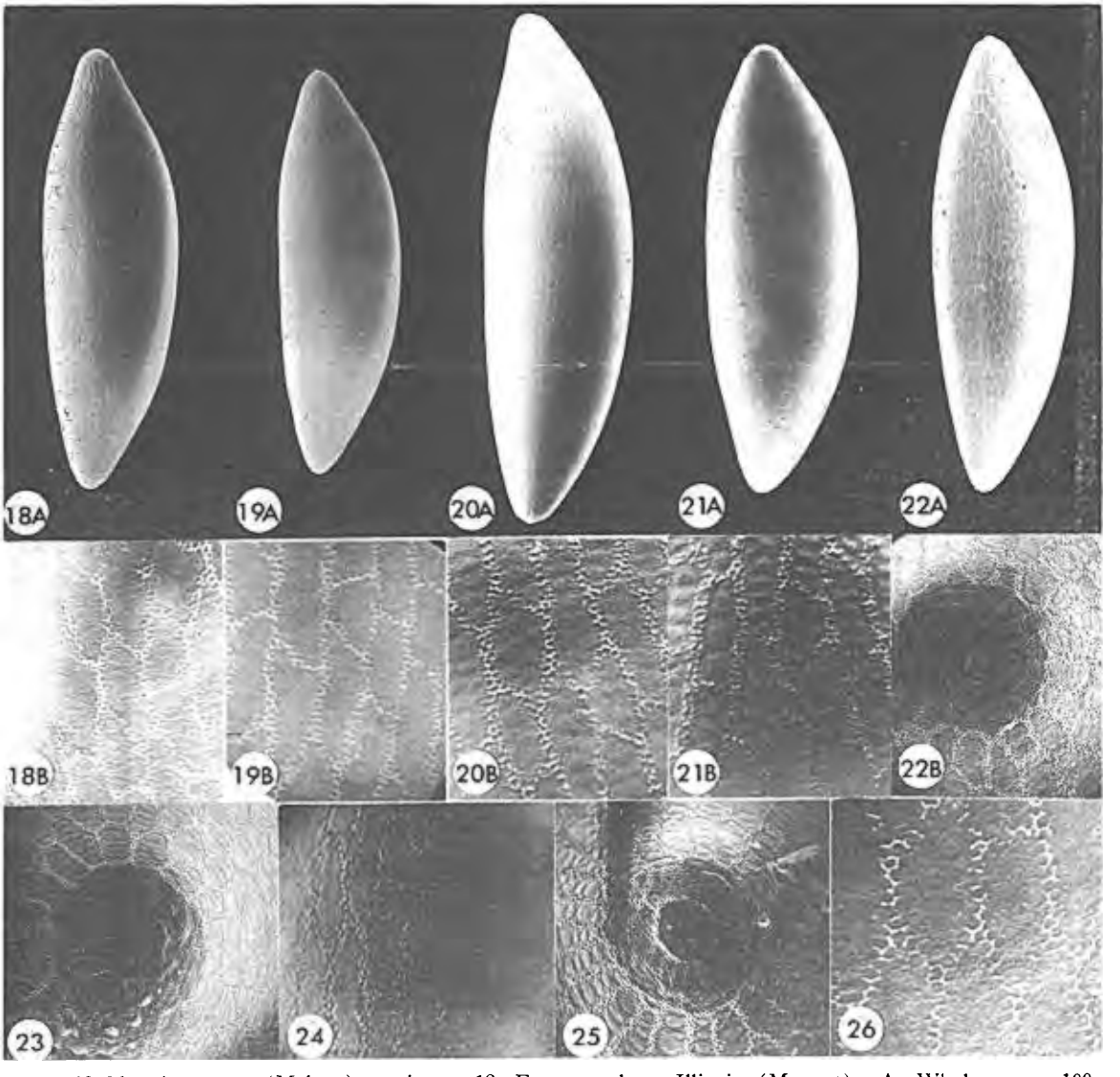
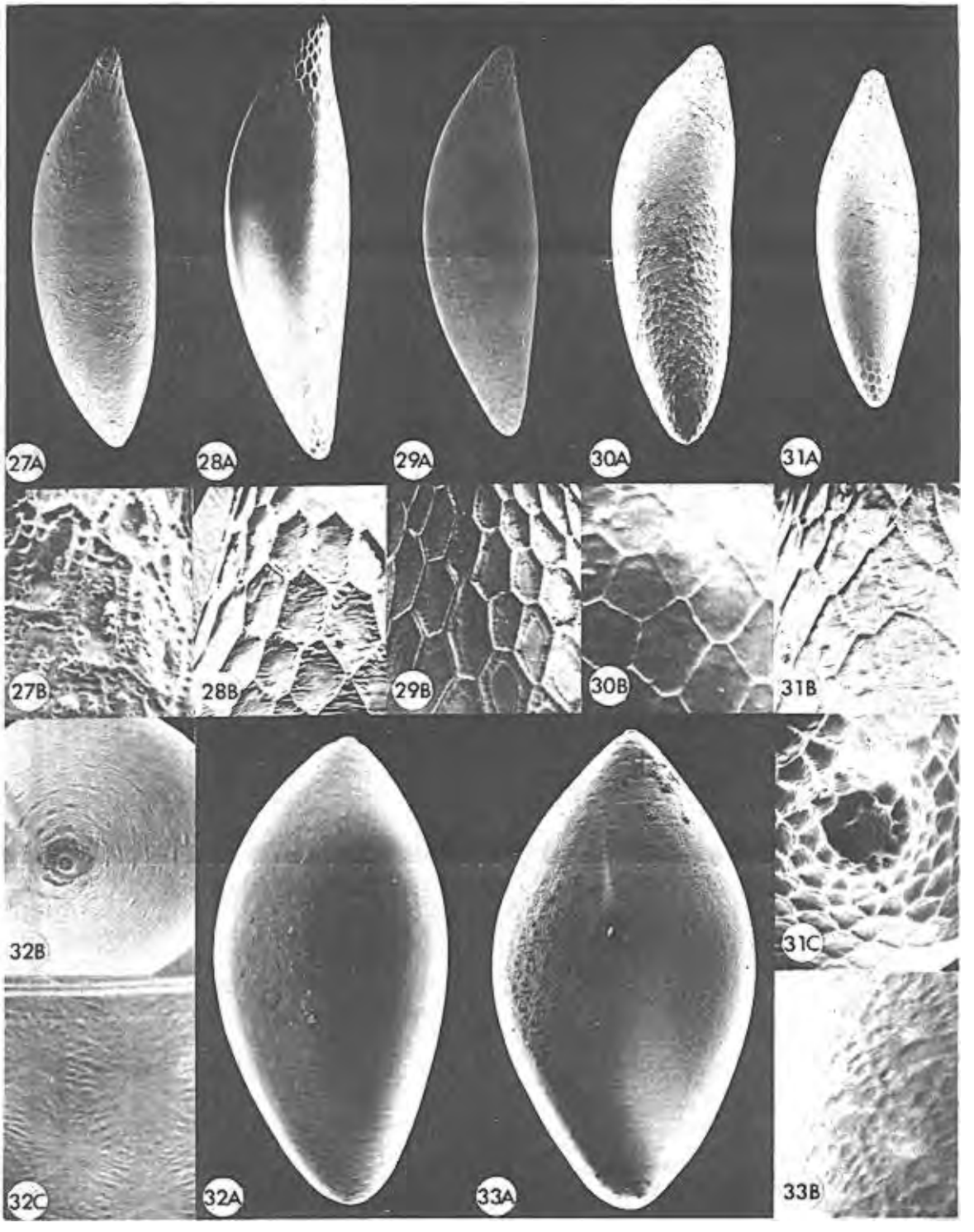


FIG. 18–26.—*A. vexans* (Meigen), variants. 18. From southern Illinois (Mermet). A, Whole egg ×100; B, chorionic sculpturing ×500. 19. From Olney. A, Whole egg ×100; B, chorionic sculpturing ×500. 20. From Piatt Co., Ill. (long form). A, Whole egg ×100; B, chorionic sculpturing ×500. 21. From Piatt Co. (short form). A, Whole egg ×100; B, chorionic sculpturing ×500. 22. From northwestern Manitoba (Flin Flon). A, Whole egg ×100; B, micropylar end ×500. 23. From northern Alabama, micropylar end ×500. 24. From northern Alabama, chorionic sculpturing ×500. 25. From Champaign Co., micropylar end ×500. 26. From Champaign Co., chorionic sculpturing ×1200.

MORPHOLOGY

Significant features by which eggs of aedine mosquitoes may be recognized include (1) shape in lateral profile, (2) presence or absence of a polygonal reticulated surface, (3) shape of polygons (impressions of secreting epithelium) in the reticulation, (4) characteristics of the margins of the polygons, (5) markings on the faces (discs) of the polygons, and (6) hue of the surface (as seen in reflected white light against a nonreflecting background). Eggs of some (Fig. 6–8, 18–22, 33 et al.) have an overall reticulation. Others (Fig. 9, 28, 31 et al.) have the reticulation clearly presented in relief terminally and indistinctly presented in the midsection. The only species shown here (Fig. 13–17) that lacks a reticulation in relief is *Aedes sticticus*, and it is typical of a large group of species that will be illustrated later.

Species representing the subgenera *Finlaya* and *Stegomyia* (Fig. 1, 4) are characterized by a dimorphic pattern of sculpturing that changes from one to the other form along each side. The venter of the eggs is reticulated (1C and 4B), and the dorsum is either heavily embossed (1B) or finely crenulated (left side 4B). *A. atropalpus* (Fig. 2) and *A. sierrensis* (Fig. 3), considered by some to be *Ochlero-*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tatus, would seem to fit elsewhere and most likely in *Finlaya* as was proposed previously (Cupp and Horsfall 1969).

The boldest reticulation is exhibited by *A. stimulans, A. grossbecki,* and *A. canadensis* (Fig. 6–8) among the species depicted. These have polygons with sharp linear margins with the discs of the polygons variously and specifically marked. Note the nearly straight lines of the *A. stimulans,* the wavy lines of *A. grossbecki,* and the laterally extended lines of *A. canadensis* (Fig. 6B, 7B, 8B). The discs of Fig. 6B and 8B are clearly different and specific. Those of Fig. 6B are marked with flat hexagonal areas of differing elevations. Those of *A. canadensis* (Fig. 8B) are covered by angular sections with raised lines separating them. The discs of the polygons of *A. grossbecki* show faint indications of subdivisions.

Fig. 5 and Fig. 9–12 are dissimilar in shape but have the same basic configuration in sculpturing of the chorion. Each unit of the reticulation is a large cell with 2 lateral extensions (best seen in Fig. 12B). Several variations between species may be seen. Sculpturing of *A. trivittatus* is barely visible with the cell walls appearing as dots connected by mere traces of elevations (Fig. 11B). The reticulation on eggs of *A. atlanticus* and *A. dupreei* is indistinct except terminally (Fig. 9, 10). Discs of the several species are variable also. Those of *A. sollicitans* and *A. atlanticus* are dotted with numerous minute excrescences (Fig. 5B, 9B). The others have one or more slightly elevated plaques (Fig. 10B, 11B, 12C).

Undoubtedly many species in the genus *Aedes* are composed of populations recognizable by differences in features of the eggs. Fig. 13–17 represent such differences in populations of *A. sticticus* and Fig. 18–22 do so for *A. vexans.* Variations in shape and size as well as differences in sculpturing are indicated. Most striking differences for *A. sticticus* may be seen in eggs from northern Wisconsin (Fig. 13) and northern Alabama (Fig. 14). They vary both in size and in pattern. Note the raised reticulation and numerous minute plaques of the Wisconsin population (Fig. 13B). Compare that with the very low relief and single raised plaque of the Alabama strain. The population from central Illinois resembles that from Wisconsin, while that from southern Illinois is similar to one of the forms from northern Alabama (Fig. 17, 15).

A. vexans (Fig. 18–26), the only other binomial taxon represented by populations from widely different localities, also has variants based on size and sculpturing. In the fall, 2 variants appear on the flood plains of rivers in central Illinois (Fig. 20, 21). Eggs from a field site fall into 2 classes based on length with little overlap in sizes. Sculpturing of the two is similar. The population from Manitoba (Fig. 22) is similar to the long form present in the fall in central Illinois. The form from Olney (southern Illinois) is similar to the short form common in central Illinois.

Seven species of *Psorophora* (Fig. 27–33) are depicted. These eggs are strongly arched dorsally (left profile) and, for the most part, are much less so ventrally (Fig. 27–29). Eggs of the subgenus *Janthinosoma, P. cyanescens* (Fig. 27), *P. horrida* (Fig. 28), *P. ferox* (Fig. 29), and *P. varipes* (Fig. 31) have carinate borders of the polygons on the anterior ends, at least. The disc of each cell is smooth, depressed, and is bounded by a ring of radiating ridges that leaves the impression that the surface is pitted (Fig. 27B and 29B). The ridges are present in the cells of *P. horrida* (Fig. 28B) without the appearance of pits. The subgenus *Grabhamia* is represented by *P. confinnis* (Fig. 30). The reticulation is clearly visible over the entire egg without emphasis terminally as in the case with *Janthinosoma* spp. The egg illustrated was inadequately cleaned of its exochorion, hence the lack of smoothness. The subgenus *Psorophora* is represented by *P. ciliata* (Fig. 32) and *P. howardii* (Fig. 33). The differences between them are subtle and difficult to use. Eggs of *P. ciliata* are less arched ventrally and lack clearly visible reticulations at all magnifications. The spiral pitting is a conspicuous feature (Fig. 32A and B) and is evident visually in white light on most eggs.

Chorionic sculpturing is created by the follicular cells as they secrete the envelope. The polygons comprising the reticulation are the outlines of the cells that were appressed to the envelope. Impressions, plaques, and ridges represent the surfaces of the secreting cells. The exochorion, which is adherent to newly deposited eggs, is the layer of spent cells extruded with the eggs. It, too, often bears distinctive features useful in identification. For example, on *Psorophora* a nipplelike process directed anteriorly extends from the center of each polygon. From some *Aedes* the exochorion is readily removed; it is strongly adherent to some, notably the species of *Finlaya.*

FIG. 27–33.—*Psorophora* spp. 27. *P. cyanescens* (Coquillett), from northern Alabama. A, Whole egg ×100; B, chorionic sculpturing near anterior end ×500. 28. *P. horrida* (Dyar & Knab), from central Kentucky. A, Whole egg ×100; B, chorionic sculpturing ×500. 29. *P. ferox* (Humboldt), from southern Illinois (Mermet). A, Whole egg ×100; B, chorionic sculpturing near anterior end ×500. 30. *P. confinnis* (Lynch-Arribalzaga), from northern Alabama. A, Whole egg with partially retained exochorion ×100; B, chorionic sculpturing near middle ×500. 31. *P. varipes* (Coquillett), from southern Illinois (Mermet). A, Whole egg ×100; B, chorionic sculpturing near anterior end ×500; C, Micropylar end ×500. 32. *P. ciliata* (F.), from southern Illinois (Mermet). A, Whole egg with 2 artifacts near middle ×100; B, micropylar end ×150; C, chorionic sculpturing near middle ×500. 33. *P. howardii* (Coquillett), from northern Alabama. A, Whole egg with artifact near middle and near micropyle ×100; B, chorionic sculpturing near middle ×500.

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