Comparative Morphology of Epithemata (Polar Chorionic Structures) in the Eggs of *Ephemerella ignita* (Ephemeroptera: Ephemerellidae)[1]

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Abstract. The eggs of the mayfly *Ephemerella ignita* (Ephemeroptera: Ephemerellidae) are characterized by a polar adhesive structure, the epithema. The epithema prevents egg drift following deposition in water by extending a network of threads. Mated females lay an aggregate of eggs that is attached to the last abdominal sternites. Within the aggregate, eggs with differently shaped epithemata are arranged in a well-defined pattern. Three types of eggs were defined by three types of epithemata, namely short, bulging, and long. In addition, histological observations confirmed the thread-like composition of the epithemata and their peculiar arrangement in each type. Short and long epithema egg types differed also by their total lengths. Structural changes in the architecture of the epithemata are discussed in relation to a different function of such devices.

The fine structure of the egg chorionic pattern in Ephemeroptera has been used, as with other insect orders, to elucidate differences and relationships among species (Alba-Tercedor & Sowa, 1987; Gaino et al., 1987, 1989; Gaino & Mazzini, 1984; Mazzini & Gaino, 1990). In addition, ultrastructural studies on mayfly eggs have indicated the adhesive function of their peculiar projections, which may be involved in preventing egg drift following deposition in water (Gaino & Mazzini, 1987, 1988). As a consequence, shell surface structures may give some insight into the devices developed by different species to survive in their respective habitats (Gaino & Mazzini, 1988).

Ootaxonomy is based chiefly on the species-specificity of chorionic architecture, which is constant within the representatives of a species. For instance, the organization of the mayfly egg has been employed to classify the order and to recognize nymph and adult stages in the same species (Koss, 1968).

The polar devices, referred to as the "epithema" (this term having been introduced first by Bengtsson, 1913) or "polar cap" (terminology after Koss & Edmunds, 1974), is a unique chorionic structure in eggs of *Ephemerella ignita* Poda at the posterior polar region and is involved in egg adhesion to the substratum.

The occurrence of eggs with adhesive polar devices of different lengths in *E. ignita* were noted earlier and were considered to be related to the position of the eggs within the ducts (Bengtsson, 1913; Degrange, 1960). This observation might have relevant implications in the field of ootaxonomy and may imply additional functions.

The primary objective of this paper is to describe and document the ultra-

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structural features of the eggs of *E. ignita* with emphasis on variations among epithemata.

Materials and Methods

Adult females of *Ephemerella ignita* involved in oviposition were collected in June 1990 along the Berlino stream near Rossiglione, Genova, at approximately 300 m elevation. Ovipositing females were identified by the green aggregate of eggs adhering to the abdomen.

Eggs were fixed in Karnovsky's (1965) fixative and rinsed in sodium cacodylate trihydrate buffer. For histological examination, eggs were dehydrated in a graded series of ethanol and embedded in metacrylate resin (JB4). Sections were stained with toluidine blue. For scanning electron microscopic (SEM) examination, selected material was dehydrated following the above-described procedure for histological study, critical-point dried using liquid CO_2 in a Bomar apparatus, attached to specimen holders by silver-conducting paint, and coated with gold-palladium in a Balzer Union evaporator. Specimens were observed with a Philips EM 505 scanning electron microscope.

The dimensions, including epithemata, were measured in a sample of 120 eggs, subdivided into three groups of 40 eggs each, according to the morphology of their epithemata. Data given are means from each group of 40 eggs.

Results

Mated females of *Ephemerella ignita* lay aggregates of eggs that are visible to the naked eye. Eggs exit the single gonopore located in the middle portion of the posterior edge of sternite 7 and aggregate. The eggs aggregate in a depression formed by flattened sternites 8 and 9 and anterior flexion of the last abdominal segments (Fig. 1). The aggregate consists of approximately 600 eggs, which are arranged differently according to their position (Fig. 1). The eggs along the exterior margin are arranged side by side to form a uniform layer. Those within the aggregate are arranged less compactly. Microscopically, the eggs differ essentially by the shape of their respective epithemata (Fig. 1A–C).

Three different categories of eggs may be recognized. Each category is positioned in a well-defined sequence, from the surface of the aggregate to its innermost region (Fig. 1). The tightly packed eggs near the periphery each have an elongated epithema (Fig. 1C) that is triangular with its major base adhering to the chorion. A second category of eggs occurs in the underneath portion of the aggregate. They are characterized by a bulging area at the top of the epithema (Fig. 1B). A third category of eggs is characterized by short epithemata, with reduced distance between the base and its roundish (Fig. $1A_1$) or flattened (Fig. $1A_2$) apex. This third category of eggs is found intermingled with the second category and at the core of the aggregate.

Eggs with Short Epithemata

Eggs in this category are the most common type in *E. ignita* (Fig. 2). They measure 213 μm in length and 132 μm in width on average and have a polar epithema of 37 μm in height. The surface of the chorion is granular with

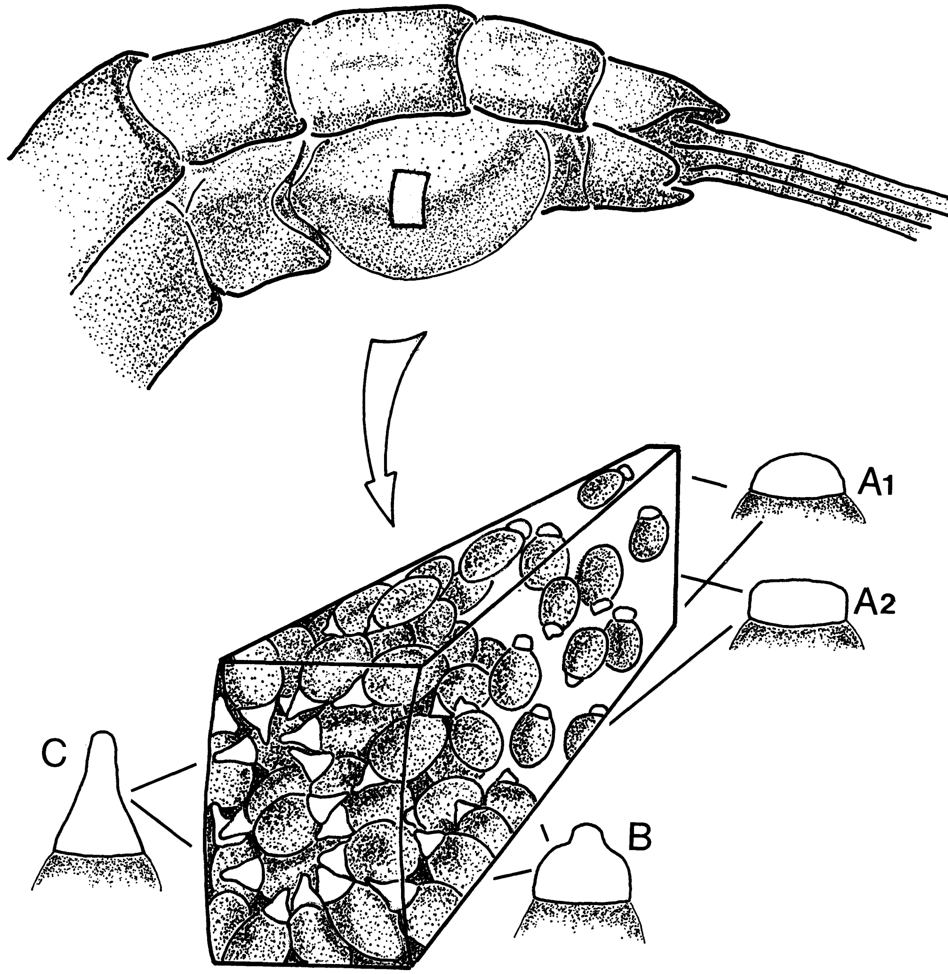
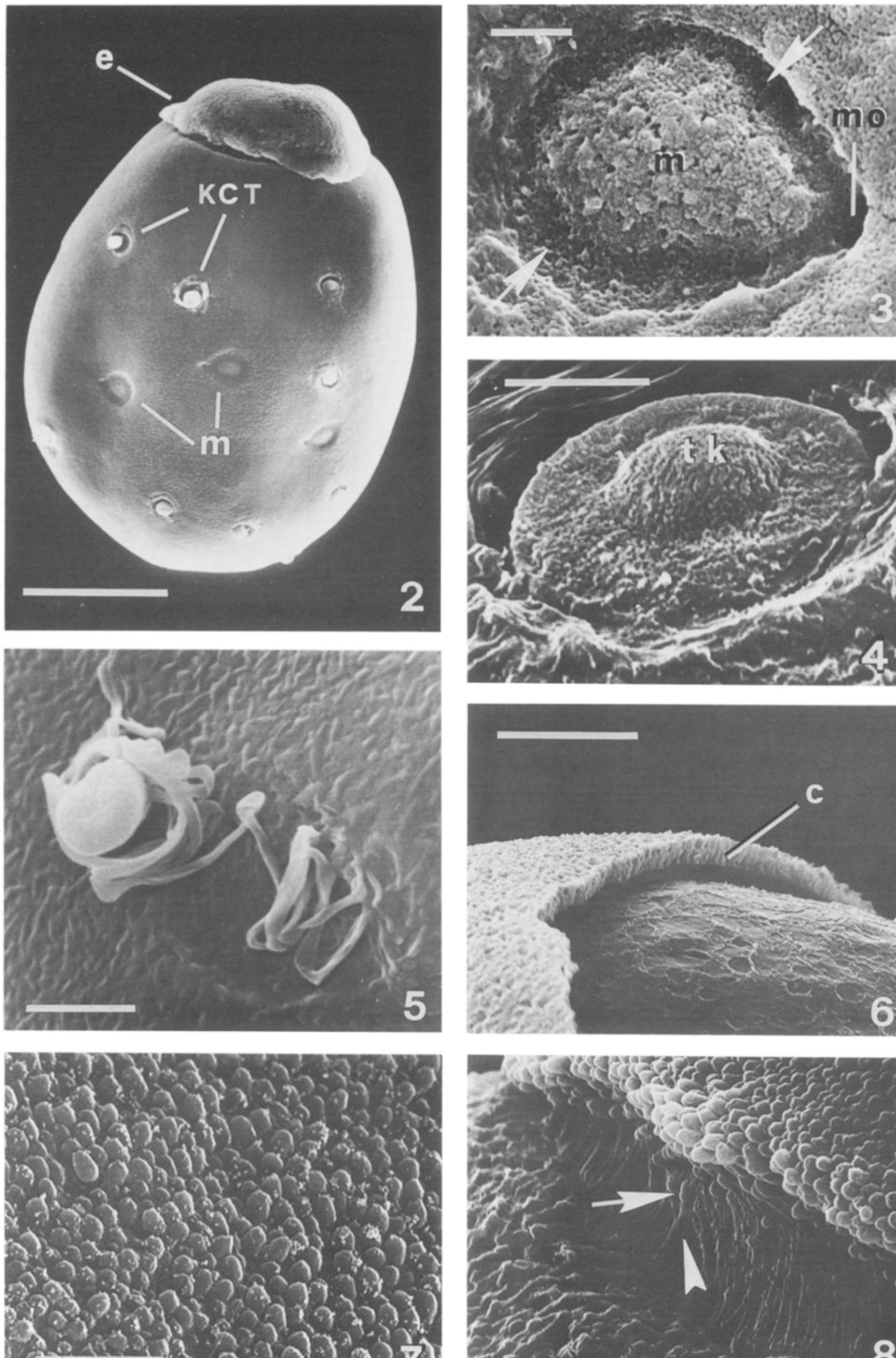
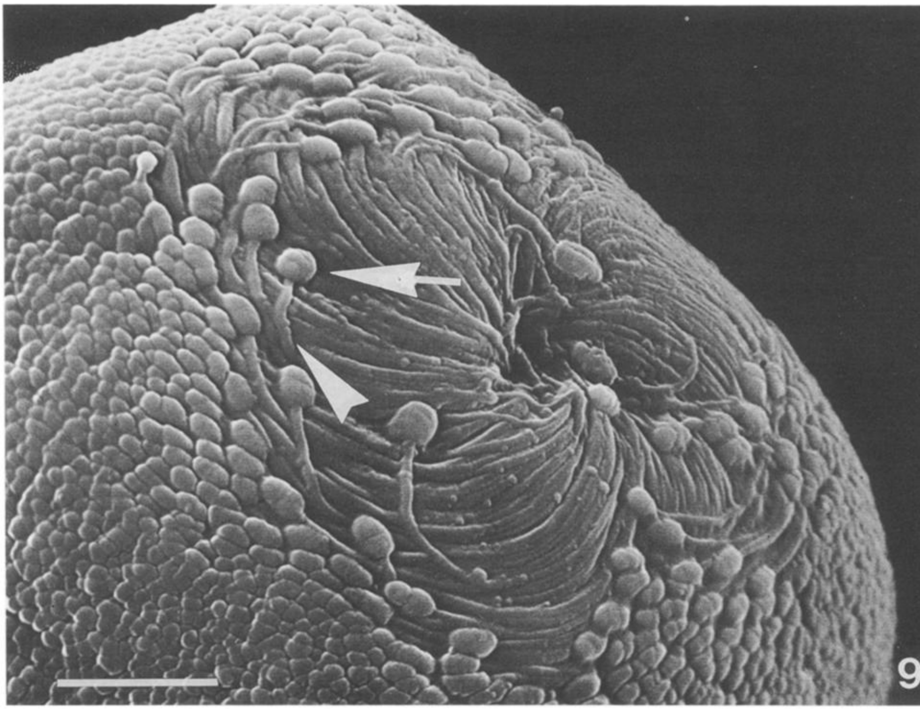


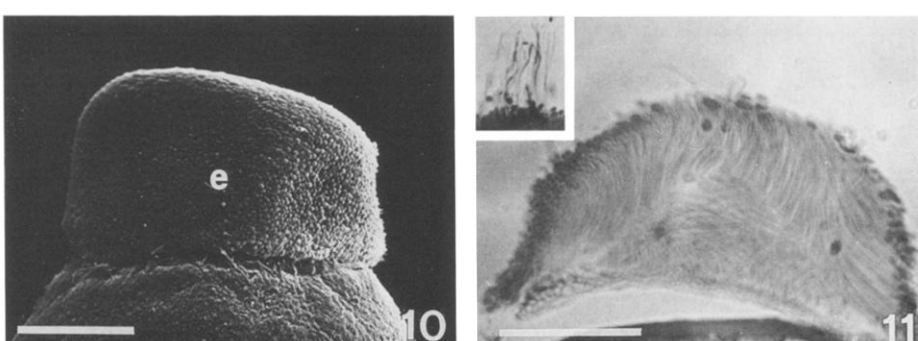
FIG. 1. Posterior abdomen of *Ephemerella ignita*. Note the egg aggregate at sternites 8 and 9. The aggregate is detailed in the inset (indicated by arrow) to show the egg arrangement from the outer surface inward. At the egg pole, a chorionic differentiation or epithema (white apical part of each egg) is shown. Epithemata differ by egg position in the aggregate and their structure (A, B, C). The distinction between A_1 and A_2 lies in the different shapes of the apical parts of the epithemata.

micropyles and scattered adhesive structures (Fig. 2). Four or more tagenoform micropyles (terminology after Koss & Edmunds, 1974) generally are located in the equatorial or subequatorial area (Fig. 2). They consist of an oval sperm guide and a micropylar opening that leads to the canal and is situated on one side (Fig. 3). The sperm guide is surrounded by a peripheral chorionic depression (Fig. 3). Adhesive structures are sparse and consist of fibrous coiled threads ending in a knob (KCTs, according to Koss & Edmunds, 1974). These attachment devices (Fig. 4) are lodged in a depression of the chorion that becomes visible when the threads extend outside (Fig. 5). The chorion is 2 μm



FIGS. 2–8. Chorionic pattern of eggs with short epithemata in *Ephemerella ignita*. Fig. 2. Overview of the egg with polar epithema (e), knob-terminated coiled threads (KCT), and micropyles





FIGS. 9–11. Chorionic pattern of eggs with short epithemata. Fig. 9. The apical part of the epithema showing the coiled configuration of the threads (arrowhead) with their terminal granules (arrow). Scale bar represents 10 μm. Fig. 10. Lateral view of the epithema (e) showing its cylindrical shape. Scale bar represents 25 μm. Fig. 11. A section of the epithema showing its network of threads with superficial extensions (inset). Scale bar represents 25 μm.

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(m). Scale bar represents 50 μm. Fig. 3. Micropyle (m) with oval sperm guide delimited by a chorionic depression (arrows) and micropylar opening (mo). Scale bar represents 2 μm. Fig. 4. Detail of the terminal knob (tk) of a KCT in its coiled configuration. Scale bar represents 2 μm. Fig. 5. A KCT in its extended configuration. Scale bar represents 5 μm. Fig. 6. The egg chorion (c) has been broken to show its thickness. Scale bar represents 10 μm. Fig. 7. Detail of the outermost surface of the epithema showing the arrangement of the granules. Scale bar represents 5 μm. Fig. 8. The basal part of the epithema showing some threads (arrowheads) each ending in a granule (arrows). Scale bar represents 5 μm.

thick (Fig. 6). The posterior pole of the egg is marked by the epithema that appears as a relatively solid cap, with a blunt (Fig. 2) or flat (Fig. 10) apex having a granular surface (Fig. 7). The composition of the epithema is evident at its base where numerous threads abutting from the chorion end in distinct granules about 2.5 μm in diameter (Fig. 8). Therefore, the pattern of the granular units reflects the arrangement of the terminal part of each thread on the surface of the epithema (Fig. 8). At the apex of the epithema, the threads become coiled with a gradual reduction in number of the terminal granules (Fig. 9). The same organization is found in apically flattened epithemata with a cylindrical shape (Fig. 10).

Histological sections of the epithema reveal a compact structure, attributable to the abundance and tight arrangement of the threads (Fig. 11). Thread extension (inset, Fig. 11) supports the view that the epithema has adhesive functions.

Eggs with Bulged Epithemata

Eggs in this category are 242 μm long and 132 μm wide on average and possess a chorionic pattern similar to those with short epithemata. The epithema is about 69 μm in height and ends in an apical bulge (Fig. 12). Thread components with distinct granules arranged on the surface are visible only at the base of the epithema (Fig. 13); the remainder of the surface reveals a sequence of ribs running toward the apex (Fig. 14); at the terminal bulge, the ribs are more compact (Fig. 14).

Histological sections confirm the basic organization of the threads as described above; with their lack of polar coiling, their tendency to fuse, and their tendency to be enclosed in an amorphous chorionic matrix (Fig. 15).

Eggs with Long Epithemata

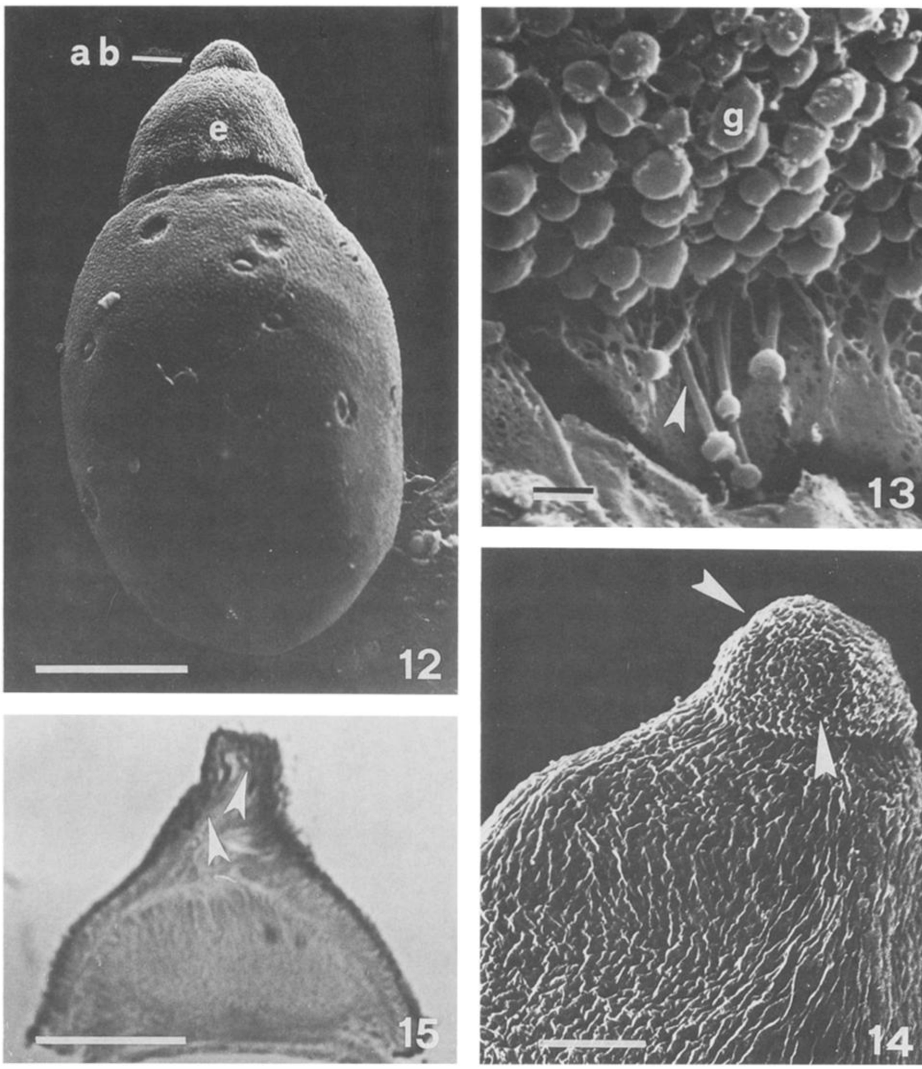
These eggs, 294 μm long and 137 μm wide on average, differ markedly from the two categories described above by length (104 μm) and organization of their epithemata (Fig. 16).

The threads tend to cluster and to form a polymorphic aggregate by fusing with the terminal granules (Fig. 17). Toward the apical part of the epithema, the granules are covered partially by a more finely granular substance (Fig. 18) and are incorporated into the chorion where they are arranged in linear sequence (Fig. 19). At the apex of the epithema, the surface is characterized by chorionic protrusions (Fig. 20).

In histological sections, the innermost core consists of an amorphous matrix with peripheral threads arranged side-by-side and fused apically to achieve a continuous border (Fig. 21).

Eggs with short epithemata constitute the most common category (60% of the total), followed by eggs with long epithemata (30%) and eggs with bulging epithemata (10%).

Egg types with short and long epithemata differ significantly by their length ranges, namely 190–240 μm and 250–350 μm, respectively. Eggs with bulged epithemata were of intermediate length: 200–280 μm (Fig. 22).

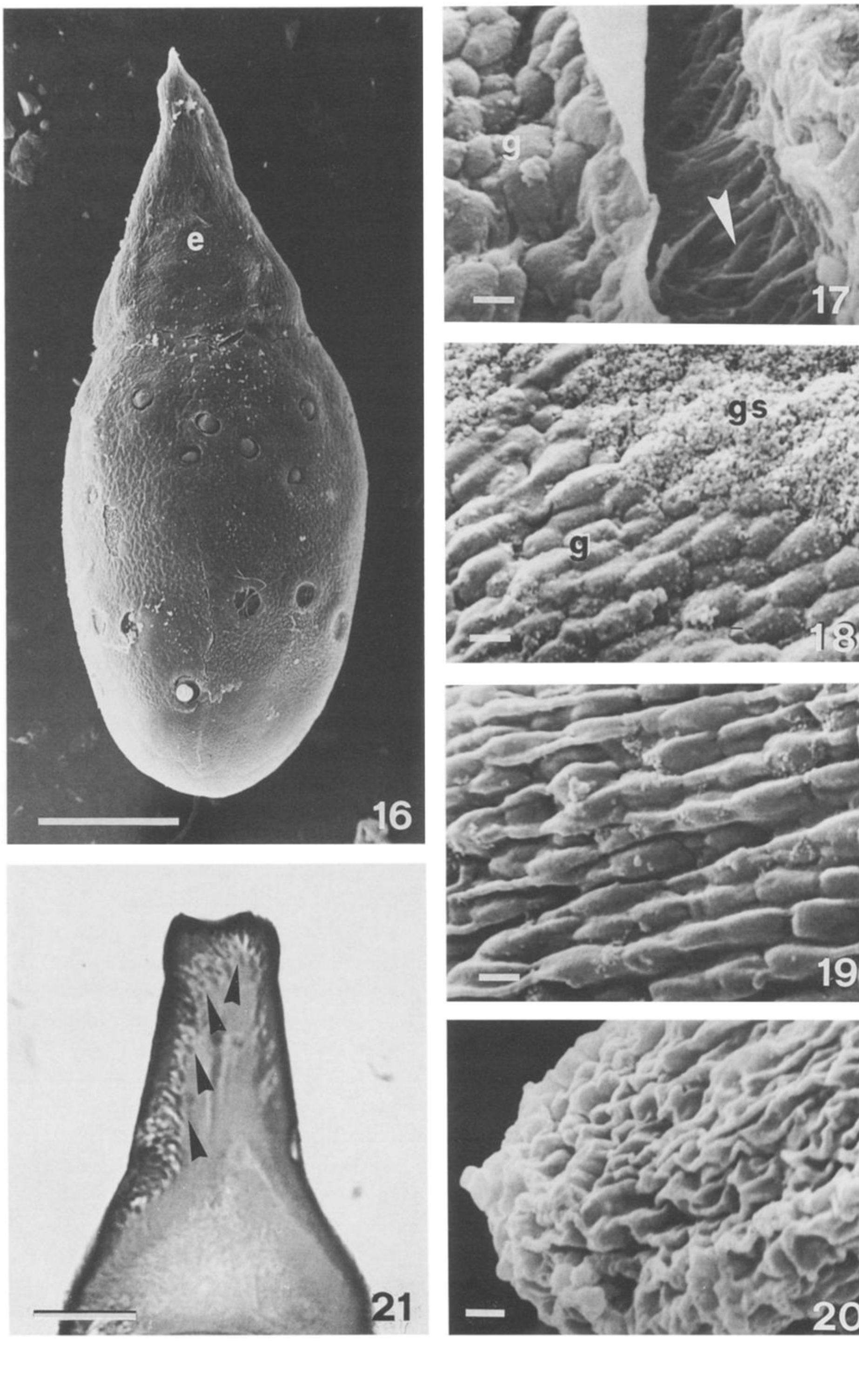


FIGS. 12–15. Chorionic pattern of eggs with bulged epithemata. Fig. 12. Overview of the egg with the apical bulge (ab) in the epithema (e). Scale bar represents 50 μm. Fig. 13. The basal region of the epithema shows the surface of the granules (g) with their threads (arrowheads). Scale bar represents 1 μm. Fig. 14. The rest of the epithema presents ribs more densely arranged in the apical bulge (arrowheads). Scale bar represents 10 μm. Fig. 15. A section of the epithema showing apical fusion of the threads (arrowheads). Scale bar represents 25 μm.

DISCUSSION

The variety of polar configuration in *Ephemerella ignita* constitutes a peculiar characteristic of this species and encourages caution in utilizing eggs for taxonomic purposes.

The occurrence of eggs with elongated epithemata has been reported previously and considered to be a result of egg location within the oviducts (Bengts-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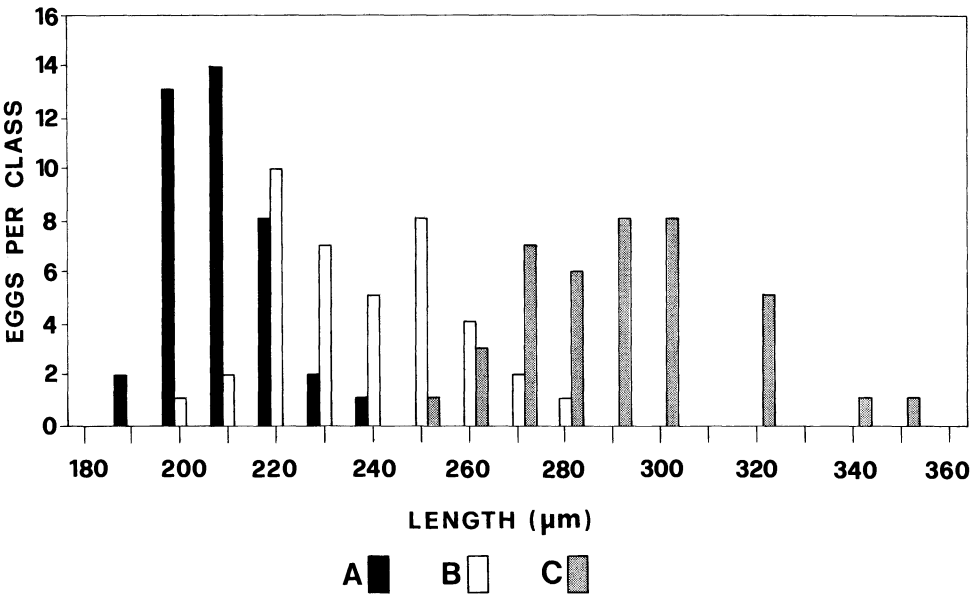

FIG. 22. Distribution of egg lengths among eggs having short epithemata (A), bulged epithemata (B), and long epithemata (C).

son, 1913; Degrange, 1960). Egg envelopes are elaborated by follicle cells and laid down in a well-defined sequence (Gaino & Mazzini, 1990; Mazzini & Gaino, 1990). We postulate that, as reported for several other insect species in which follicle cells gradually develop special regions of the chorion (Bilinski & Jankowska, 1987; Margaritis et al., 1980; Mazur et al., 1980), the polar position of the epithemata requires differentiation of follicle cells in this region. Differences in both shape and organization of the epithema are related to changes in chorionic synthesis in the ovarioles. This phenomenon might reflect reduced activity in elaboration and deposition of the classical thread component. These considerations suggest further that eggs with bulged and long epithemata, owing to the peripheral position of the latter in the aggregate, should represent the final product of the ovarioles. Following ovulation, these eggs, which differ from the classical model (with short epithemata) in the shape of their epithemata, are grouped compactly to form a peripheral layer enveloping the entire

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FIGS. 16–21. Chorionic pattern of eggs with long epithemata. Fig. 16. Overview of egg with epithema (e). Scale bar represents 50 µm. Figs. 17–20. Sequence illustrating the organization of the long epithema. Fig. 17. Basal region with threads (arrowhead) and partly fused granules (g). Scale bar represents 1 µm. Fig. 18. Granular substance (gs) partially covering the granules (g). Scale bar represents 1 µm. Fig. 19. Linear arrangement of interconnected granules. Scale bar represents 1 µm. Fig. 20. Apical part of the epithema with chorionic elaborations. Scale bar represents 1 µm. Fig. 21. Section of long epithema showing the peripheral border constituted of fused threads (arrowheads). Scale bar represents 25 µm.

mass. The small number of recognized categories might explain their absence in common descriptions of egg morphology for *E. ignita*.

The adhesive function of the epithema has been described previously (Degrange, 1960; Koss & Edmunds, 1974). Despite its compact appearance, the cap of threads swells in water and exposes the adhesive elements that attach the eggs to the substratum. Their activity is strengthened by the few scattered knob-terminating coiled threads.

Attachment structures of mayflies prevent egg drift following deposition. The unique arrangement of such devices with different structure and position on the shell surface (Gaino & Mazzini, 1987, 1988) reflects an adaptive mechanism for many mayfly species.

Scanning electron microscopy (SEM) analysis of the eggs of *E. ignita* revealed that differences in epithema length were related to structural organization that probably reflects a different adhesive function. In short epithema eggs, the numerous threads shift toward the extended configuration; in the other categories, thread components tend to cluster peripherally. This latter condition seems to be static, thus indicating that eggs with bulged and long epithemata play a protective role by holding the egg mass together.

According to Koss & Edmunds (1974), the polar cap of Ephemerellidae is constituted of uncoiled threads. By contrast, SEM studies indicated that short epithemata arise from threads collectively coiled about the pole of the egg. Such a feature is similar to that of the polar cap of *Caenis* as described in an SEM study by Malzacher (1982). This observation emphasizes the difficulties of categorizing such attachment structures without adequate supporting light and ultrastructural analysis. Occurrence of polar caps at one or both poles, as described for several groups of Ephemeroptera, may indicate convergent character states related to adaptive advantages of developing various adhesive structures for survival.

Literature Cited

Alba-Tercedor, J. & Sowa, R. 1987. New representatives of the *Rhithrogena diaphana*-group from continental Europe, with a redescription of *R. diaphana* Navás, 1917 (Ephemeroptera: Heptageniidae). *Aquat. Insects*, 9: 65–83.

Bengtsson, S. 1913. Undersökningar öfver äggen hos Ephemeriderna. *Entomol. Tidskr.*, 34: 271–320.

Biliński, S. M. & Jankowska, W. 1987. Oogenesis in the bird louse *Eomenacanthus stramineus* (Insecta, Mallophaga) I. General description and structure of the egg capsule. *Zool. Jb. Anat.*, 116: 1–12.

Degrange, C. 1960. Recherches sur la reproduction des Ephéméroptères. *Trav. Lab. Hydrobiol. Piscic. Univ. Grenoble*, 51: 7–193.

Gaino, E. & Mazzini, M. 1984. Scanning electron microscope study of the eggs of some *Habrophlebia* and *Habroleptoides* species (Ephemeroptera, Leptophlebiidae). *In* Landa, V., Soldan, T. & Tonner, H., eds., *Proceedings of the 4th International Conference on Ephemeroptera, Bechyné*, Czechoslovak Academy of Sciences, Čecké Budějovice, pp. 193–202.

1987. Scanning electron microscopy of the egg attachment structures of *Electrogena zebrata* (Ephemeroptera: Heptageniidae). *Trans. Am. Microsc. Soc.*, 106: 114–119.

1988. Fine structure of the chorionic projections of the egg of *Rhithrogena kimminsi* Thomas (Ephemeroptera: Heptageniidae) and their role in egg adhesion. *Int. J. Insect Morphol. Embryol.*, 17: 113–120.

1990. Follicle cell activity in the ovarioles of *Habrophlebia eldae* (Ephemeroptera: Leptophlebiidae). *Trans. Am. Microsc. Soc.*, 109: 300–310.

GAINO, E., BELFIORE, C. & MAZZINI, M. 1987. Ootaxonomic investigation of the genus *Electrogena* (Ephemeroptera, Heptageniidae). *Boll. Zool.*, 54: 169–175.

GAINO, E., DEGRANGE, C., MAZZINI, M. & SOWA, R. 1989. Etude en microscopie à balayage des oeufs de quelques espèces de *Rhithrogena* Eaton groupe *alpestris* (Ephemeroptera, Heptageniidae). *Vie Milieu*, 39: 219–229.

KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.*, 27: 137A–138A.

KOSS, R. W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. *Ann. Entomol. Soc. Am.*, 61: 696–721.

KOSS, R. W. & EDMUNDS, G. F. 1974. Ephemeroptera eggs and their contribution to phylogenetic studies of the order. *Zool. J. Linn. Soc.*, 55: 267–349.

MALZACHER, P. 1982. Eistrukturen europäischer Caenidae (Insecta, Ephemeroptera). *Stuttg. Beitr. Naturk. D. Ser. A (Biol.)*, 356: 1–15.

MARGARITIS, L. H., KAFATOS, F. C. & PETRI, W. H. 1980. The eggshell of *Drosophila melanogaster*. I. Fine structure of the layers and regions of the wild-type eggshell. *J. Cell Sci.*, 43: 1–35.

MAZUR, G. D., REGIER, J. C. & KAFATOS, F. C. 1980. The silkmoth chorion: morphogenesis of surface structures and its relation to synthesis of specific proteins. *Dev. Biol.*, 76: 305–321.

MAZZINI, M. & GAINO, E. 1990. Oogenesis and involvement of chorionic structures in ephemeropteran taxonomy. *In* Campbell, I. C., ed., *Mayflies and Stoneflies. Proceedings of the 5th International Ephemeroptera Conference and the 9th International Plecoptera Conference,* Kluwer Academic Publishers, Dordrecht, pp. 95–104.

Vancouver Meetings

The 1992 Annual Meeting of the American Microscopical Society (AMS) will be held with meetings of the American Society of Zoologists (ASZ), Animal Behavior Society, The Canadian Society of Zoologists, The Crustacean Society, and the International Association of Astacology at the Hyatt Regency and Four Seasons Hotels in Vancouver, B.C., Canada, during the traditional post-Christmas period, 27–30 December. Several symposia and workshops are planned. AMS will sponsor a workshop on low-cost image analysis for light microscopy in teaching and research. Socials, commercial exhibits, a job placement service, and a babysitting service also are planned.

The meetings are hosted by The University of British Columbia, with John Phillips and Geoffrey Scudder co-chairing the Local Committee. For more information contact: Laura Jungen, Smith, Bucklin & Associates, 401 North Michigan Avenue, Chicago, Illinois 60611-4267, Telephone (312) 644-6610, FAX (312) 321-6869; OR Julian P. S. Smith III, AMS Secretary, Department of Biology, Winthrop University, Rock Hill, South Carolina 29733, Telephone (803) 323-2111, FAX (803) 323-2347, BITNET: FBI00JPS@UNCCVM.