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ULTRASTRUCTURE OF THE EGGS OF *CULICOIDES CIRCUMSCRIPTUS*, *CULICOIDES GEJGELENSIS*, AND *CULICOIDES IMICOLA* (DIPTERA: CERATOPOGONIDAE)[1]

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ABSTRACT. The eggs of *Culicoides circumscriptus* Kieffer, *Culicoides gejgelensis* Dzhafarov, and *Culicoides imicola* Kieffer collected in Israel are described and illustrated by scanning electron micrographs. Eggs of all 3 species were morphologically similar, generally sausage- or cigar-shaped, with slight dorsal–ventral curvature and longitudinal rows of tubercle pillars covered with a thin adhesive layer. Tubercle pillars were scarce on *C. circumscriptus* and *C. imicola* eggs, but were common on *C. gejgelensis* eggs, forming longitudinal plastrons with an associated hydrofuge meshwork. All 3 species had micropyle domes and associated aeropyles at the anterior end of their eggs.

INTRODUCTION

Despite the worldwide abundance and importance of *Culicoides* species, little has been reported on their egg ultrastructure. Some of the descriptive efforts from the early 20th century dealing with *Culicoides* egg morphology and oviposition behavior are reviewed by Hinton (1981) in a short paragraph summarizing the Ceratopogonidae. Little effort has been made recently to use the scanning electron microscope (SEM) to study the ultrastructure of *Culicoides* eggs. Campbell and Kettle (1975) used light and SEM microscopy to describe eggs of *Culicoides brevitarsis* Kieffer before and after oviposition. Nunamaker et al. (1987) used SEM techniques to describe and illustrate the ultrastructure of eggs of *Culicoides variipennis* (Coquillett).

We were able to obtain eggs from 3 *Culicoides* species in Israel. The eggs were examined ultrastructurally and their morphology is described herein on the basis of scanning electron micrographs.

MATERIALS AND METHODS

All eggs were obtained from field-collected gravid females captured in black light suction DuToit (1944) traps hung in a cattle shed at the Volcani Center experimental dairy farm, Bet Dagan (32°00′N, 34°49′E), Israel. Females of *Culicoides circumscriptus* Kieffer were collected during July, August, and December 1992. Females of *Culicoides imicola* Kieffer were collected during July, August, and September 1992, and females of *Culicoides gejgelensis* Dzhafarov were collected during July 1992.

Individual gravid females oviposited egg clusters in the laboratory on wet filter paper. At least 6 egg clusters were collected for each species. Eggs were allowed to embryonate and then preserved in alcoholic Bouin's fixative (80% ethanol [150 ml], concentrated formalin [60 ml], glacial acetic acid [15 ml], picric acid crystals [1 g]) and mailed to the Florida Medical Entomology Laboratory in Vero Beach, FL. There they were placed in small petri dishes, washed twice in 80% ethanol to remove picric acid, dehydrated through a graded ethanol series, and critical-point dried. Specimens were then placed on SEM stubs covered with sticky tape, final dried over calcium chloride for 10 min, and sputter coated with gold/palladium before immediate examination in a Hitachi S-510 SEM (Rockville, MD). Approximately 30 eggs from each species were examined by SEM.

Measurements were made from micrographs first transformed into computer image files with a Hewlett Packard ScanJet IIP (Boise, ID) scanner, then imported into Sigma Scan/Image software (vers. 3.90, Jandel Scientific, San Rafael, CA). Eggs of *C. circumscriptus* and *C. gejgelensis* were selected randomly for measurement from micrographs of egg clusters. Eggs of *C. imicola* were selected randomly for measurement directly on the SEM screen. All eggs were generally boat-shaped in lateral view with a convex dorsal surface. The posterior end was more tapered than the anterior end. The terminology used in descriptions follows Hinton (1981) and Nunamaker et al. (1987). An additional term, "micropylar dome" is defined by Linley et al. (1991).

DESCRIPTIONS

Culicoides circumscriptus
(Figs. 1 and 2)

Size and overall appearance. Thinly sausage-shaped with a slight dorsal–ventral curve beginning

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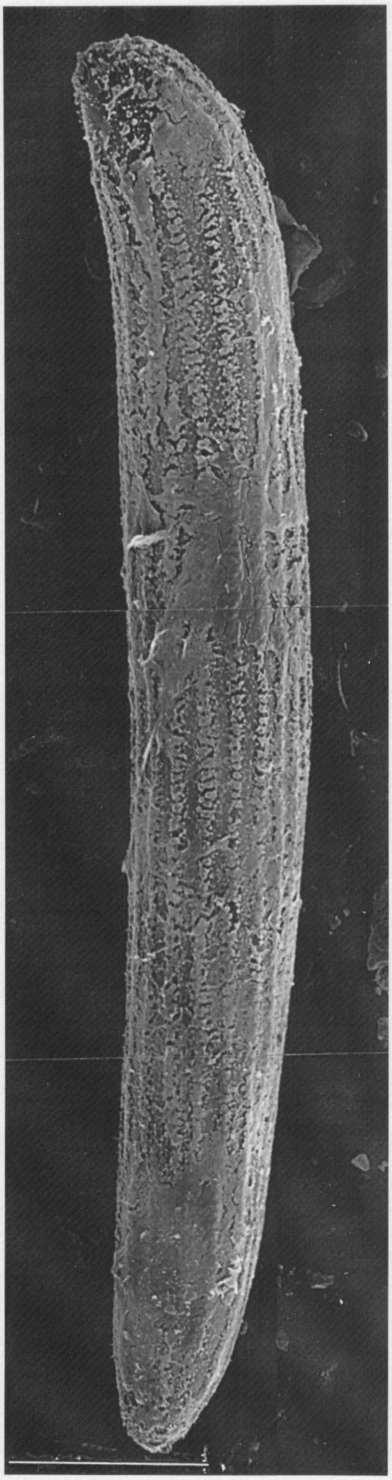

Fig. 1. Egg of *Culicoides circumscriptus.* Lateral view of entire egg, anterior end at top, ventral surface to the left. Scale, 50 µm.

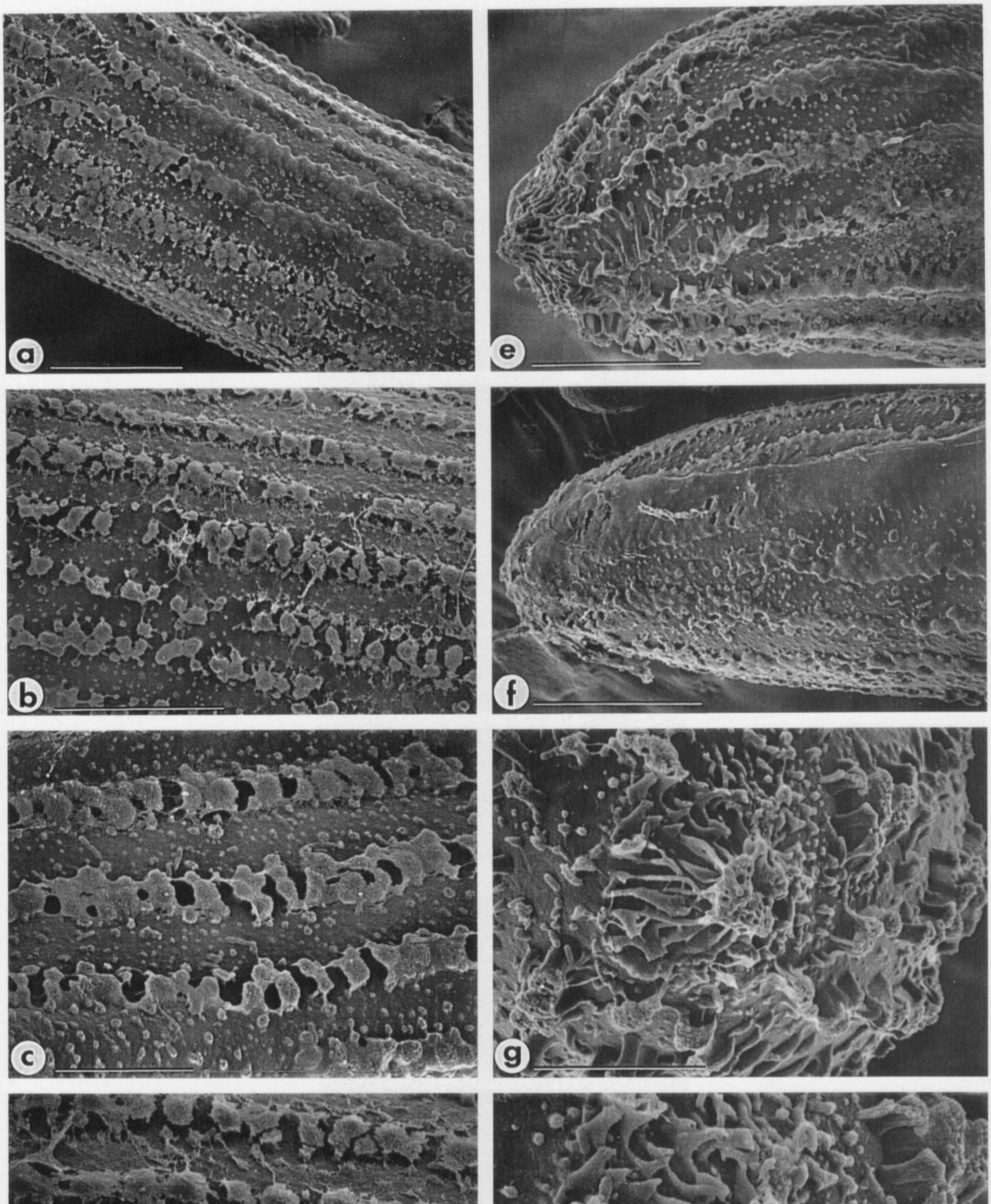

Fig. 2. Egg of *Culicoides circumscriptus.* a. Ventral posterior surface showing lines of adhesive along surface of large tubercles. b. Detail, ventral posterior surface without adhesive. c. Ventral chorion, anterior of midline showing lateral lines of large tubercles with small tubercles on the chorion in between. d. Detail, ventral chorion without adhesive. e. Anterior end. f. Posterior end showing thick longitudinal layers of adhesive. g. Detail, anterior micropylar dome. h. Detail, anterior micropylar dome showing aeropyle openings. Scale, 20 μm (a, b, e, f), 10 μm (c, d, g, h).

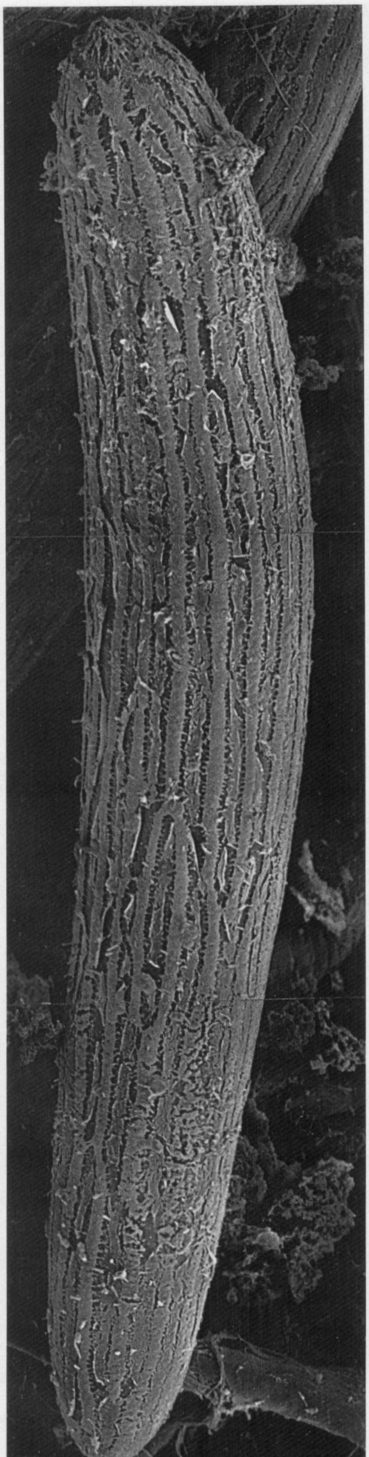

Fig. 3. Egg of *Culicoides gejgelensis*. Lateral view of entire egg, anterior end at top, ventral surface to the left. Scale, 50 μm.

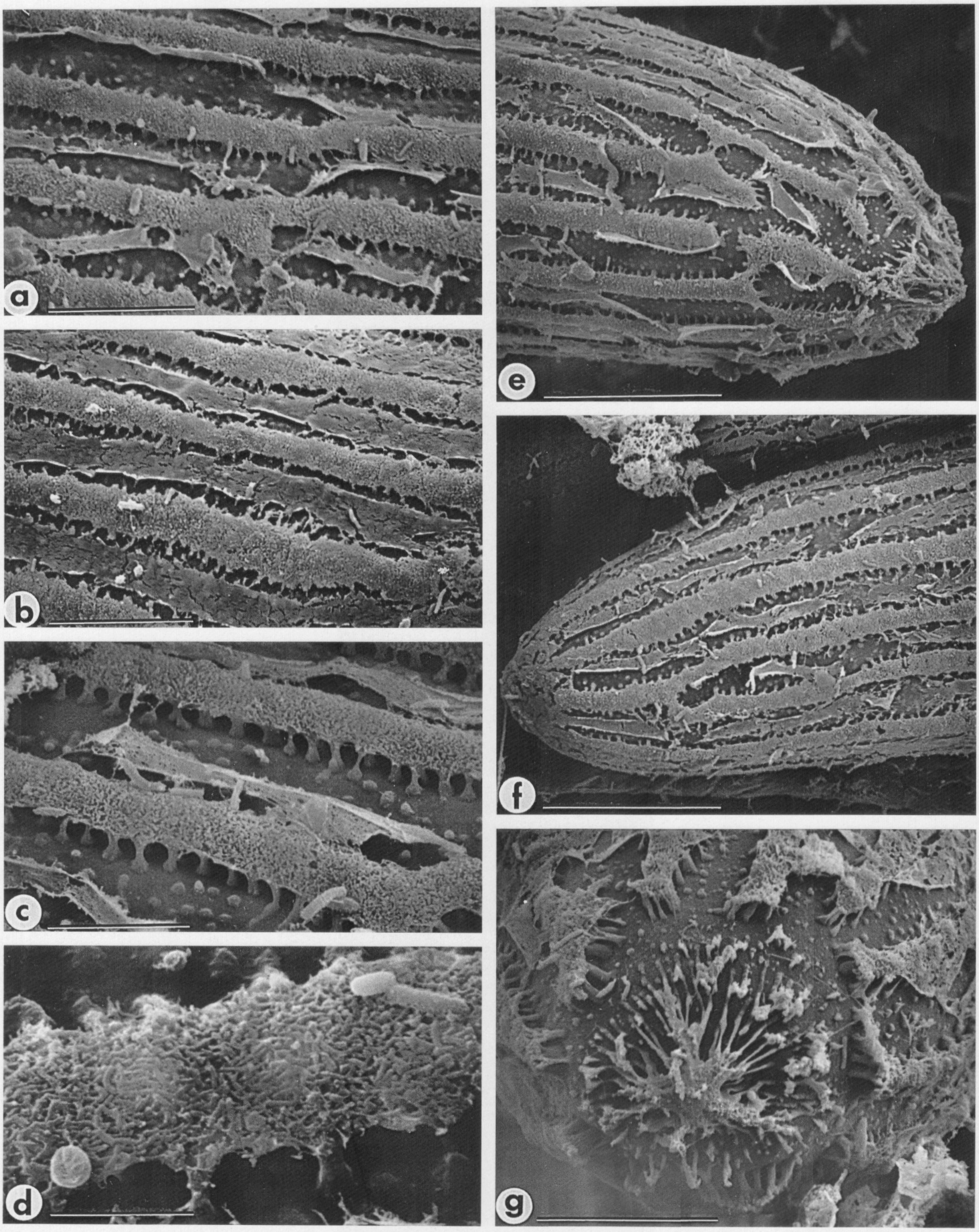

Fig. 4. Egg of *Culicoides gejgelensis.* a. Chorion showing longitudinal tubercle tracks topped by hydrofuge hairs. b. Chorion showing longitudinal tubercles with layers of adhesive in between. c. Tubercle pillars topped by networks of hydrofuge hairs. d. Detail, longitudinal tubercle strip showing hydrofuge meshworks. e. Anterior end. f. Posterior end. g. Detail, anterior micropylar dome with aeropyle openings. Scale, 200 μm (e, f), 10 μm (a, b, g), 5 μm (c), 2 μm (d).

Fig. 5. Egg of *Culicoides imicola.* Lateral view of entire egg, anterior end at top, ventral surface to the right. Scale, 50 μm.

in the anterior third, dimensions as in Table 1. Surface with longitudinal rows of large tubercles covered with a thin layer of adhesive (Figs. 1 and 2a–2d).

Chorion. Covered with symmetrical longitudinal rows of large tubercles (Figs. 1 and 2a–2e) covered uniformly with a thin layer of adhesive particularly evident at the posterior end where adjacent tubercle rows are connected by a thin layer of adhesive (Figs. 1 and 2f) and small tubercles are present between the longitudinal rows.

Anterior and posterior ends. Anterior micropylar dome with multiple aeropyle openings (Figs. 2e, 2g, 2h). The surface at both ends with a thin coating of adhesive.

Culicoides gejgelensis
(Figs. 3 and 4)

Size and overall appearance. Sausage-shaped with a slight dorsal–ventral curve beginning anterior of midline, dimensions as in Table 1. Surface with tubercle pillars, some covered with a thin layer of adhesive (Figs. 3 and 4a–4d).

Chorion. Covered with numerous symmetrical longitudinal rows of tubercle pillars (Figs. 3 and 4a–4f) topped by hydrofuge hairs forming plastrons (Fig. 4c). Covered uniformly with a dense hydrofuge meshwork forming plastrons (Figs. 4c, 4d). Between tubercle rows, thin layers of adhesive were lifted from the surface (Figs. 3, 4a, 4c, 4e, 4f).

Anterior and posterior ends. Anterior micropylar dome with multiple aeropyle openings (Figs. 4e, 4g).

Culicoides imicola
(Figs. 5 and 6)

Size and overall appearance. Cigar-shaped with a slight dorsal–ventral curve beginning anterior of the egg midline, dimensions as in Table 1. Surface with sparsely spaced circular tubercles, many covered with a thin layer of adhesive (Figs. 5 and 6a–6f).

Chorion. Covered with symmetrical longitudinal rows of sparsely spaced circular tubercle pillars (Figs. 5 and 6a–6f). Pillars particularly evident around micropylar dome (Figs. 6g, 6h). Stretching over the pillars and between the tubercle rows is a thin layer of adhesive that appears to crack and peel away from the surface between the tubercle rows (Figs. 5 and 6b, 6d). Smaller tubercles appear on the chorion between the large tubercle rows (Figs. 6b, 6c).

Anterior and posterior ends. Small anterior micropylar dome with aeropyle openings (Figs. 6e, 6g, 6h).

DISCUSSION

Eggs of the three *Culicoides* species described herein were morphologically similar. It was our hope that egg ultrastructure and morphology could

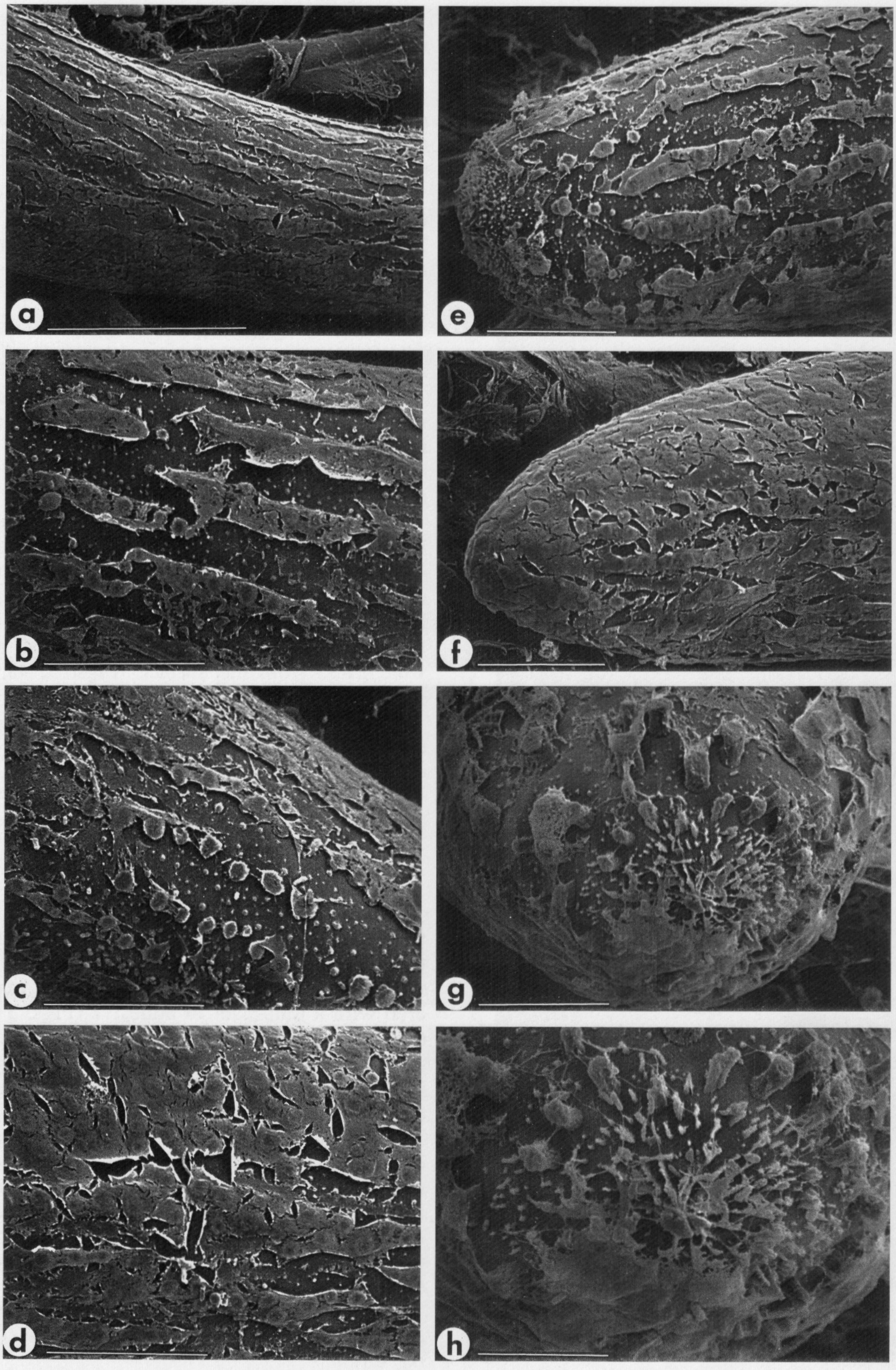

Fig. 6. Egg of *Culicoides imicola*. a. Ventral posterior surface showing lateral tubercle rows covered by adhesive. b. Open chorion, anterior third of egg showing tubercle pillars covered by adhesive. c. Open chorion showing large tubercle rows with small tubercles in between. d. Middle of egg showing cracked layer of adhesive. e. Anterior end. f. Posterior end. g. Anterior micropylar dome. h. Detail, anterior micropylar dome. Scale 50 μm (a), 20 μm (b–f), 10 μm (g, h).

Table 1. Dimensions (in μm) of the eggs of 3 species of *Culicoides*.

Species (number)	Length		Width		Length to width ratio	
	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
Culicoides circumscriptus (10)	402.6 (2.8)	384.4–412.6	54.4 (1.2)	47.5–60.1	7.4 (0.2)	6.8–8.6
Culicoides gejgelensis (11)	321.3 (4.6)	284.9–340.6	47.0 (1.1)	41.0–52.4	6.9 (0.1)	6.1–7.5
Culicoides imicola (10)	363.1 (5.5)	334.3–391.4	65.2 (0.7)	62.9–68.6	5.6 (0.07)	5.3–6.0

be used to separate *Culicoides* species and perhaps even species complexes, such as those belonging to the *C. imicola* complex. Such techniques have been used successfully to separate mosquitoes of the *Anopheles quadrimaculatus* (Say) complex (Linley et al. 1993a) and geographic populations of *Anopheles aquasalis* Curry (Linley et al. 1993b). However, it is evident from the micrographs produced here and elsewhere (Campbell and Kettle 1975, Nunamaker et al. 1987), that egg morphology and ultrastructure may not be useful for the accurate identification and separation of most *Culicoides* species.

The ultrastructure of *C. circumscriptus* and *C. imicola* eggs was indistinguishable. However, on *C. gejgelensis* eggs, tubercle pillars were common along longitudinal tracts. These pillars were topped with a dense hydrofuge meshwork that likely trapped air along the surface of the egg. Eggs of *C. gejgelensis* are oviposited in a wetter environment than those of *C. circumscriptus* or *C. imicola,* both of which oviposit in damp organic soil and are adapted to dry breeding sites (Braverman et al. 1974). *Culicoides gejgelensis* females oviposit in standing water associated with mountain rivers and marshes (Glukhova 1989). The plastron networks found along the surface of *C. gejgelensis* eggs are likely to be an important respiratory or hydrostatic adaptation that allows this species to oviposit directly into water.

Eggs of all three species were covered with a thin layer of adhesive. The eggs of many *Culicoides* species are laid in clutches of 10 to several hundred (Hinton 1981). *Culicoides circumscriptus* females lay clutches of 45 to 401 eggs (Becker 1960) and *C. imicola* lays about 60 eggs per clutch (Braverman and Linley 1994). Eggs are generally coated with a jellylike spumaline that is believed to cement the eggs together and to the substrate (Hinton 1981). Scanning electron micrographs of *C. circumscriptus* egg masses made for our study, but not included in the present manuscript, show eggs cemented together along the dorsal–ventral curvature of the egg. It is evident from the SEM micrographs published here that the layer of adhesive is stretched over the tubercle pillars to totally encase the egg and produce an air pocket between the longitudinal tubercle rows and the chorion surface. This may provide an important means of respiration for eggs laid in wet environments or for eggs that are flooded by rain or dew.

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