



## Ootaxonomic investigation of the Italian species of the genus *Electrogena* (Ephemeroptera, Heptageniidae)

ELDA GAINO

Istituto di Zoologia, Università di Genova,  
via Balbi 5.16126 Genova (Italy)

CARLO BELFIORE

Istituto e Museo di Zoologia, Università di Napoli,  
via Mezzocannone 8, 80134 Napoli (Italy)

MASSIMO MAZZINI

Dipartimento di Biologia evolutiva, Università di Siena,  
via Mattioli 4, 53100 Siena (Italy)

### ABSTRACT

The egg chorion of the Italian species of the genus *Electrogena* was investigated using the scanning electron microscope (SEM). The eggs examined showed distinctive chorionic patterns. *Electrogena grandiae*, *E. lateralis* and *E. zebrata* showed attachment structures characterized by knob-terminated coiled threads (KCTs). These structures were uniformly scattered over the egg surface in *E. grandiae*, while in *E. lateralis* they differed in size and position. In *E. zebrata*, the attachment structures were of uniform size and formed a dense mat over the whole egg surface. In contrast, no specialized sculptures were found in *E. fallax* and *E. gridellii*. The different chorionic patterns allowed specific identifications. The affinities and the taxonomic position of the species examined are discussed.

**KEY WORDS:** Ootaxonomy; Egg chorion; Scanning electron microscopy; *Electrogena*; Ephemeroptera.

### ACKNOWLEDGEMENTS

This research was financially supported by: CNR under the project «Gruppo Nazionale di Biologia Naturalistica», Contrib. n° 3350340; M.P.I. under the project «Gruppo Biogeografia del Mediterraneo Occidentale» and M.P.I. 60%. The authors also wish to thank Mr. G. Gatti for his valuable technical assistance.

### INTRODUCTION

The genus *Electrogena* was recently proposed on the basis of electrophoretic studies (Zurwerra & Tomka, 1985). It encompasses all the species of the so-called «*lateralis*-group» that were previously considered a part of the genus *Ecdyonurus*.

Five species of *Electrogena* are present in Italy, namely *E. grandiae*, *E. lateralis*, *E. gridellii*, *E. zebrata* and *E. fallax*. The taxonomic position of the latter two species was recently reviewed by Gaino & Belfiore (in press).

The object of this study was to investigate the egg chorion of the Italian species by scanning electron microscopy (SEM) in order to describe their egg morphologies, and to obtain further data for taxonomic and phylogenetic purposes. In fact, SEM has provided good evidence for species-specific chorionic patterns in Ephemeroptera (Flowers, 1980; Gaino & Mazzini, 1984; Kopelke, 1980; Landa & Soldàn, 1982; Malzacher, 1982; Mazzini & Gaino, 1985; Pescador & Peters, 1982) and in many other insects (for a review see Hinton, 1981). As a consequence, also according to data previously obtained with optical microscopy (Degrange, 1960; Koss, 1968; Koss & Edmunds, 1974; Sowa & Soldàn, 1984), the egg chorion of mayflies may be used, in addition to morphology of the nymph and adult, to distinguish among species.

### MATERIALS AND METHODS

#### Source of eggs

Eggs were removed from preserved specimens of the following species: *Electrogena grandiae* (Belfiore): mature nymphs and imagines collected in Latium, leg. C. Belfiore; *E. lateralis* (Curtis): mature nymphs and imagines from Latium and Piedmont, leg. C. Belfiore and E. Gaino; *E. zebrata* (Hagen): mature nymphs from Corsica (streams Puto and Catena), E. Gaino collection; *E. fallax* (Hagen): imagines from Sardinia (stream Mannu), leg. C. Belfiore; *E. gridellii* (Grandi): mature nymphs collected in the river Rosandra by Trieste, leg. C. Belfiore.

#### Scanning electron microscopy

The eggs, stored in alcohol, were dried by the critical point method, using liquid CO<sub>2</sub> in a Bomar apparatus, mounted on a specimen holder, placed in a vacuum evaporator and coated with a layer of gold-palladium alloy in a Balzer Union evaporator. Egg surface observations were carried out using a Philips 505 scanning electron microscope.

#### Terminology

The basic descriptive terminology used by Koss & Edmunds (1974) is followed in the present paper.

### RESULTS

Comparison of the chorion of the Italian species of the genus *Electrogena* revealed some important differences in morphological features. The eggs may be divided into two groups based on chorionic morphology. In the first group, which consists of *E. grandiae*, *E. lateralis* and *E. zebrata*, the chorion is characterized by specialized attachment structures, while in the

second group, which consists of *E. fallax* and *E. gridellii*, the chorion is quite smooth.

The micropyles, generally located in the equatorial or subequatorial area, consist of two parts: a chorionic depression called sperm guide, and a tunnel-like micropylar canal.

*Electrogena grandiae* (Belfiore, 1981)

The eggs measure about 150  $\mu\text{m}$  in length and 110  $\mu\text{m}$  in width. The external surface of the shell is marked by a pattern of tubercles and coiled threads (Fig. 1). The tubercles are round in shape; they vary from 0.7 to 1.3  $\mu\text{m}$  in diameter and are scattered over the entire cell surface (Fig. 2). On average, there are about 30-32 tubercles per 100  $\mu\text{m}^2$  the distance between adjacent tubercles ranging from 5 to 15 nm. At high magnification, the chorion shows a granular, homogeneous ground matrix which also covers the tubercles (Fig. 3).

The granular matrix adheres to the entire chorion surface except the knob-terminated coiled threads (KCTs: Koss & Emunds, 1974) which emerge from the granular matrix (Fig. 3) and are uncovered. Each of these peculiar attachment structures measures about 2.5  $\mu\text{m}$ . The attachment structures are sparsely scattered over the egg surface (Figs. 1 and 2); their density, about 2 per 100  $\mu\text{m}^2$ , is very low compared to that of the tubercles. Each thread, formed by bundles of fibres, is coiled about its own place and some micrographs show their outstretched conformation (Fig. 4).

The micropyles are visible in the subequatorial area. Four micropyles are normally found, two of which are usually close together (Fig. 5). The sperm guide is oval, about 11  $\mu\text{m}$  long and 7  $\mu\text{m}$  wide, with a well-defined border formed by a thickened rim bearing several randomly distributed tubercles (Fig. 5).

*Electrogena lateralis* (Curtis, 1834)

The eggs measure about 130  $\mu\text{m}$  in length and 95  $\mu\text{m}$  in width. The chorionic pattern is very similar to that previously described for *E. grandiae*. The eggs are characterized by a granular ground matrix, round-shaped tubercles and KCTs (Fig. 6). However, there are some important differences between *E. lateralis* and *E. grandiae*. The tubercles (0.7 to 1.0  $\mu\text{m}$  in diameter) are more numerous, about 45-50 per  $\mu\text{m}^2$ , and their dense concentration outlines the sperm guide opening (Fig. 7). Two kinds of KCTs are distinguishable on the basis of size and distribution. The elements of the first group are smaller (about 3  $\mu\text{m}$  in diameter) and more uniformly distributed, while those of the second group are larger (about 5  $\mu\text{m}$  in diameter) and concentrated at one egg pole only (Fig. 6). These smaller attachment threads are less numerous than in *E. grandiae*.

Four micropyles are found in the equatorial or subequatorial zone. Their sperm guide is ovoidal and measures about 10  $\mu\text{m}$  in length and 7  $\mu\text{m}$  in width. The micropylar rim appears thickened by the dense concentration of chorionic tubercles (Fig. 7).

*Electrogena zebrata* (Hagen, 1864)

The eggs measure about 150  $\mu\text{m}$  in length and 120  $\mu\text{m}$  in width. The chorion is characterized by geometrically arranged attachment structures of large KCTs; covering the whole egg surface (Fig. 8).

Higher magnification shows that these attachment structures, each measuring about 10  $\mu\text{m}$ , are arranged in a particular configuration in which the coiled threads are separated from each other by a ring of protuberances (Fig. 9). The terminal knob usually covers the coiled thread (Fig. 9), and may assume a triangular shape (Fig. 10). This feature is probably due to the tendency of the covering part to contract during dehydration and other procedures utilized for preserving SEM material.

The micrographs show two micropyles in the subequatorial zone. The sperm guide is ovoidal, it measures about 10  $\mu\text{m}$  in length and 6.4  $\mu\text{m}$  in width, and shows a micropylar opening (Fig. 11) set at the junction point between the sperm guide and the micropylar canal.

*Electrogena fallax* (Hagen, 1864)

The eggs measure about 165  $\mu\text{m}$  in length and 120  $\mu\text{m}$  in width. The chorion is quite smooth and lacks any differentiated sculptures (Fig. 12). At high magnification, the chorionic surface shows a rough pattern of small spherules (Fig. 13), having a diameter of 0.2  $\mu\text{m}$ , that may cluster together (Fig. 14).

Two micropyles are found in the subequatorial area (Fig. 12). The sperm guide is ovoidal and measures about 8  $\mu\text{m}$  in length and 6  $\mu\text{m}$  in width.

*Electrogena gridellii* (Grandi, 1953)

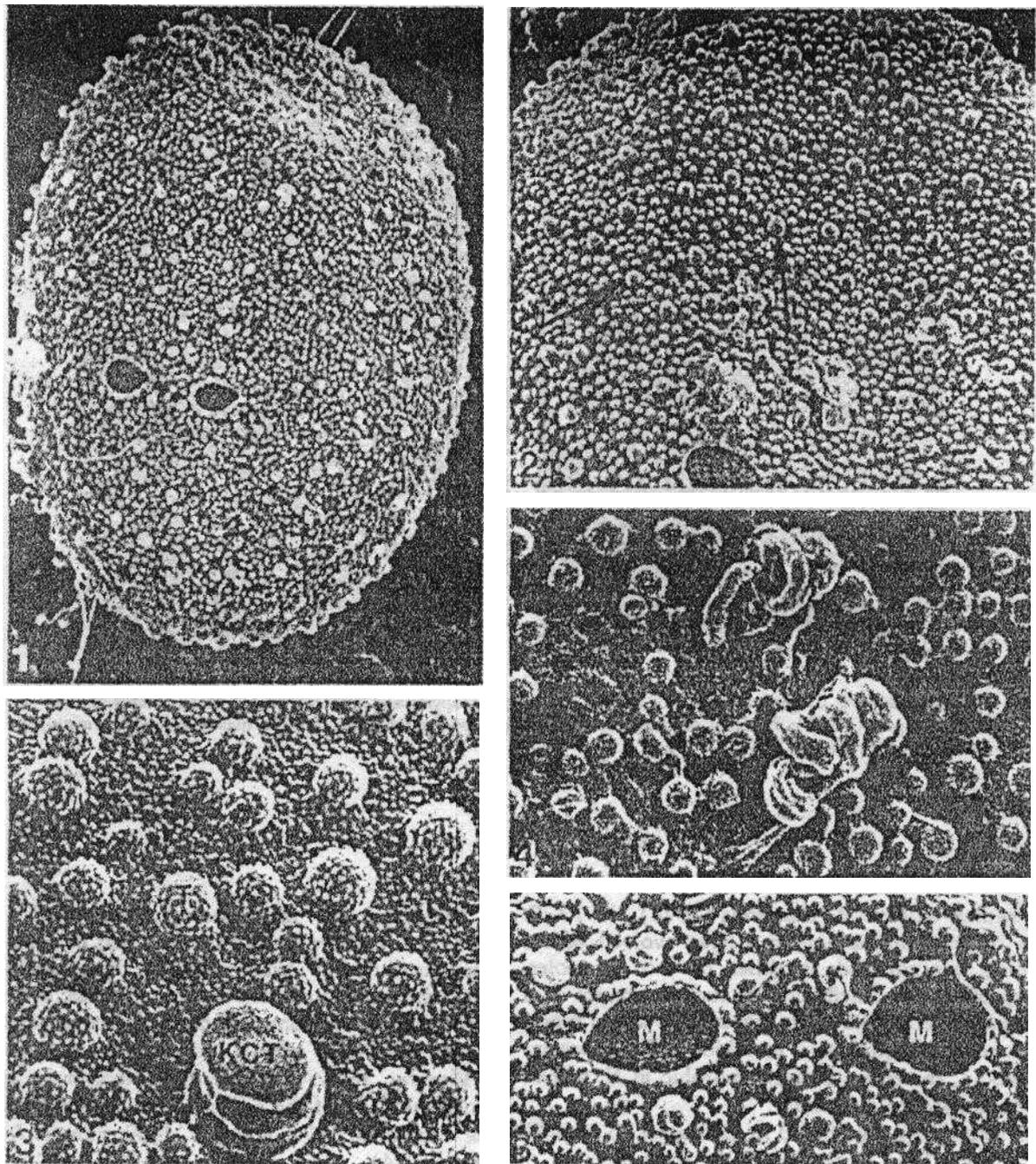
The eggs measure about 150  $\mu\text{m}$  in length and 100  $\mu\text{m}$  in width. The chorion of this species is quite smooth (Fig. 16) and resembles that of *E. fallax*. At high magnification, however, the chorion envelope appears to be covered by a granular ground matrix. The latter consists of numerous irregularly shaped granules, each measuring about 0.3  $\mu\text{m}$ , and often packed together to form clumps (Fig. 16).

Some micropyles are visible in the equatorial or subequatorial area (Fig. 15). The sperm guide is ovoidal: it measures about 8  $\mu\text{m}$  in length and 6  $\mu\text{m}$  in width and the micropylar opening is situated at the side (Fig. 17), as in all other species of the genus *Electrogena*.

## DISCUSSION

Scanning electron microscopic studies of egg chorionic patterns differentiate all Italian species of the genus *Electrogena*, even the very closely related *E. grandiae* and *E. lateralis* (see Belfiore, 1981).

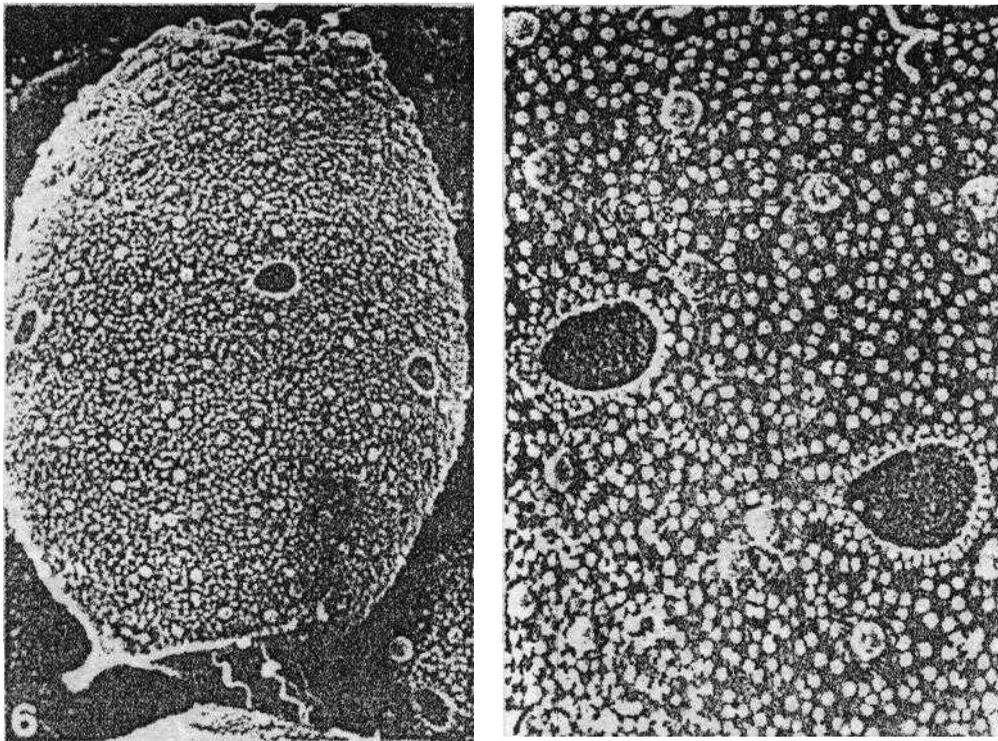
Two different types of chorion sculpturing occur in the Italian species of *Electrogena*. The first type shows the persistence of attachment structures with knob-terminated coiled threads (KCTs). In fact, in the Hep-



Figs. 1-5 - *Electrogena grandiae*. 1) general outline of the egg with two micropyles in the subequatorial area (x 570); 2) chorionic sculpturing of tubercles (stars) and knob-terminated coiled threads (arrows) (x 1100); 3) tubercles (T) covered by granular ground matrix and a thread (KCT) coiled about its own plate (x 6800); 4) two outstretched knob-terminated coiled threads (x 4600); 5) two micropyles (M) with chorionic tubercles surrounding their rims (x 2000).

tageniidae, the most common egg type shows chorionic tubercles and distinct coiled threads with well developed terminal knobs (Koss & Edmunds, 1974). *E. grandiae*, *E. lateralis* and *E. zebra* conform to the more common type and show some species-specific chorionic sculptures. The presence of KCTs is considered an important advantage for the eggs, as such structures could serve for anchorage upon deposition (KOSS,

1973). In *E. lateralis* and in *E. grandiae*, the KCTs are small and scattered over the egg surface. In *E. lateralis* some of them reach larger dimensions and are concentrated at one pole of the egg. This particular polar arrangement may assume the same role played by the attachment threads of polar caps, orienting the eggs with relation to resistance to water flow (Koss & Edmunds, 1974).



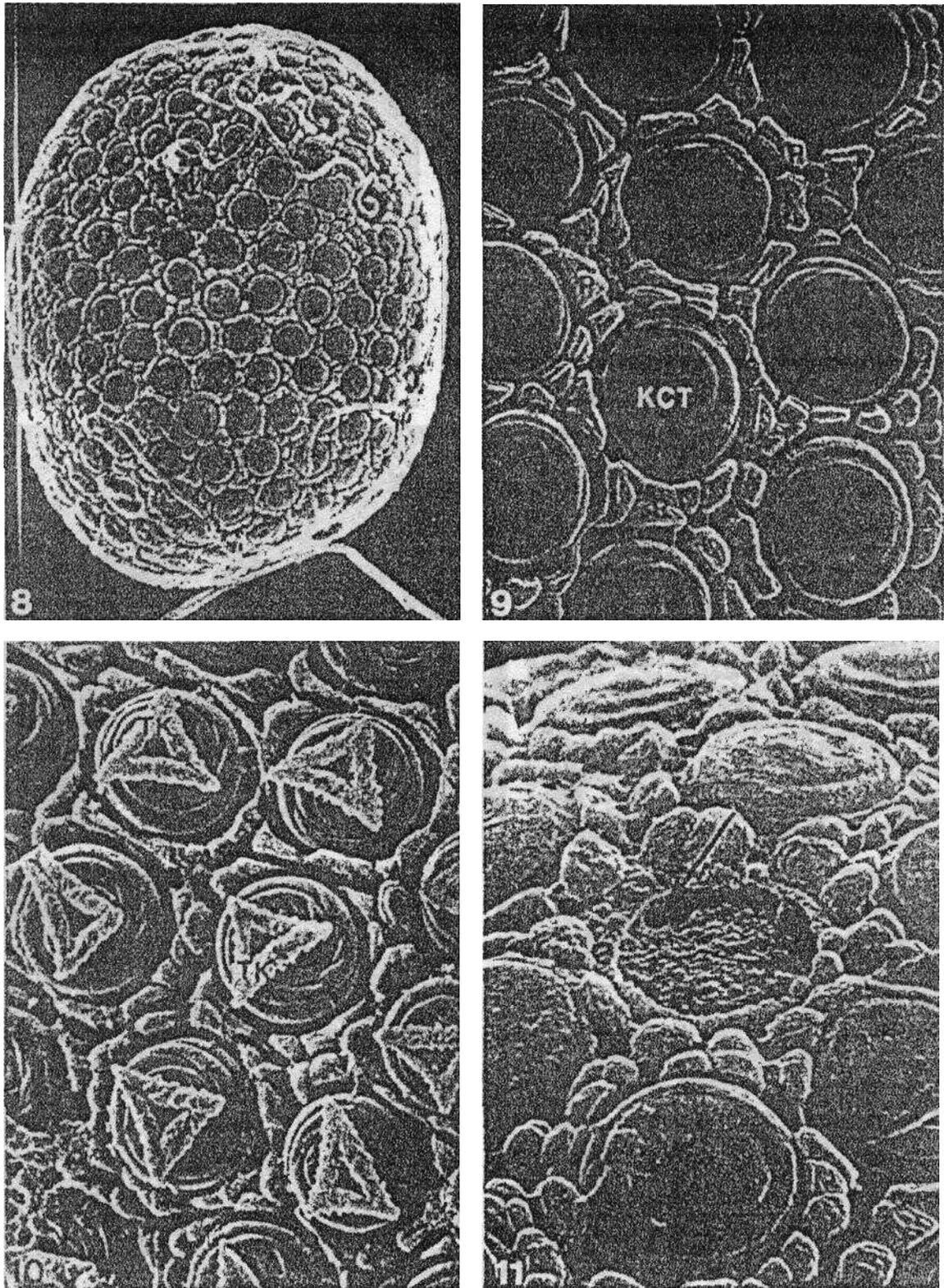
Figs. 6-7 • *Electrogena lateralis*. 6) general outline of the egg showing micropyles and knob-terminated coiled threads of major size (arrow) concentrated at one pole (x 650); 7) detail of chorionic sculpturing showing tubercles arranged mainly around the micropylar rim and knob-terminated coiled threads (KCTs) (x 2000).

According to Koss & Edmunds (1974), the KCTs would be apomorphic and may have evolved before becoming concentrated at either of the egg poles. Consequently, the distribution of such structures in a dense mat covering the entire egg surface would represent a relatively primitive condition. The character state would, therefore, be plesiomorphic in *E. zebrata* and apomorphic in *E. lateralis*.

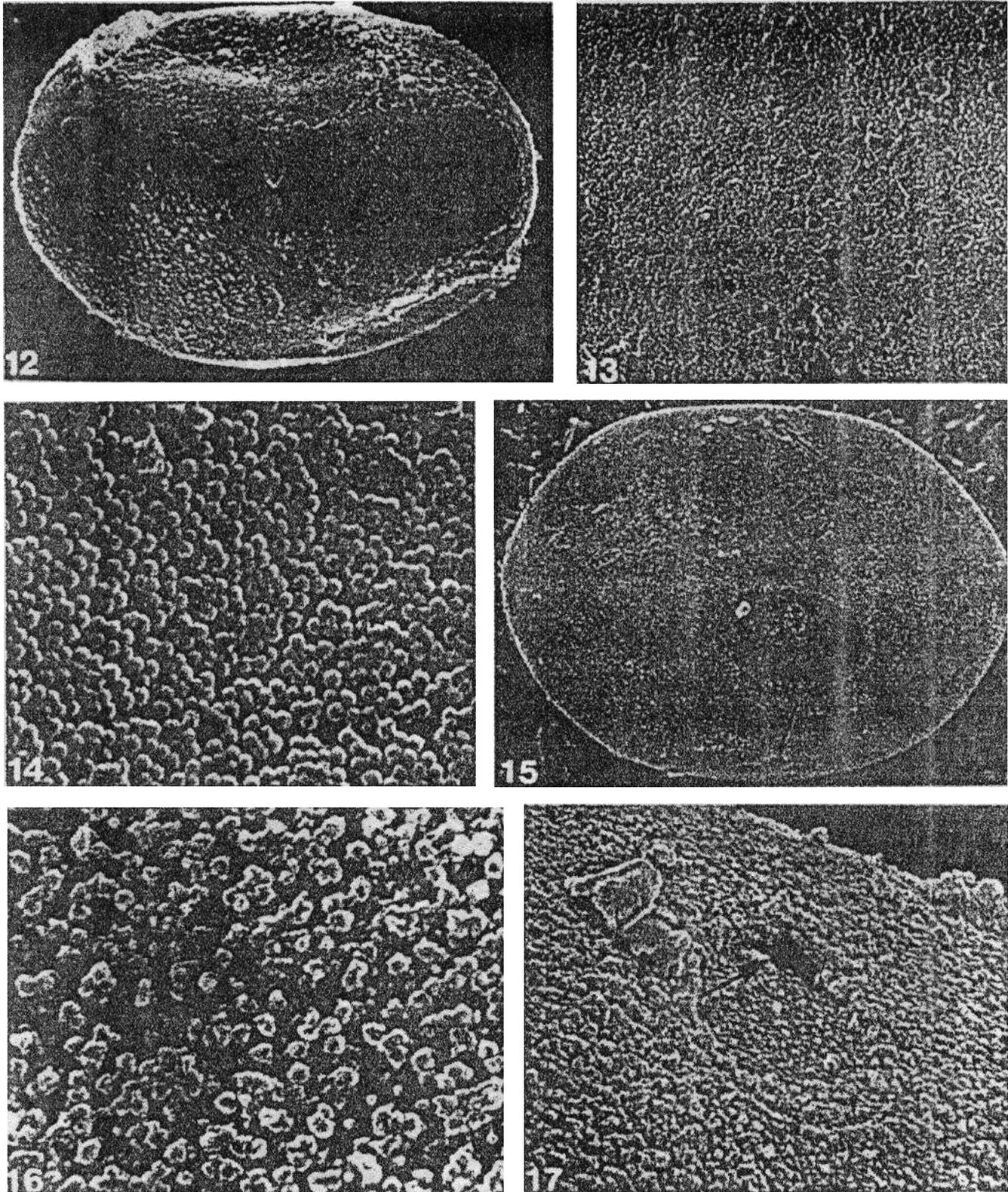
The second egg type found in *Electrogena* (*E. gridellii* and *E. fallax*) lacks particular attachment structures. A chorion devoid of these structures is uncommon in the European Heptageniidae. In fact, such a condition was observed only in the genus *Epeorus* (Degrange, 1960; Koss, 1968). The chorion organization in *E. fallax* and *E. gridellii* may be derived from a divergent line, where attachment structures may have been lost to produce a smooth chorion. Unfortunately, data are still lacking for the egg chorion of many other European species of *Electrogena*. As a consequence, a comparative evaluation of the phylogenetic relationships among these species remains difficult. It should also be considered, however, that *E. fallax* and *E. zebrata* are often found to live together. The presence or absence of attachment structures, therefore, cannot be considered a response to selective pressure exerted by habitat conditions.

Considering the taxonomic position of *E. zebrata*, Gaino & Belfiore (in press) noted that its penis is very similar to that of *Afronurus kugleri*, thus suggesting that *E. zebrata* could possibly be related to the latter genus. In contrast, the comparison of egg morphologies between *Electrogena zebrata* and the examined species of *Afronurus*, shows constant differences in the arrangement and size of the KCTs on the egg chorions (Koss & Edmunds, 1974; Kopelke, 1980; Flowers & Pescador, 1984).

In conclusion, this study shows that chorionic sculpturing is species-specific also in the Italian species of *Electrogena* and can be utilized, together with nymph and adult morphological characters, for systematic purposes. In addition, KCT distribution suggests that the eggs of *E. zebrata* may conserve a plesiomorphic state, while in *E. grandiae* and in *E. lateralis* the pattern of these attachment structures would represent a more advanced step. In particular, the concentration of KCTs at one egg pole demonstrates a more developed apomorphic condition in the latter species. As the most common egg type found in the Heptageniidae shows attachment structures, its lack in *E. fallax* and *E. gridellii* represents a rather surprising change in the established egg shape of *Electrogena*.



Figs. 8-11 - *Electrogena zebrata*. 8) general outline of the eggs (x 570); 9) detail of chorionic arrangement of knob-terminated coiled threads (KCT) separated from each other by a ring of protuberances (P) (x 2000); 10) triangular shape of terminal knob (TK) (x 2000). 11) micropyle showing micropylar opening (arrow) (x 3100).



Figs. 12-17 - 12) *Electrogena fallax*. General outline of the egg (x 570); 13) chorionic sculpturing (x 3500), 14) detail of chorionic pattern with clustered spheres (x 14000). 15) *Electrogena gridellii*. General outline of the egg with micropyles (arrows) (x 570); 16) chorionic pattern of irregular-shaped granules (x 12000); 17) detail of the micropyle showing micropylar opening (arrow) (x 4500).

## REFERENCES

- Belfiore C., 1981 - On the Italian species of the *Ecdyonurus lateralis* Group (Ephemeroptera, Heptageniidae). *Aquatic Insects*, 3: 171-178.
- Degrange Ch., 1960 - Recherches sur la reproduction des Ephéméroptères. *Trav. Lab. Hydrobiol. Pisc. Univ. Grenoble*, 51: 7-193.
- Flowers R. W., 1980 - A review of the nearctic *Heptagenia* (Heptageniidae, Ephemeroptera), pp. 93-102. *In*: J. F. Flannagan & K. E. Marshall (eds.). *Advances in Ephemeroptera biology*. Plenum Press, New York. London, pp. 552.
- Flowers R. W., Pescador M. L., 1984 - A new *Afronurus* (Ephemeroptera: Heptageniidae) from the Philippines. *Int. J. Entomol.*, 26: 362-365.
- Gaino E., Belfiore C., - On the type specimens of *Electrogena zebrata* Habrophlebia fusca (Curtis) and *H. consiglioi* Biancheri (Ephemeroptera; Leptophlebiidae). *Int. J. Insect. Morphol. Embryol.*, 14: 327-334.
- Gaino E., Mazzini M., 1984 - Scanning electron microscope study of the eggs of some *Habrophlebia* and *Habroleptoides* species (Ephemeroptera, Leptophlebiidae). *In*: V. Landa *et al.* (eds.). *Proc. IV Int. Conf. Ephemeroptera*, pp. 193-202.
- Hinton H. E., 1981 - *Biology of insect eggs*, Pergamon Press, Oxford, pp. 1125.
- Kopelke J. P., 1980 - Morphologische Studien an den Eiern der Eintagsfliegen (Ephemeroptera) aus der Emergenz der zentralafrikanischen Bergbaches Kalengo. *Mitt. Schweiz. Entomol. Ges.*, 53: 297-311.
- Koss R. W., 1968 - Morphology and taxonomic use of Ephemeroptera eggs. *Ann. Entomol. Soc. Amer.*, 61: 696-721.
- Koss R. W., 1973 - The significance of the egg stage to taxonomic and phylogenetic studies of the Ephemeroptera. *In*: W. L. Peters & J. G. Peters (eds.), *Proc. I. Int. Confer. Ephemeroptera*, pp. 73-78.
- Koss R. W., Edmunds G. F., 1974 - Ephemeroptera eggs and their contribution to the phylogenetic studies of the order. *Zool. J. Linn. Soc.*, 55: 267-349.
- Landa V., Soldán T., 1982 - *Ecdyonurus samalorum* sp. n. from Czechoslovakia. (Ephemeroptera, Heptageniidae). *Acta ent. bohemoslov.*, 79: 31-36.
- Malzacher P., 1982 - Eistrukturen europäischer Caenidae (Insecta, Ephemeroptera). *Stutt. Beitr. Nat. Ser. A*, 356: 1-15.
- Mazzini M., Gaino E., 1985 - Fine structure of the egg shells of *Electrogena zebrata* Habrophlebia fusca (Curtis) and *H. consiglioi* Biancheri (Ephemeroptera; Leptophlebiidae). *Int. J. Insect. Morphol. Embryol.*, 14: 327-334.
- Pescador M. L., Peters W. L., 1982 - Four new genera of Leptophlebiidae (Ephemeroptera: Atalophlebiinae) from southern South America. *Aquatic Insects*, 4: 1-19.
- Sowa R., Soldán T., 1984 - Two species of *Rhithrogena* Eaton (Ephemeroptera, Heptageniidae) from Central Europe. *In*: V. Landa *et al.* (eds.), *Proc. IV Int. Conf. Ephemeroptera*, pp. 75-84.
- Zurwerra A., Tornka I., 1985 - *Electrogena* gen. nov., eine neue Gattung der Heptageniidae (Ephemeroptera). *Ent. Ber. Luzern*, 13: 50-104.