Description of the Egg of *Aedeomyia squamipennis* (Diptera: Culicidae)

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ABSTRACT The egg of *Aedeomyia squamipennis* (Lynch Arribalzaga) is described with the aid of scanning electron micrographs. This study allows separation of the eggs of *Ad. squamipennis* from the eggs of other mosquitoes inhabiting similar aquatic vegetation.

KEY WORDS *Aedeomyia squamipennis*, egg, scanning electron micrographs, Panama

Aedeomyia squamipennis (Lynch Arribalzaga) is reported from South America, Trinidad, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Belize, Mexico, and Cuba (Knight and Stone 1977, Knight 1978). Tyson (1970) reported that the aquatic stages of *Ad. squamipennis* are associated with vegetation such as *Pistia stratiotes* (L.), *Azolla* sp., *Salvinia* sp., *Utricularia* sp., and *Eichhornia crassipes* (Mart.) Solms. Our interest in *Ad. squamipennis* focuses on the vertical transmission of arbovirus. In 1969, Justines isolated Gamboa virus (family Bunyaviridae, genus Bunyavirus) from adult *Ad. squamipennis* collected on the Chagres River, Panama (Justines 1985). Viral isolations from *Ad. squamipennis* in Panama contributed to the establishment of the Gamboa serogroup by Calisher et al. (1981). Galindo et al. (1983) isolated Gamboa virus from males and females of *Ad. squamipennis* reared from larvae collected in Panama during the impoundment phase of the Bayano Hydroelectric Project. Dutary et al. (1987, 1989) implicated *Ad. squamipennis* in the transovarial transmission of Gamboa virus by isolating the virus directly from triturated egg batches as well as from all instars and pupae of this mosquito. Criteria for the identification of *Ad. squamipennis* eggs were established by Dutary et al. (1987), who described the egg mass as seen with a dissecting microscope. Those authors obtained eggs from caged, blood-engorged *Ad. squamipennis* females that oviposited on aquatic vegetation known to be free of any mosquito eggs. Species identification was confirmed by hatching the eggs, rearing larvae to the adult stage, and visually identifying *Ad. squamipennis*.

The detailed morphology of the egg has not been reported previously, and we provide here a complete description based on scanning electron micrographs (SEM).

Materials and Methods

All eggs used in this study were collected in the Republic of Panama from the Chagres River near the Juan Mina Field Station (9° 10′ N; 79° 39′ W). Aquatic vegetation belonging to the following genera was examined for *Ad. squamipennis* eggs: *Pistia* sp., *Ludwigia* sp., and *Salvinia* sp. A hand lens was used in the field to verify identification of the mosquito eggs. As soon as they were collected in the field, batches of eggs were preserved in screw-cap vials containing 30% ethanol. Within 24 h of collection, the preservative was replaced with fresh 30% ethanol.

Identification of the eggs was based on comparisons with eggs obtained from caged, blood-engorged *Ad. squamipennis* females that oviposited on aquatic vegetation placed in pans in the mosquito cage. In addition, field-collected eggs were brought to the laboratory, hatched, and larvae were reared for identification. *Ad. squamipennis* is the only species of the genus *Aedeomyia* in the New World.

Pieces of aquatic vegetation with attached eggs were glued to stubs with metallic silver paint, gold coated, and examined in a SEM (Hitachi S-510). The morphological terminology follows Harbach and Knight (1980).

Results

Description of Egg. *Shape, Overall Appearance.* Egg is shiny black, and covered in a very loose outer chorion, which is removed easily (Fig. 1a). Shape is asymmetrical in lateral view, widest at about anterior third, ventral side flat or very slightly concave, dorsal side strongly curved, both ends rounded (Fig. 1a). Anterior end in ventral view is markedly swollen, posterior end tapered, micropylar apparatus inconspicuous, barely visible (Fig. 2a). Egg length 344.9–390.1 μm (mean ± SE = 373.2 ± 9.9 μm, $n = 4$).

Inner Chorion. Surface with no discernible pattern indicating chorionic cell boundaries (Fig. 1a),

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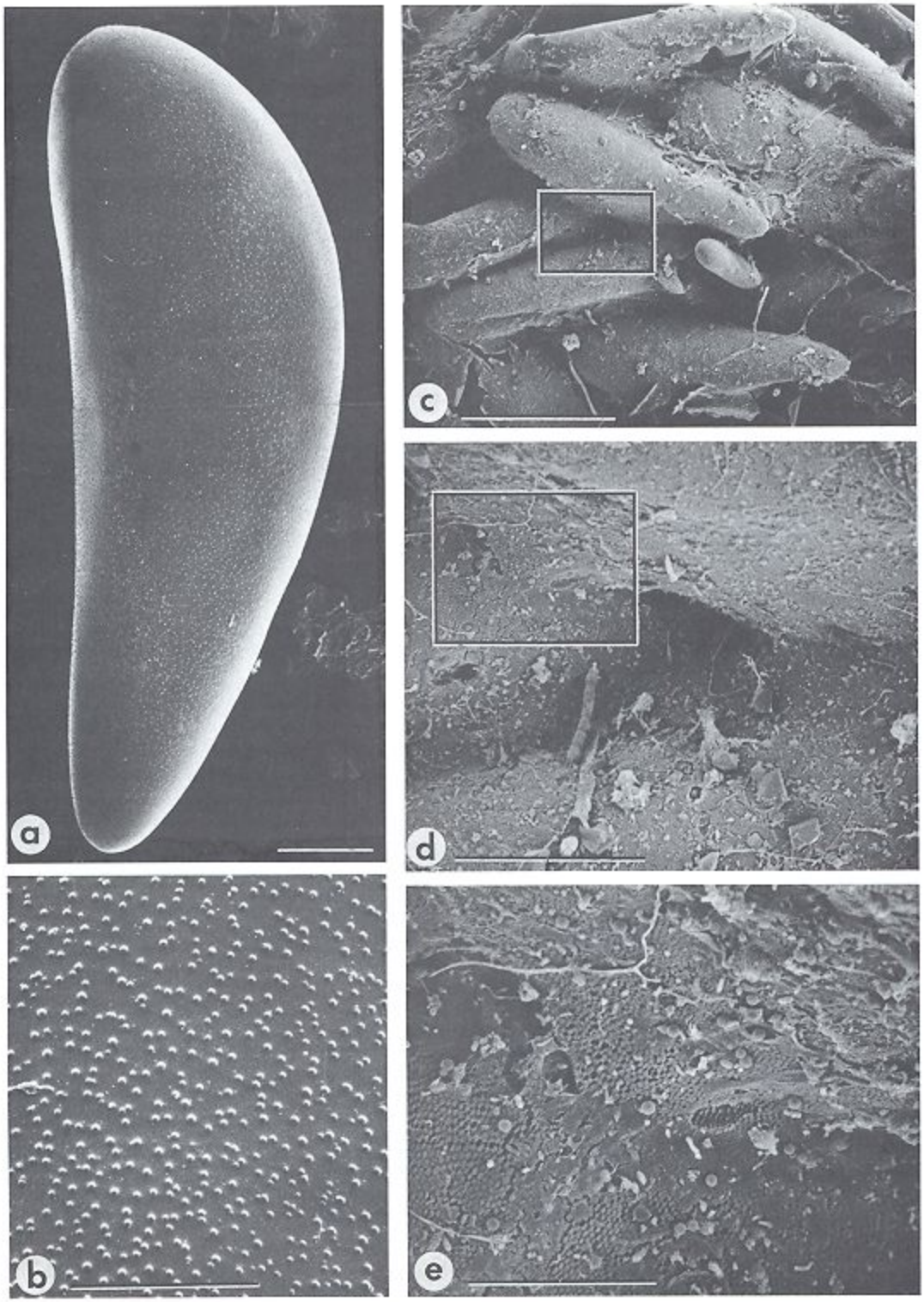

Fig. 1. (a) Whole egg, lateral view, outer chorion removed, anterior end at top, dorsal side at right. (b) Detail, surface of inner chorion. (c) Several eggs in an egg mass, showing outer chorion forming veil-like cover. (d) Area outlined in (c) enlarged, showing fusion of outer chorion to form continuous sheet between eggs. (e) Enlarged view of area outlined in (d), showing structural detail of fused outer chorion. Scale = 200 μm (c), 50 μm (a and d), 20 μm (b and e).

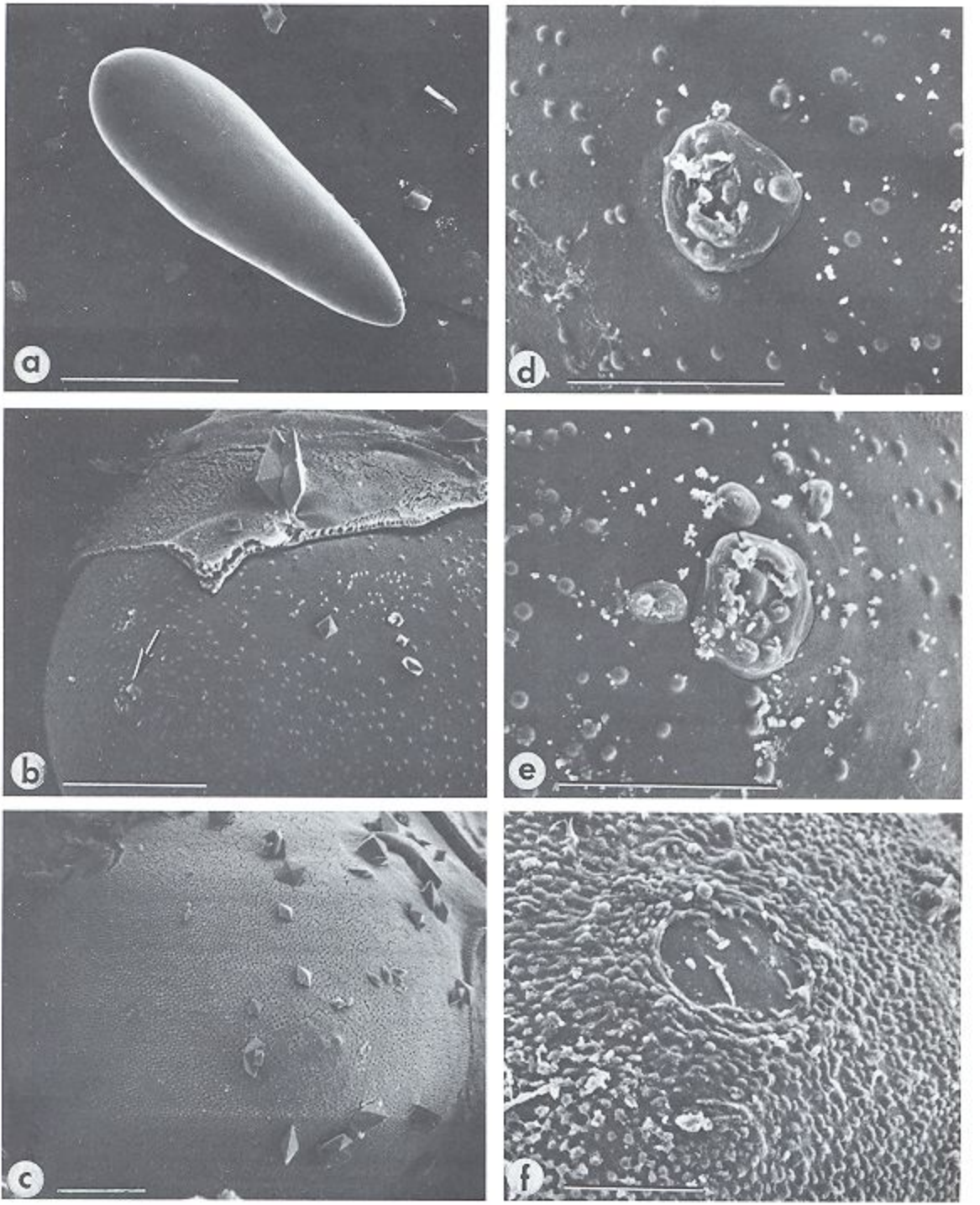

Fig. 2. (a) Whole egg, outer chorion removed, ventral view, anterior end at top left. (b) Anterior end, showing micropyle on inner chorion (arrow), outer chorion with torn edge visible at top. (c) Posterior end, outer chorion intact. (d) Detail of micropylar disk on inner chorion, outer chorion not present. (e) Same as (d), but with a few large tubercles on inner chorion around disk. (f) Site of detached disk at anterior end, showing structure of surrounding outer chorion. Scale = 500 μm (a), 20 μm (b and c), 10 μm (d–f).

covered overall with fairly sparse, randomly distributed small, round, low tubercles (Fig. 1 a and b), diameter 0.31–1.11 μm, (mean = 0.69 ± 0.03 μm, n = 35).

Outer Chorion. Very loosely attached to each egg, easily peeled or rubbed off, and with remarkable ability to form a continuous, fused sheet covering contiguous eggs in a batch, apparently by anastomosis between adjacent eggs. Micrographs at increasing magnifications (Fig. 1 c–e) clearly show a continuous layer, with structure visible at high magnification (Figs. 1e and 3 a–h) unified, with no discontinuity between adjacent eggs (Fig. 4). Detailed structure of outer chorion complex, most of outer surface with very closely packed, more or less rounded, abutting, mushroom-shaped tubercles, their caps not separated by interstitial pores, but with occasional cracks which are probably fixation artifacts (Fig. 3a). Diameter of caps 0.33–0.74 μm (mean = 0.57 ± 0.01 μm), surfaces flat or slightly concave, with small nodules (Fig. 3 b and h), each cap borne on a somewhat narrower pillar (Fig. 3b and c), height 0.69–1.02 μm (mean = 0.87 ± 0.02 μm), each connected to adjacent pillars by thin horizontal struts (Fig. 3c). Internally, each pillar arises from a nodule on outer side of basal layer (Fig. 3e), also with nodular inner surface where it lies in contact with the inner chorion (Fig. 3d). No observable structural correspondence between surface of inner chorion and overlying inner layer of outer chorion (Fig. 3d). Close-packed arrangement of caps in outer chorion typical of most areas on egg, but anterior surfaces often with quite open arrangement (Fig. 3f), or somewhat more tightly arrayed (Fig. 3g) with tubercles much less elevated, more nodular, and connected to adjacent ones by thin bridges.

Anterior End, Micropyle. Micropylar apparatus inconspicuous, micropylar disk forming only a small discontinuity on inner chorion (arrow, Fig. 2b), its position marked by a small opening among low, nodular tubercles that more typically form outer chorion at anterior end (Fig. 2f). Disk itself more or less round, slightly raised, diameter 6.5–8.7 μm (mean = 7.4 ± 0.7 μm, n = 3), with a few low tubercles present on the disk (Fig. 2d) or a few slightly larger ones on immediately surrounding chorion (Fig. 2e).

Posterior End. Rounded, covered with closely packed tubercles of outer chorion (Fig. 2c).

Other mosquitoes collected in the same habitat during this study included species of *Anopheles, Culex, Mansonia,* and *Uranotaenia.*

Discussion

Structurally, the egg of *Ad. squamipennis* is remarkable among known mosquito eggs, particularly with respect to the outer chorion. In other species this layer often incorporates elaborate exterior ornamentation (for example, Linley and Chadee 1991, Linley et al. 1991) and is attached quite firmly to the inner chorion, although it can be removed (Craig 1955). In *Ad. squamipennis,* the outer chorion forms a loose veil over the egg, which appears not to adhere to the inner layer and to have some mobility in being able to slide over the inner egg surface. The lack of adhesion is not an artifact of preparation, as might be suggested by Fig. 2b, because some micrographs (not shown) show the intact sheath pulled partially off the egg in the manner of a loose pillowcase. In addition, the most extraordinary property of the outer chorion is its apparent ability to anastomose between adjacent eggs (Figs. 1 c–e and 4), so that a continuous sheath is formed over the egg mass or at least over several adjacent eggs. *Ad. squamipennis* females deposit their eggs in loosely organized masses on the upper surface of aquatic vegetation at or very near the waterline, with most of the eggs lying very close together and more or less parallel to the leaf surface. The surface of a freshly oviposited egg mass is shiny with a film of water, indicating that the outer chorionic sheath is wettable. This thin film presumably allows for gaseous exchange essential for respiration. A similar situation exists in the rosette-like egg masses of *Mansonia* mosquitoes. Lincoln (1965) has shown for *Ma. africana* (Theobald) that the watery film associated with the mass is essential for survival of the eggs. What is not clear currently is how the outer chorionic sheath of adjacent *Ad. squamipennis* eggs forms. Micrographs of individual eggs show that each is enshrouded completely at the time of oviposition, so it must be presumed that as the eggs are pushed close together their outer chorionic sheaths unite to form a veil-like continuous layer. Another possibility is that the veil-like material is not the outer chorion, but a product of a female accessory gland. All female mosquitoes that have been examined have accessory glands, but it is not known whether the accessory glands secrete the veil. It is seen clearly in the micrographs that the veil-like material exhibits complex structural layers, and the pillar-like form of the tubercles, each with its expanded cap, is similar to the more conventional outer chorionic structures of other mosquito species previously examined, such as *Tripteroides bambusa* (Yamada) (Linley and Chan 1991). We conclude that the veil-like sheath serves the important function of holding the egg mass together and helping to cement it to the substrate.

Eggs of *Ad. squamipennis* can be distinguished from other mosquito eggs in aquatic vegetation by the following four criteria: (1) *Aedeomyia* eggs are oriented more or less parallel to the substrate; eggs are ovoid, without a terminal spine; eggs are enveloped in a shiny hydrophilic film. (2) Eggs of *Mansonia* spp. are oriented perpendicularly to the substrate, forming a rosette; eggs with a terminal spine; eggs not enveloped in a shiny hydrophilic film. (3) Eggs of *Culex* spp. and *Uranotaenia* spp. are deposited singly or in a free-floating raft; eggs

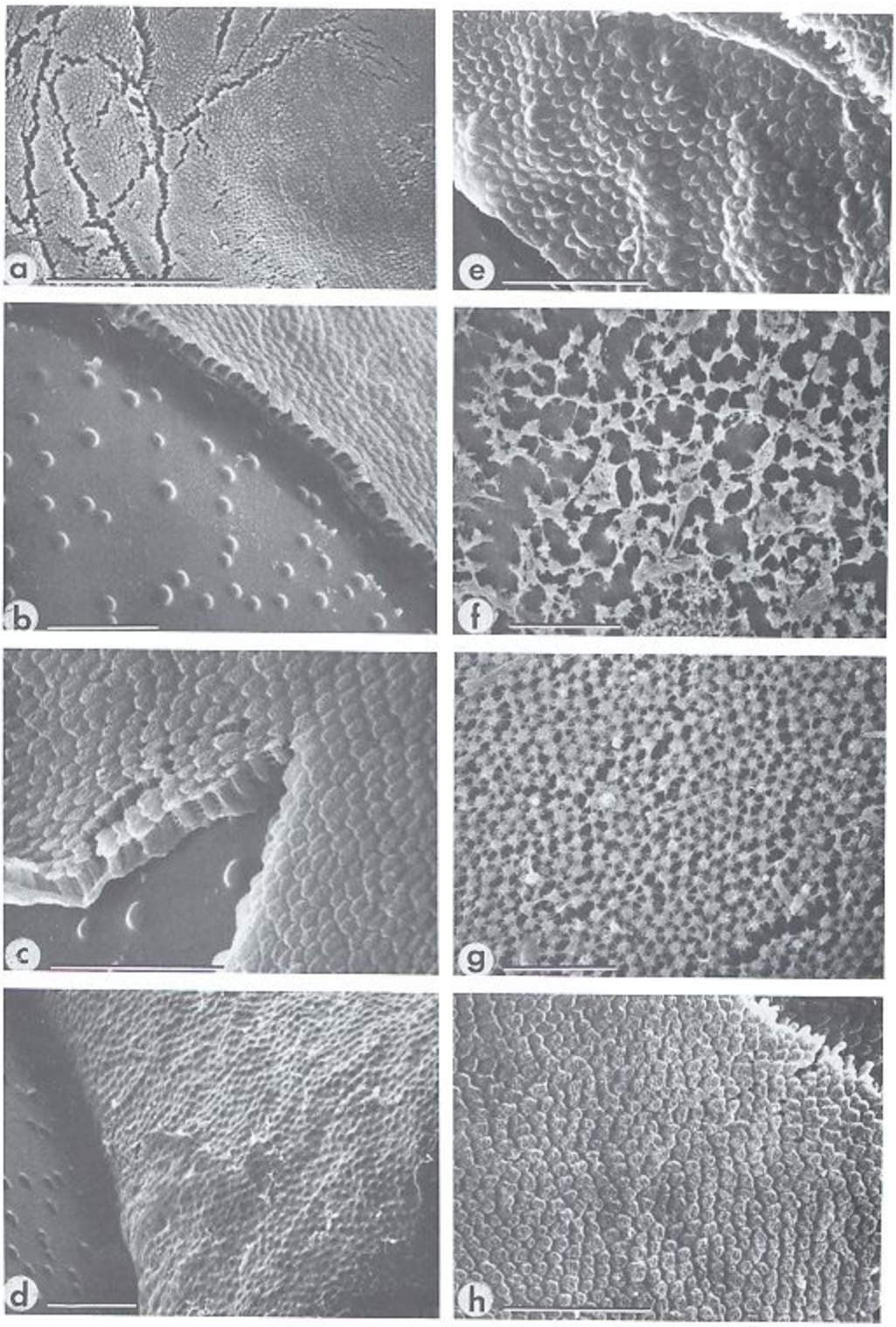

Fig. 3. (a) Outer chorion surface, with tubercles tightly packed, cracks showing pillarlike structure of tubercles. (b) Surface of inner chorion (bottom left), with torn edge of outer chorion showing pillarlike tubercles and outer chorion surface (upper right). (c) Extreme detail of torn edge of outer chorion, showing struts connecting pillars. (d) Inner chorion (left), with outer chorion (right) folded back to reveal structure of the inner side of its basal surface. (e) Outer surface of basal layer of outer chorion. (f) Very loosely packed tubercles in outer chorion at anterior end of egg. (g) Moderately closely packed tubercles in outer chorion near anterior end of egg. (h) Detail, typical closely packed surface of outer chorion. Scale = 2 μm (a), 5 μm (b–h).

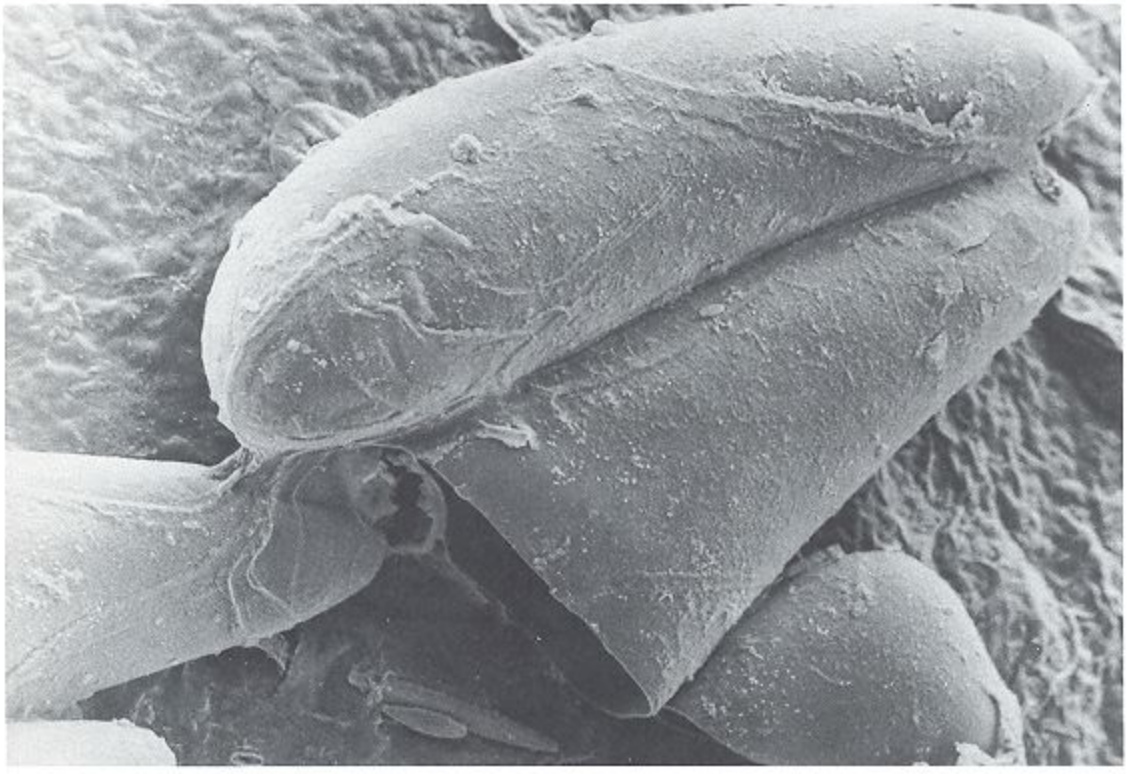

Fig. 4. Low-magnification view of unhatched (above) and hatched (below) eggs showing loosely attached outer chorion in the form of a continuous sheet connecting adjacent eggs. Length of egg = ≈350 μm.

never have lateral floats. (4) Eggs of *Anopheles* spp. have lateral floats and are oviposited singly.

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