

OOGENESIS AND INVOLVEMENT OF CHORIONIC STRUCTURES IN EPHEMEROPTERAN TAXONOMY

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ABSTRACT

The ovarian development of *Habrophlebia eldae* Jacob & Sartori 1984 (Ephemeroptera: Leptophlebiidae) was examined ultrastructurally with a view to characterize the follicle cell-oocyte interaction in relation to vitellogenesis. The transition from the previtellogenic to the vitellogenic stage with differentiation of the follicular epithelium including its junctional system, and the development of the oocyte were observed. Postvitellogenic development entails deposition of egg envelopes as follows: first the vitelline layer, then the endochorion, the exochorion and lastly a layer of mucous-like material containing fibrils and dense bodies of paracrystalline structure.

Scanning electron microscope (SEM) investigations carried out on eggs of 21 species of Ephemeroptera belonging to the Leptophlebiidae and Heptageniidae, pointed out a fine chorionic decoration that differs according to taxonomic position. In fact the organization of the shell surface, the fine morphology of both micropyles and attachment structures could be used for taxonomic purposes in addition to other morphological data on adults and nymphs.

INTRODUCTION

Ephemeropteran egg structure may be approached from two points of view:

1. the events taking place during egg maturation such as uptake of nutritional substances and synthesis of egg envelopes and
2. fine chorionic organization for taxonomic purposes.

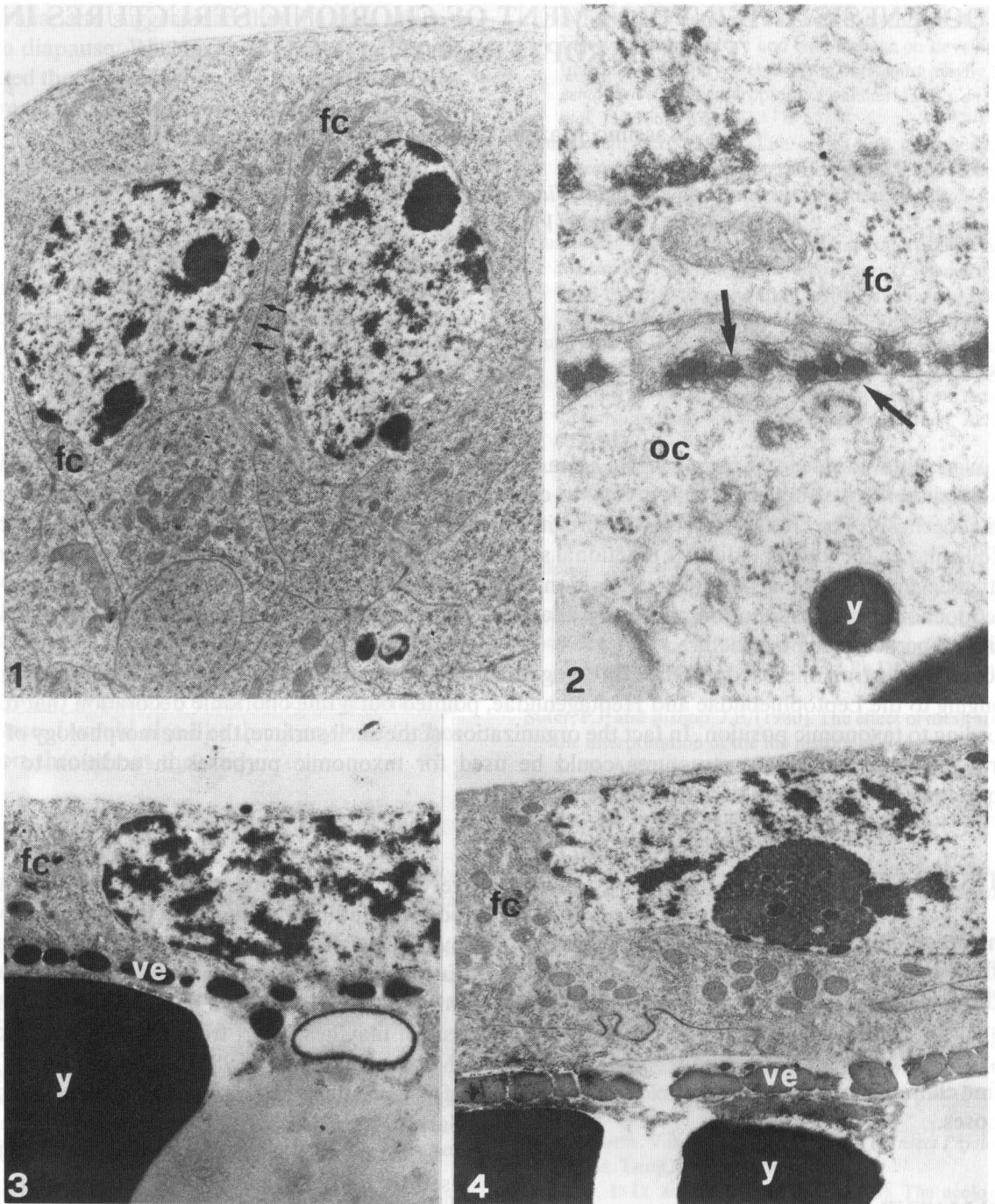
EGG MATURATION

Ephemeroptera are characterized by panoistic ovarioles lacking nurse cells or trophocytes; the oocytes are nourished only by the follicular epithelium. Apart from the paper by Soldán (1979), the only data available about mayfly reproduction are

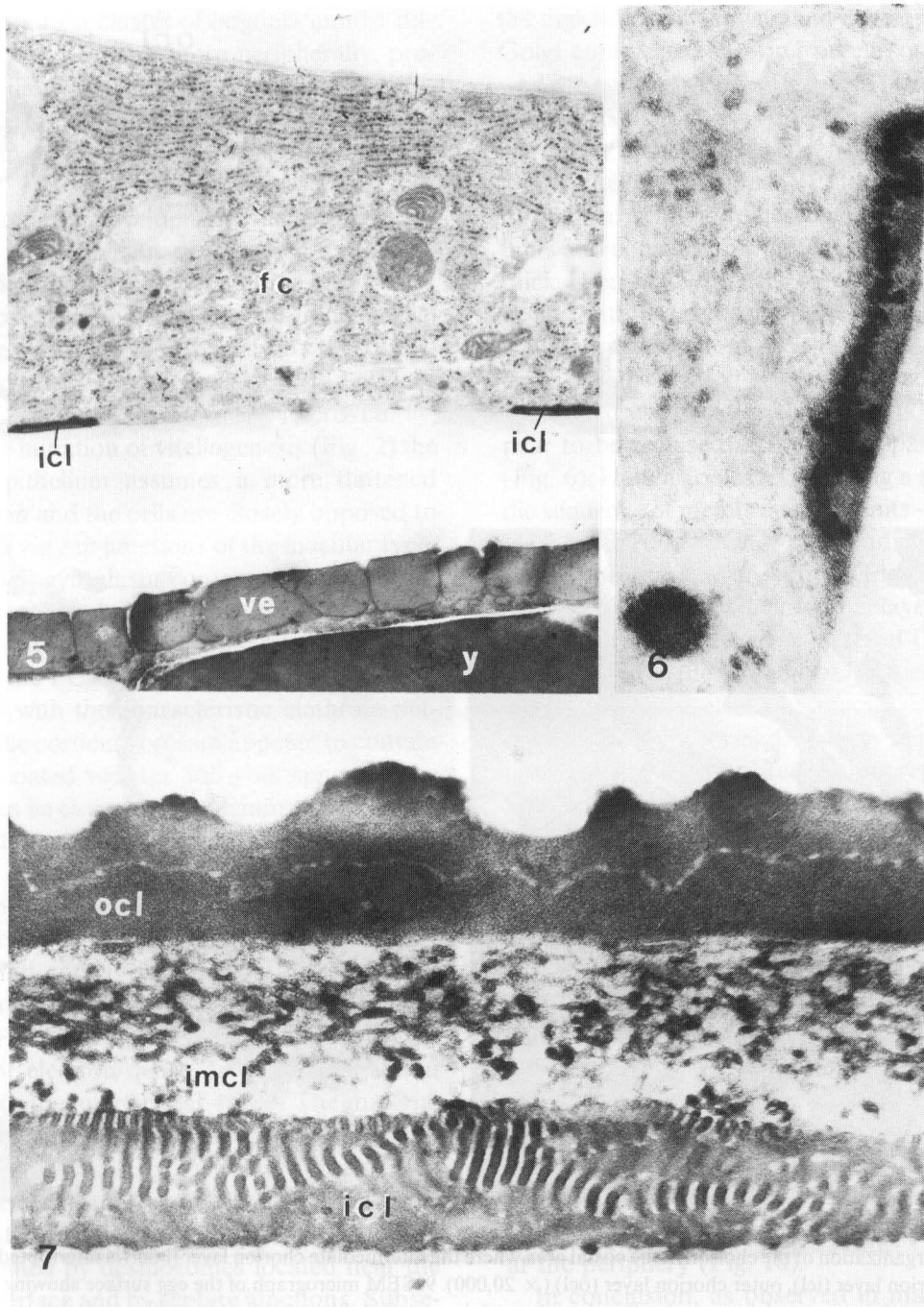
very old. In this work, classical cytological methods were utilized to describe the structure of the ovary in different developmental stages in six European species. Practically no ultrastructural information has yet been published on the oogenesis of mayflies, apart from some of our findings (Mazzini and Gaino 1985) on the mature egg shell of a leptophlebiid (*Habrophlebia eldae* Jacob & Sartori, as *H. fusca*).

Transmission electron microscopy (TEM) was used to study oogenesis in *H. eldae*, taking into particular account the morphological events involving both the developing oocytes and the follicular cells as a model of a panoistic ovary (Figs 1, 2).

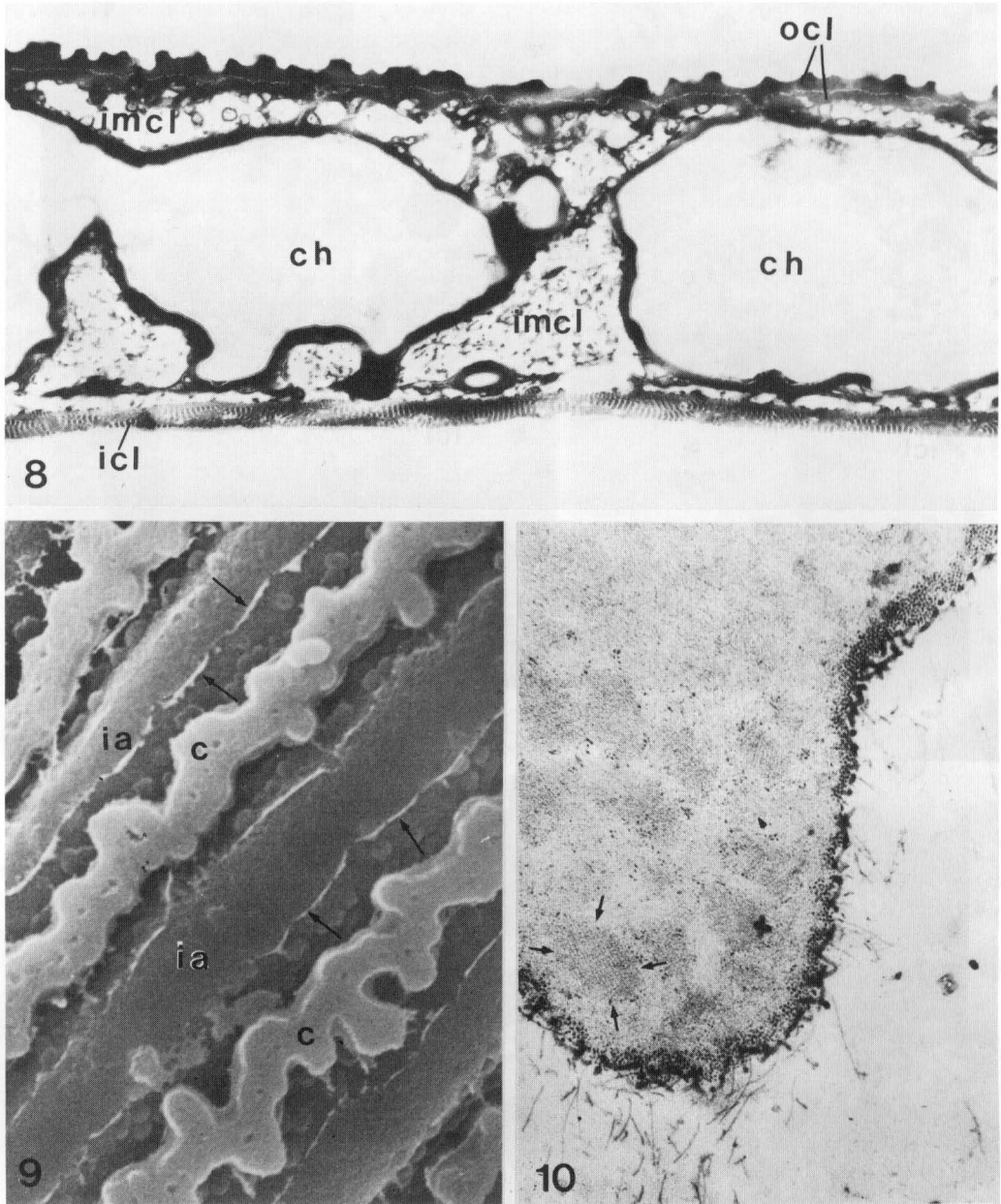
Panoistic ovarioles of this species consist of a number of follicles arranged in a linear sequence of increasing size. The apical portion of the ger-



Figs 1-4. 1. Germarium region showing two follicle cells (fc) very close to each other. Arrows indicate a junction ($\times 12,000$). 2. Electron dense amorphous material (arrows) evident at the follicle cell-oocyte interface (fc-oc); yolk sphere (y), ($\times 48,000$). 3. Electron dense material realized upon the oocyte by follicle cell (fc) forms the vitelline envelope (ve); yolk sphere (y) ($\times 13,000$). 4. Vitelline envelope (ve) composed of units forming a discontinuous sheet; follicle cell (fc), yolk spheres (y) ($\times 13,000$).



Figs 5-7. 5. Vitelline envelope (ve) and precursor material of inner chorion layer (icl) secreted by follicle cells (fc); yolk sphere (y) ($\times 18,000$). 6. High magnification of the apical part of a follicle cell during the inner chorion layer synthesis showing material associated with electron dense bodies ($\times 108,000$). 7. Organization of the chorion in the intercostal area showing the inner chorion layer (icl) and the intermediate chorion layer (imcl) supporting the outermost bilayered coat (ocl) ($\times 72,000$).



Figs 8–10. 8. Organization of the chorion in the costal area where the intermediate chorion layer (imcl) is interrupted by chambers (ch); inner chorion layer (icl), outer chorion layer (ocl) ($\times 20,000$). 9. SEM micrograph of the egg surface showing the chorionic pattern of costae (c) separated from each other by intercostal areas (ia). Strands of mucous-like material are visible in the intercostal areas (arrows) ($\times 3,000$). 10. Fine organization of the mucous-like material lined by the fibrillar coat and containing bodies with a paracrystalline structure (arrows) ($\times 38,000$).

marium contains a cluster of oogonia amidst differentiating oocytes and, more peripherally, pre-follicular cells. In each ovariole while the last follicle is in vitellogenic growth, the preceding one is blocked in previtellogenesis or early vitellogenesis.

Early in ovarian development, oocytes become associated with a follicular epithelium formed by tightly packed cells (Fig. 1). A close interface is sustained between oocyte (OC) and follicle cells. These latter are anchored basally to the tunica propria and interlocked apically with the plasma membrane of the oocyte via a few microvilli.

Upon the initiation of vitellogenesis (Fig. 2) the follicular epithelium assumes a more flattened configuration and the cells are closely opposed to one another via gap junctions of the macular type. Follicular cell cytoplasm contains numerous mitochondria and also ribosomes which are free and associated with the endoplasmic reticulum. Thin sections at the FC/OC interface reveal numerous coated pits with the characteristic clathrate pattern while the cortical ooplasm appears to contain numerous coated vesicles and yolk spheres. The yolk spheres lie close to the oolemma (Fig. 2) and are formed by the coalescence of small vesicles into larger aggregates. Most of the cell volume is occupied by a large nucleus with uniformly dispersed chromatin. Intercellular space between follicle cells and oocyte plasma membrane appears upon initiation of the vitelline envelope (VE) deposition (Fig. 3). This event is indicated by the presence of electron-dense amorphous material realized into the intercellular space. The material is first disposed in the form of electron dense ellipsoidal granules (Fig. 3) which subsequently fuse to form irregularly shaped structural units (Fig. 4). At this developmental stage the follicle cells are joined by desmosomes located near the FC/OC interface and by septate junctions. Subsequently their contact surface increases owing to a more oblique angle to the longitudinal axis of the egg. The elongate nucleus, with its major axis parallel to the oocyte surface, is characterized by one or sometimes two prominent nucleoli and occupies a major portion of the cell volume. Rough endoplasmic reticulum is very abundant as

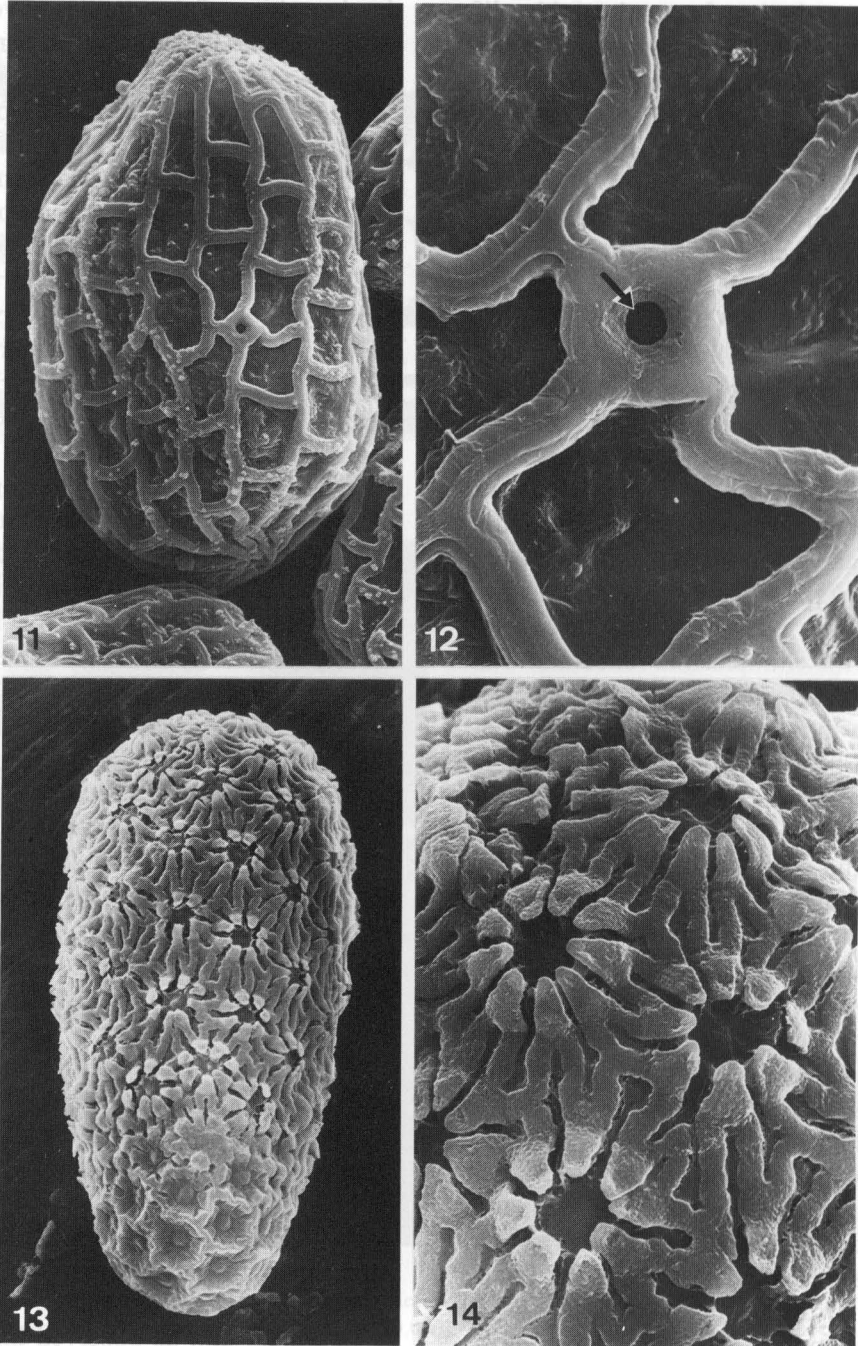
the deposition of the vitelline envelope proceeds. Golgi complexes, showing precursor material of vitelline envelope, are more numerous during this and subsequent phases of development than during earlier stages of vitellogenesis.

The vitelline envelope (VE) formation proceeds through a gradual coalescence of the structural units and consists on a uniform coat about $0.4\ \mu\text{m}$ thick (Fig. 5). Synthesis of chorionic envelopes begins with the elaboration of a portion adjacent to the vitelline coat, the inner chorion layer (Fig. 5). This portion is characterized by material associated with the electron-dense bodies that appear to be released at the apical part of the FC (Fig. 6). Here it coalesces realizing a sort of periodic sequence of membrane-like units – each lamella about 17 nm thick – perpendicularly placed with respect to the FC surface (Fig. 7). The superimposed intermediate chorionic layer consists of loosely structured fibrillar material (Fig. 7). This coat supports the outermost bilayered chorion, which is highly electron opaque. As a consequence the fully formed chorion can be divided morphologically into three distinct regions that, in *H. eldae* are differently organized according to the chorionic pattern of this species.

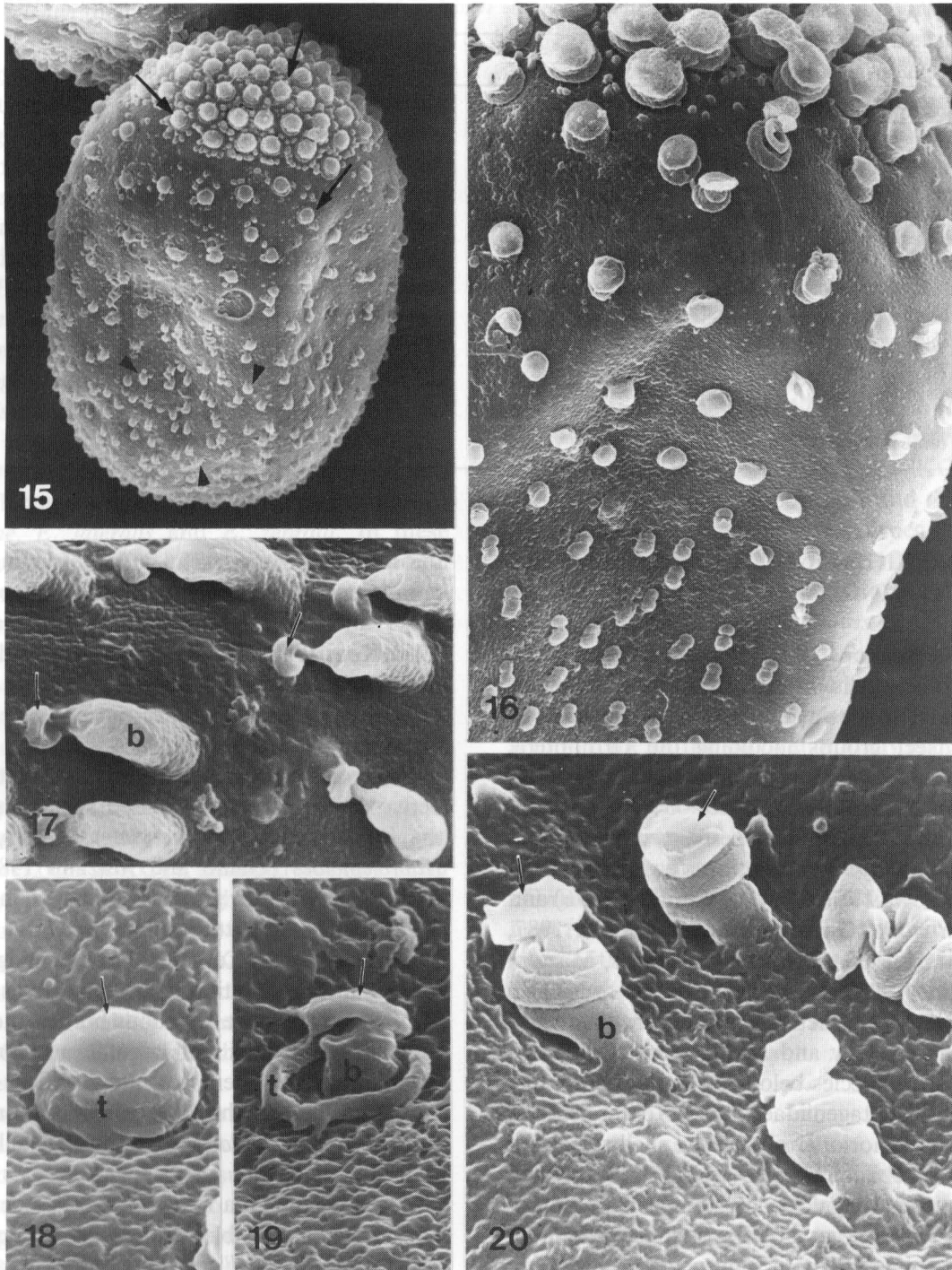
At SEM level, a series of longitudinal costae, separated by intercostal areas, arise from the egg surface (Fig. 9). The intermediate chorionic layer of each costa shows a regular sequence of chambers separated by columns (Fig. 8).

According to previous observations (Mazzini and Gaino 1985), the external surface of the chorion, in many of the species examined, is covered with a mucous-like layer composed of material of variable thickness; in *H. eldae* it also fills the chambers and shows a paracrystalline-like structure in the internal area and fibrillar material at the periphery (Fig. 10).

In conclusion, as observed in many pterygote insects (see Mazzini *et al.* 1984), in Ephemeroptera the egg shell layers are secreted by the follicle cells (FCs) in a well defined order in successive secretory cycles. Exochorion formation by the FCs represents the most conspicuous phase in the differentiation program of these cells.



Figs. 11–14. 11–12. SEM view of the whole egg of *Thraulius bellus* showing (Fig. 11. $\times 408$) the chorionic pattern of ridges and (Fig. 12. $\times 1,920$) the micropyle (arrow) located in the equatorial zone. 13–14. SEM view of the whole egg of *Choroterpes picteti* showing (Fig. 13. $\times 464$) the decoration of the chorion and (Fig. 14. $\times 1,440$) the arrangement of ridges arising from the egg surface to encircle a central cavity.



Figs 15–20. 15. SEM view of the egg of *Rhithrogena alpestris* showing the chorionic pattern of KCTs arising directly from the chorion (arrows) and from basal excrecences (arrow heads) ($\times 468$). 16. SEM view of the egg of *Rhithrogena allobrogica* showing the different size and morphology of the KCTs ($\times 900$). 17–20. SEM views of the chorion of some species of the *Rhithrogena alpestris* group, showing KCT attachment structures (arrows) with the thread (t) supported by a basal excrecence (b): 17. *Rhithrogena kimminsi* ($\times 1,980$). 18. *R. landai* with thread coiled ($\times 8,100$). 19. *R. landai* with thread partly extended ($\times 8,100$). 20. *R. alpestris* ($\times 66,750$).

Table 1. A list of species whose eggs were studied with the scanning electron microscope

| Leptophlebiidae | Heptageniidae |
|---|--|
| <i>Habroleptoides modesta</i> (Hagen) | <i>Electrogena lateralis</i> (Curtis) |
| <i>Habroleptoides umbratilis</i> (Eaton) | <i>Electrogena grandiae</i> (Belfiore) |
| <i>Habroleptoides auberti</i> (Biancheri) | <i>Electrogena zebra</i> (Hagen) |
| <i>Habroleptoides budtzi</i> (Ebsen-Petersen) | <i>Electrogena fallax</i> (Hagen) |
| <i>Habroleptoides pauliana</i> (Grandi) | <i>Electrogena gridellii</i> (Grandi) |
| <i>Habroleptoides berthelemyi</i> (Thomas) | |
| <i>Habrophlebia eldae</i> Jacob and Sartori | <i>Rhithrogena alpestris</i> Eaton |
| <i>Habrophlebia lauta</i> Eaton | <i>Rhithrogena landai</i> Sowa and Saldán |
| <i>Habrophlebia consiglioi</i> Biancheri | <i>Rhithrogena vaillanti</i> Sowa and Degrange |
| | <i>Rhithrogena kimminsi</i> Thomas |
| | <i>Rhithrogena allobrogica</i> Sowa and Degrange |
| <i>Choroterpes picteti</i> (Eaton) | |
| <i>Thraulius bellus</i> Eaton | |

TAXONOMIC SIGNIFICANCE OF EGG SCULPTURING

SEM observations of the impressions produced by the follicle cells on the external surface of the egg have led to the development of ootaxonomy in many insect groups including the Orthoptera, Phasmatodea, Lepidoptera and Diptera (e.g. Hinton 1981, Mazzini *et al.* 1984).

Numerous SEM studies performed on ephemeropteran eggs by various authors (e.g. Alba-Tercedor and Sowa 1987, Sowa and Soldán 1986) and our own work (Gaino and Mazzini 1984, 1987, Gaino *et al.* 1987, Gaino *et al.* in press, Mazzini and Gaino 1985) revealed that in this order also the chorion is characterized by decorations varying in morphology and arrangement. Our SEM studies of 21 species belonging to the Leptophlebiidae and Heptageniidae (Table 1) demonstrated longitudinal chorionic ribs on the shell surface of the leptophlebiids. These may also be connected to delineate irregular polygonal areas, as in *Thraulius bellus* (Figs 11, 12), or forming a more complex network of conical projections encircling a central cavity, as in *Choroterpes picteti* (Figs 13, 14). In contrast the chorion of heptageniids bears more specialized structures which are used as adhesive devices (Figs 15–20).

Shell decorations and the fine morphology of

the micropyles are useful in constructing taxonomic keys. This certainly agrees with previous observations of light microscopists (Degrange 1960, Koss 1968, 1973, Koss and Edmunds 1974) which emphasized that chorionic pattern may be utilized together with other morphological data for taxonomic purposes.

Ootaxonomic characters allow us to distinguish between eggs of closely related species, such as the Italian species of the genera *Habrophlebia*, *Habroleptoides* (Gaino and Mazzini 1984) and *Rhithrogena alpestris* group (Gaino *et al.* in press).

The chorionic pattern of *Habrophlebia* species is characterized by a system of ribs running across the egg in the direction of its major axis. In *H. eldae* these ribs have a sinuous border due to the irregular projections and indentations along their outside margin. There are many orifices on the dorsal surface of the ribs, which are probably aeropyles. In *H. lauta* these ribs have small finger-shaped expansions along their margins which project into the area between two adjacent ribs. No orifices are visible on the dorsal side of the ribs. In *H. consiglioli* each rib shows lateral spiniform expansions.

The chorionic pattern of *Habroleptoides* consists of a network of polygonal ridges covering the egg surface only in *H. auberti*. In the species *H. umbratilis*, *H. modesta*, *H. budtzi*, *H. pauliana* and

H. berthelemyi the chorion is completely covered by closely spaced thin raised ribs. In *H. modesta* each rib is slightly twisted. In *H. budtzi* the chorionic pattern is very close to that of *H. umbratilis* but the eggs are very elongated and are pointed at both ends. Chorionic ribs of *H. pauliana* and *H. berthelemyi* are respectively thinner and wider if compared to those of *H. umbratilis*. From an ootaxonomic point of view this suggests that, apart from *H. auberti*, the other species of *Habro-leptoides* are very close to each other.

A comparison of the Italian species of the genus *Electrogena*, encompassing all the species of the *lateralis*-group (Zurverra and Tomka 1985), revealed some important morphological differences (Gaino *et al.* 1987). The eggs may be divided into two different chorionic types. The first includes *E. grandiae*, *E. lateralis* and *E. zebrata*. In these species the chorion is characterized by special attachment structures, knob terminated coiled threads (KCTs). Two kinds of KCTs are distinguishable in *E. lateralis*; the first are smaller and more uniformly distributed and the second are larger and concentrated at one egg pole. In *E. grandiae* there are attachment structures of the second kind and these are sparsely scattered over the egg surface. In *E. zebrata* the chorion is characterized by geometrically arranged attachment structures of large KCTs, arranged side by side on the egg surface. Each thread is covered by a thin transparent terminal knob through which its coiled shape may be seen. KCTs are involved in adhesion to the substrate. In fact each individual knob, after breaking at one side, triggers the spring-like uncoiling of the threads. The resultant outstretched configuration reveals that the threads consist of many fine twisted fibres closely packed together. Finally the KCTs project from the chorion and reach the substratum with their round-ended knobs (Gaino and Mazzini 1987). The eggs of the second type, those of *E. fallax* and *E. gridellii*, show a chorion devoid of any differentiated sculptures. However a rough pattern of spherules in *E. fallax* and numerous irregularly shaped granules in *E. gridellii* enable specific identification.

The species of the *Rhithrogena alpestris* group

show two kinds of chorionic projections (Figs 15–20). The first is characterized by large KCTs concentrated at one egg pole (Fig. 15). They may also be scattered towards the equatorial region, where they reduce in size (Fig. 16). The second is characterized by smaller projections, composed of a basal excrescence supporting a KCT (Figs 17–20). The morphology and dimensions of the latter projections together with other chorionic features constitute a good character set for distinguishing *R. alpestris*, *R. landai*, *R. vaillanti*, *R. allobrogica* and *R. kimminsi* (Gaino *et al.* in press). The coiled thread is usually on top of each excrescence (Figs 17, 20) but in *R. landai* every thread is coiled around the excrescence (Figs 18, 19). In *R. kimminsi* the KCTs are concentrated at one egg pole only and the rest of the chorion is covered by rows of peg-like projections supporting KCTs (Fig. 17). Like the KCTs and the other projections supporting them in those species of the *R. alpestris* group examined, there is SEM evidence that the peg-like structures are involved in adhesion to the substrate.

The ability of the eggs to attach to the substrate is important for the survival of many ephemeropteran species. While some evolved an adhesion layer on the chorion, others have evolved more complex structures like those described above. The basal boss supporting the threads could assist in orienting the extended thread in the position of least resistance to water flow. Permanent attachment of the eggs is probably assured by the polar KCTs which have thicker, stronger threads and wide terminal knobs.

CONCLUSION

Ultrastructural studies by SEM and TEM highlight the peculiar trends occurring during the maturation of ephemeropteran eggs. Apart from enabling us to understand basic biological phenomena, this approach brings to light some taxonomic considerations about the systematic value of chorionic structure which will provide a new set of characters for phylogenetic studies.

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