31 Eggs of the European Ephemerellidae (Ephemeroptera)

Denise Studemann, Peter Landolt and Ivan Tomka
University of Fribourg, Institute of Zoology, Department of Entomology, CH-1700 Fribourg, Switzerland

The eggs of 12 European and two American species of Ephemerellidae were investigated using the scanning electron microscope. Chorionic patterns are stable within a species and eggs extracted from subimago and imago are similar. The chorionic sculpturing is distinct for each species, except for D. paradinasi — T. major *and* E. ignita — E. aurivillii. *The examination of the eggs has not revealed specific generic characters.*

Introduction

In their study, Koss and Edmunds (1974) describe the general features of mayfly eggs and use the description of the eggs of many species to provide a phylogenetic tree of the genera of the Ephemeroptera. However, only a few European species were involved in that study. In the last ten years the eggs of some European mayfly species have been studied using scanning electron microscopy: *Baetis* (Kopelke and Müller-Liebenau 1981a, 1981b, 1982), Caenidae (Malzacher 1982), *Habrophlebia* and *Habroleptoides* (Gaino and Mazzini 1984; Mazzini and Gaino 1985), *Electrogena* (Gaino et al. 1987), *Arthroplea* (Studemann et al. 1987) and Siphlonuridae (Studemann et al. 1988; Studemann and Tomka 1991).

The 12 European species of Ephemerellidae can easily be identified. On the other hand, their affinity to the different genera is not clearly evident. With the morphological characters of the larvae and the imagos given in Allen and Edmunds (1962, 1963a, 1963b, 1965) for the American species, it is often difficult to attribute the European species to a particular genus. The purpose of this study is to evaluate the contribution of the egg structure to their taxonomy.

Materials and Methods

The species studied in this paper are listed in Table 1. The taxa *Drunella*, *Ephemerella*, *Serratella*, *Torleya*, *Attenella* and *Eurylophella* are treated as genera, after Hubbard (1990). The female imagos were captured or reared by the authors except *Eurylophella karelica*, which came from the collection of Dr. M. Keffermüller, and *Attenella margarita*, which was collected by Dr. T. Fink. This last species and *Serratella tibialis* are distributed in North America, *Ephemerella aurivillii* in North America and North Europe, while all the remaining species are restricted to Europe (Table 1).

Most of the eggs were extracted from frozen female imagos and fixed first in a 100 mM sodium-cacodylate/25 per cent glutaraldehyde solution and then transferred after four hours to a solution of 100 mM sodium-cacodylate/1 per cent OsO_4. Some eggs were extracted from female imagos (or subimagos) stored in 80 per cent alcohol and put successively in the following solutions: 96 per cent alcohol, mixture of alcohol/acetone, 100 per cent acetone. The next steps were identical for all the eggs: dehydration by the critical point method using liquid CO_2, mounting on metallic holder covered with adhesive paper and coating with a 75 nm layer of gold-palladium in a vacuum evaporator. The investigations were carried out by a Hitachi H700 scanning electron microscope (= SEM). For each species the observations were made on at least 20 eggs and the measurements were taken on four to eight eggs.

Results

According to Koss and Edmunds (1974) the eggs of the Ephemerellidae (except those of *Eurylophella*) have an ovoid form and possess:

- one polar cap of type I (non-coiled, single unit cap) (Fig. 7),
- knob-terminated coiled threads (= KCT) dispersed on the egg surface (Figs. 7, 9),
- micropyles of type III (tagenoform and entirely chorionic micropyles) (Fig. 8) with an ovoid chorionic sperm guide and a micropylar opening leading to the micropylar canal, which cannot be seen on the SEM — photographs,
- a chorion with very various patterns of chorionic sculpturings.

In the genus *Eurylophella*, the eggs have a cylindrical form and they lack a polar cap and KCT. The dimensions of the eggs studied, the number and dimension of the micropyles, as well as the number of KCT are given in Table 2.

Table 1. Species studied with their taxonomical position in the Ephemerellidae and geographical distribution.

Taxonomy of the species	Geographical distribution
Ephemerellae	
Drunella (Needham, 1905)	
paradinasi G. del Tanago & G. de Jalon, 1983	Iberian Peninsula
Ephemerella Walsh, 1862	
aurivillii (Bengtsson, 1909)	North Europe, North America
ignita (Poda, 1761)	Europe
ikonomovi Puthz, 1971	Northern Mediterranean
mesoleuca (Brauer, 1857)	Europe
mucronata (Bengtsson, 1909)	Europe
notata Eaton, 1887	Europe
Serratella Edmunds, 1859	
albai G. del Tanago & G. de Jalon, 1983	Iberian Peninsula
hispanica (Eaton, 1887)	Iberian Peninsula
tibialis McDunnough, 1924	North America
Torleya Lestage, 1925	
major (Klapalek, 1905)	Europe
Timpanogae	
Attenella Edmunds, 1971	
margarita Needham, 1927	North America
Eurylophella Tiensuu, 1935	
iberica Keffermüller & Da Terra, 1978	Iberian Peninsula
karelica Tiensuu, 1935	North Europe

Table 2. Measurements on the eggs of the Ephemerellidae.

Species	Egg length with polar cap (µm)	No. of KCT	No. of micropyles	Micropyle length (µm)
D. paradinasi	200	8-12	1-2	11
E. aurivillii	215	10-14	6-12	10
E. ignita	250	16-28	4-8	9
E. ikonomovi	340	20-28	8-12	18
E. mesoleuca	300	10-16	4-6	14
E. mucronata	190	8-12	2-6	15
E. notata	300	12-16	6-8	12
S. albai	230	8-12	6-10	11
S. hispanica	270	6-10	2-4	14
S. tibialis	180	10-12	2-6	10
T. major	200	10-14	2-4	12
A. margarita	240	12-18	12-16	10
E. iberica	160	none	1-2	15
E. karelica	200	none	1-2	14

Description of the Eggs

Drunella paradinasi (Figs. 1-3). The surface of the chorion is flat, slightly rough with smooth discs, a little smaller than the micropyles, all over the egg.

Ephemerella aurivillii (Figs. 4-6). The chorion appears very smooth with many micropyles and KCT.

Ephemerella ignita (Figs. 7-9, 40-43). The eggs, gathered together in a greenish ball, are carried by the female imago until released into the water. The surface of the eggs is smooth and sprinkled with micropyles and KCT without any special arrangement. The eggs present the same features in different populations, even if the imagos or the larvae are a little different: the imagos of the Swiss population are brown (eggs Figs. 7-9, 40), those of a Spanish population (Prov. Soria) are yellow

(egg Fig. 42), those of a Corsican population are red (egg Fig. 43) and the larvae of another Spanish population (Prov. Huesca) has striped legs (egg Fig. 41).

Ephemerella ikonomovi (Figs. 10-12). The chorion reveals rings under the surface of the egg. The KCT are bigger and more numerous near the polar cap. The micropyles are very large.

Ephemerella mesoleuca (Figs. 13-15, 44-45). Except for the polar cap, the whole egg is covered with raised discs (diameter about 25 µm), which seem to be rings when the fine layer is torn (Fig. 14). Near the polar cap the chorion is marked by about four spherical tubercles.

Ephemerella mucronata (Figs. 16-18). The chorion has a slightly rough surface, divided by narrow depressions in many sectors (sometimes hexagonal), smaller than the micropyles.

Ephemerella notata (Figs. 19-21). The chorion is covered with scales about twice as large as the micropyles.

Serratella albai (Figs. 22-24). The egg surface is flat, slightly rough with smooth discs, larger than the micropyles. The base of the polar cap is furnished with a crown of about 20 tubercles (Fig. 23) Most of the micropyles are situated in the equatorial region of the egg.

Serratella hispanica (Figs. 25-27). The chorion is entirely covered with skull-caps more or less as large as the micropyles in diameter. Most of the KCT are situated near the base of the polar cap (Fig. 26).

Serratella tibialis (Figs. 28-29). The egg surface is smooth without any special chorionic sculptures.

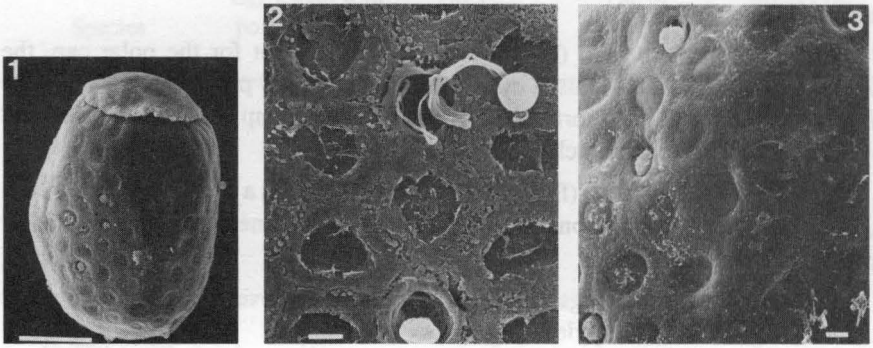
Torleya major (Figs. 30-32). The slightly rough chorion has smooth discs, smaller than the micropyles, all over the surface.

Attenella margarita (Figs. 33-34). The chorion is covered with a fine reticulation consisting of smooth lines separating rough pentagonal or hexagonal surfaces. Most of the micropyles are situated in the equatorial region of the egg. All the micropylar openings are orientated in the same direction, perpendicular to the polar cap. The sperm guide ends under the chorion surface.

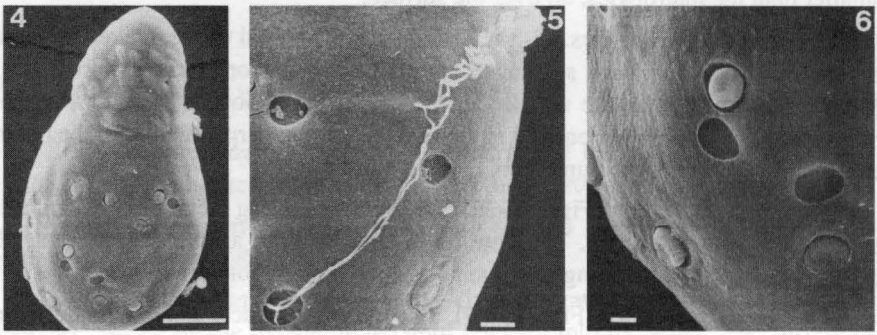
Eurylophella iberica (Figs. 35-36). The cylindrical egg lacks a polar cap and other attachment structures. The chorion is covered with a reticulation made of broken raised lines separating rough surfaces of different polygonal forms.

Eurylophella karelica (Figs. 37-39). The egg is cylindrical and devoid of polar cap and other attachment structures. The chorion presents longitudinal narrow depressions and fine transversal lines so that the chorion appears covered with rectangular scales. Most of the eggs possess one (eventually two) micropyles situated in the equatorial region.

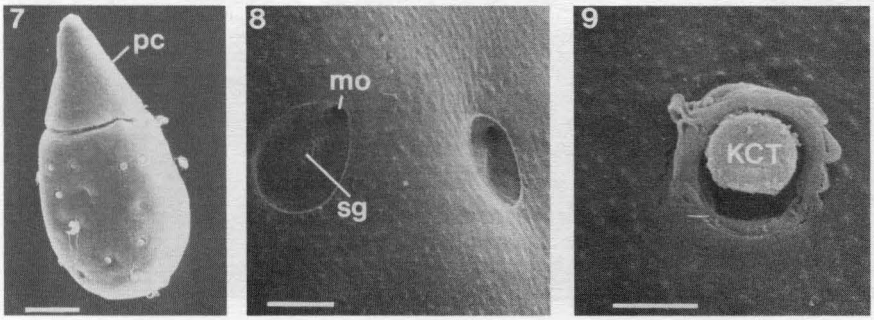
Figures 1-3. **Egg of *Drunella paradinasi*. 1: general view, 2: KCT, 3: chorion with KCT and micropyles. Scale line 1: 50 μm, scale line 2-3: 5 μm.**



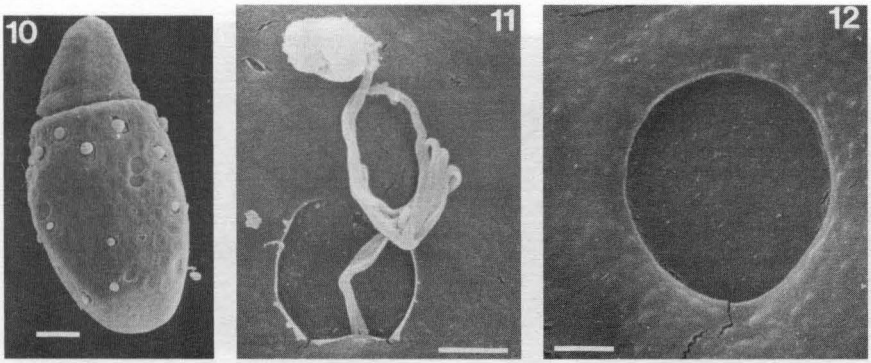
Figures 4-6. **Egg of *Ephemerella aurivillii*. 4: general view, 5: chorion with one detached KCT, 6: chorion with KCT and micropyles. Scale line 4: 50 μm, scale line 5: 10 μm, scale line 6: 5 μm.**



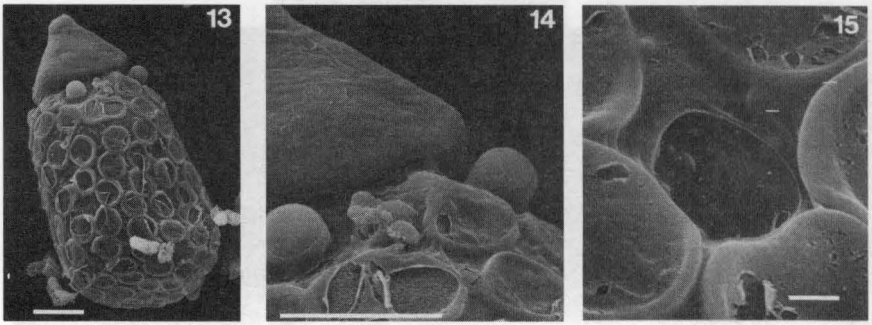
Figures 7-9. Egg of *Ephemerella ignita*. 7: general view (pc = polar cap), 8: micropyles (mo = micropylar opening; sg = sperm guide), 9: KCT. Scale line 7: 50 µm, scale line 8-9: 5 µm.



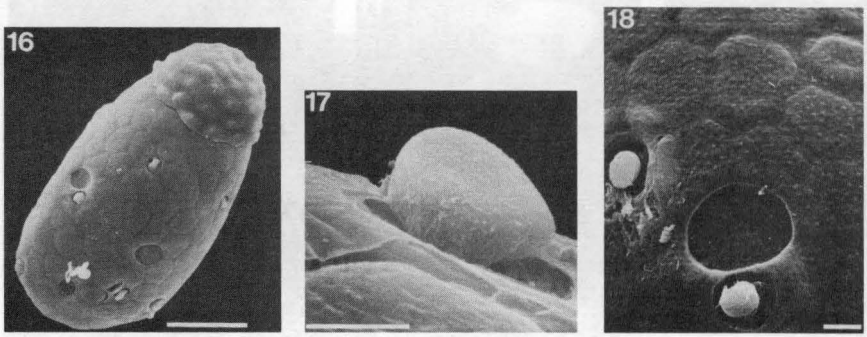
Figures 10-12. Egg of *Ephemerella ikonomovi*. 10: general view, 11: KCT, 12: micropyle. Scale line 10: 50 µm, scale line 11-12: 5 µm.



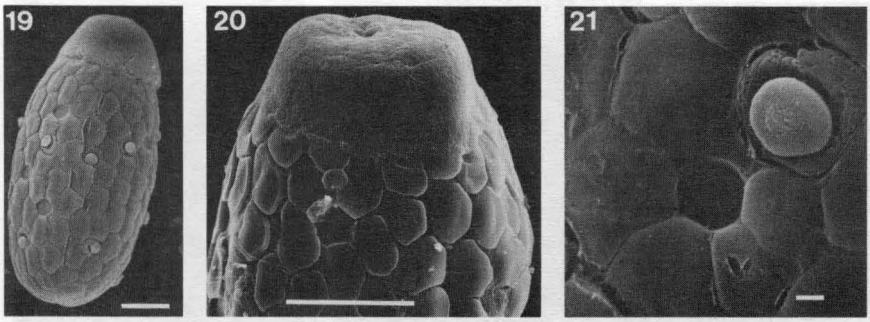
Figures 13-15. Egg of *Ephemerella mesoleuca*. 13: general view, 14: base of polar cap, 15: micropyle. Scale line 13: 50 µm, scale line 14-15: 5 µm.



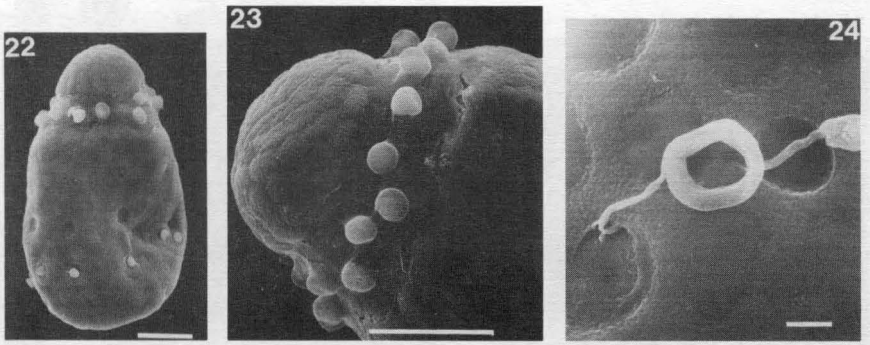
Figures 16-18. Egg of *Ephemerella mucronata*. 16: general view, 17: KCT in lateral view, 18: chorion with micropyle and KCT. Scale line 16: 50 µm, scale line 17-18: 5 µm.



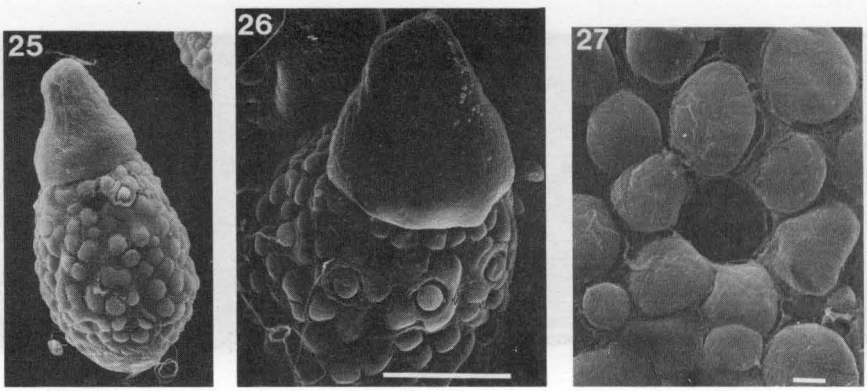
Figures 19-21. Egg of *Ephemerella notata*. 19: general view, 20: polar cap, 21: micropyle and KCT. Scale line 19: 50 µm, scale line 20-21: 5 µm.



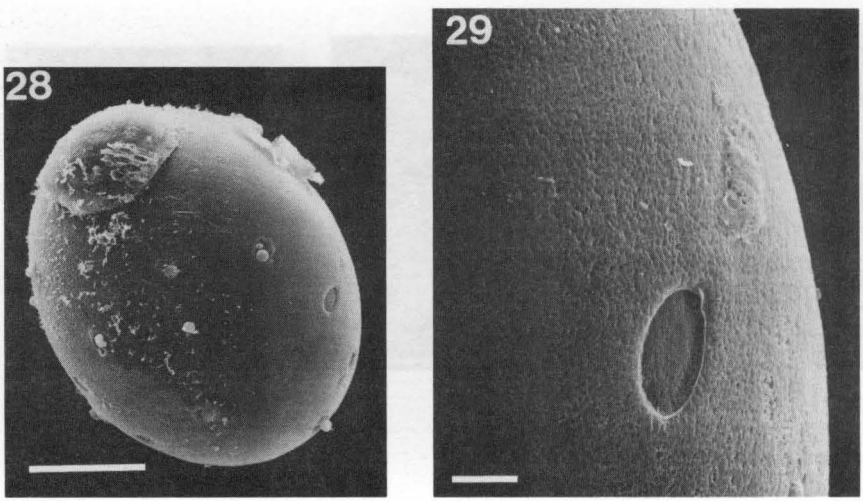
Figures 22-24. Egg of *Serratella albai*. 22: general view, 23: polar cap with crown of tubercles, 24: KCT and micropyle. Scale line 22: 50 µm, scale line 23-24: 5 µm.



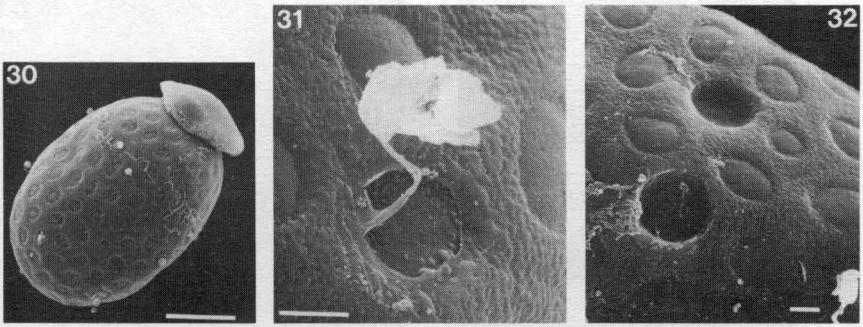
Figures 25-27. Egg of *Serratella hispanica*. 25: general view, 26: apical view, 27: micropyle. Scale line 25: 50 μm, scale line 26-27: 5 μm.



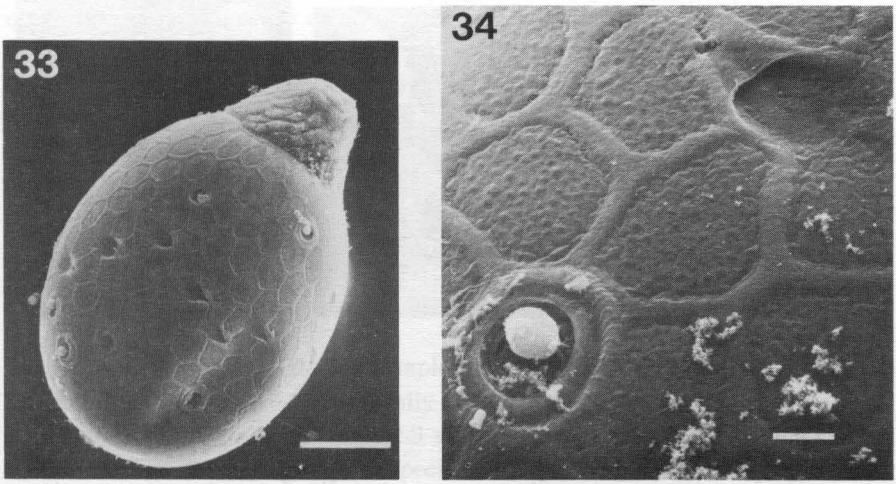
Figures 28-29. Egg of *Serratella tibialis*. 28: general view, 29: micropyle. Scale line 28: 50 μm, scale line 29: 5 μm.



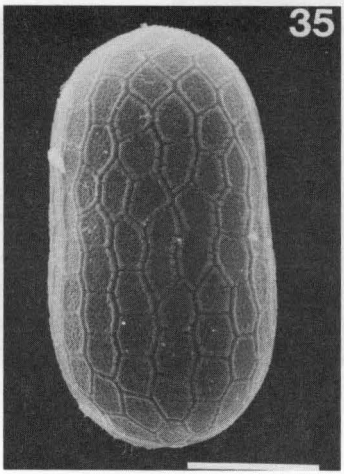
Figures 30-32. Egg of *Torleya major*. 30: general view, 31: KCT, 32: chorion with micropyles. Scale line 30: 50 µm, scale line 31-32: 5 µm.

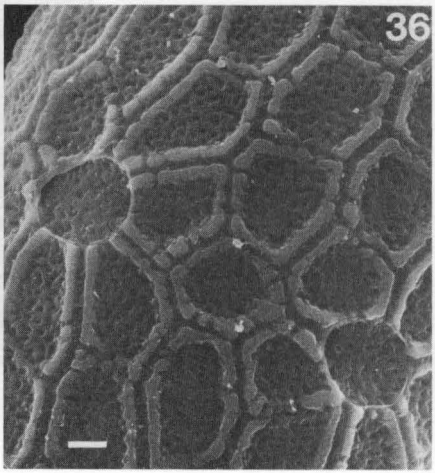


Figures 33-34. Egg of *Attenella margarita*. 33: general view, 34: chorion with micropyle and KCT. Scale line 33: 50 µm, scale line 34: 5 µm.

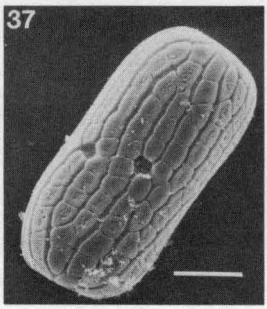


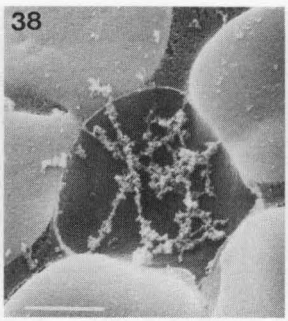
Figures 35-36. Egg of *Eurylophella iberica*. 35: general view, 36: chorion with micropyles. Scale line 35: 50 µm, scale line 36: 5 µm.

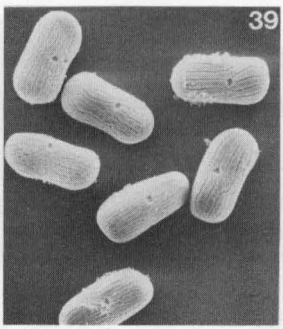




Figures 37-39. Egg of *Eurylophella karelica*. 37: general view, 38: micropyle, 39: seven eggs with micropyle. Scale line 37, 39: 50 µm, scale line 38: 5 µm.







Discussion

Intraspecific Variation

The chorionic sculpturing of the eggs of Ephemerellidae is often characteristic and stable within a species. The eggs of *Ephemerella ignita* present the same features in the four populations investigated (Figs. 40-43). The most variable structure is the polar cap, which can be more or less flat (Fig. 40) even though the eggs originate from one single female imago. According to Gaino and Bongiovanni (1992), the eggs of *Ephemerella ignita* with a long, bulging polar cap form a peripheral layer enveloping the egg ball and holding the egg mass together. Contrary to the results obtained for the genus *Baetis* (Kopelke and Müller-Liebenau 1981b, 1982), the chorionic sculpturing is almost the same if the eggs are extracted from the subimago or from the female imago (Figs. 44-45). In some cases the eggs (extracted from subimagos or imagos) were covered with a fine envelope concealing the chorionic sculpturing. It would be interesting to investigate the eggs (form of the polar cap, presence of the chorionic envelope) along the whole oviduct as well as after oviposition.

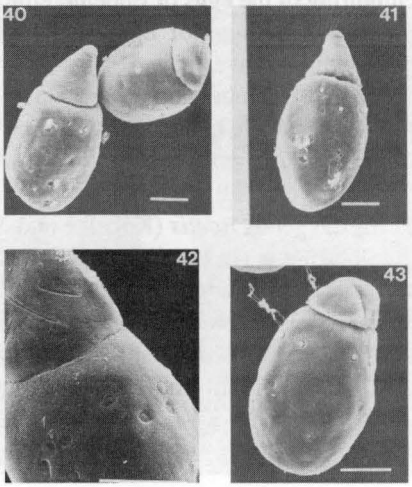
Interspecific Variation

In most of the cases the eggs can be used to separate the species of the European Ephemerellidae and are good complements to the larval and imaginal morphology. However, in *Ephemerella ignita* and *E. aurivillii*, easily distinguishable in the larval and imaginal stages, the eggs are very similar. The same can be observed for *Torleya major* and *Drunella paradinasi*. The chorionic sculpturing is species-specific in the genera *Habroleptoides* and *Habrophlebia* (Gaino and Mazzini 1984; Mazzini and Gaino 1985), *Electrogena* (Gaino et al. 1987), *Caenis* (Malzacher 1982) and in most of the *Baetis* species (Kopelke and Müller-Liebenau 1981a, 1981b, 1982). On the other hand, the nine European *Siphlonurus* species as well as the two European *Parameletus* species have almost the same egg features (Studemann et al. 1988, 1992).

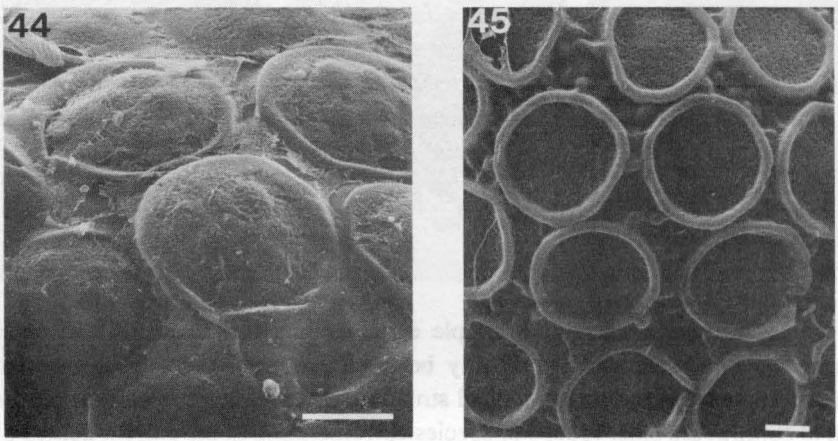
Generic Variation

The genus *Eurylophella* has simple eggs, lacking the polar cap and other attachment structures, so it can easily be separated from the other genera of Ephemerellidae. In contrast, no typical structures or chorionic sculpturing can be used to confirm the other groups of species corresponding to the current genera of the European Ephemerellidae. Similar results were obtained by Bae and McCafferty

Figures 40-43. Eggs of *Ephemerella ignita,* general view. 40: population from Switzerland, 41: population from Spain (Prov. Huesca), 42: population from Spain (Prov. Soria), 43: population from Corsica. Scale line 50 µm.



Figures 44-45. Chorion of the egg of *Ephemerella mesoleuca*. 44: egg extracted from a subimago, 45: egg extracted from an imago. Scale line 10 µm.



(1991) for the genera of Potamanthidae. As the affinity of the European species of the Ephemerellidae to the American genera is not evident using morphological egg, larval and imaginal characters, the taxonomy of the European Ephemerellidae is in need of revision. Biochemical investigations (isoenzyme electrophoresis) under way in our laboratories may contribute to a clarification of this issue.

Acknowledgements

We thank Dr. Maria Keffermüller, Dr. Adam Glazaczow and Dr. Thomas Fink for providing material. Dr. Martin Müller and Dr. René Hermann are thanked for assistance with the SEM. We are grateful to Prof. Dr. Gerolf Lampel for his critical comments of the manuscript, to Mr. Hubert Gachoud for his collaboration in the photographic field, as well as to Mrs. Sheila Werhonig and Mr. Roger Macherel for their technical help. This study was supported by the Swiss National Science Foundation (grant no. 3.506-0.86).

References

Allen, R.K. and G.F. Edmunds, Jr. 1962. A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae). V. The subgenus *Drunella* in North America. *Misc. Publ. Entomol. Soc. Amer.* 3: 147-179.

Allen, R.K. and G.F. Edmunds, Jr. 1963a. A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae). VI. The subgenus *Serratella* in North America. *Ann. Entomol. Soc. Amer.* 56: 583-600.

Allen, R.K. and G.F. Edmunds, Jr. 1963b. A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae). VII. The subgenus *Eurylophella* in North America. *Can. Entomol.* 95: 597-623.

Allen, R.K. and G.F. Edmunds, Jr. 1965. A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae). VIII. The subgenus *Ephemerella* in North America. *Misc. Publ. Entomol. Soc. Amer.* 4: 243-282.

Bae, Y.J. and W.P. McCafferty. 1991. Phylogenetic systematics of the Potamanthidae (Ephemeroptera). *Trans. Amer. Entomol. Soc.* 117: 1-143.

Gaino, E., C. Belfiore and M. Mazzini. 1987. Ootaxonomic investigation of the Italian species of the genus *Electrogena* (Ephemeroptera, Heptageniidae). *Boll. Zool.* 54: 169-175.

Gaino, E. and E. Bongiovanni. 1992. Comparative morphology of epithemata (polar chorionic structures) in the eggs of Ephemerella ignita (Ephemeroptera: Ephemerellidae). *Trans. Amer. Microsc. Soc.* 111: 255-265.

Gaino, E. and M. Mazzini. 1984. Scanning electron microscope study of the eggs of some *Habrophlebia* and *Habroleptoides* species (Ephemeroptera, Leptophlebiidae). P. 193-202 in V. Landa, T. Soldán and M. Tonner (Eds.), *Proceedings of the Fourth International Conference on Ephemeroptera*. Czech. Acad. Sci., Budejovice.

Hubbard, M.D. 1990. *Mayflies of the World. A Catalogue of the Family and Genus Group Taxa (Insecta: Ephemeroptera). Flora and Fauna Handbook 8.* Gainesville: Sandhill Crane Press, Inc.

Kopelke, J.P. und I. Müller-Liebenau. 1981a. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Ephemeroptera, Baetidae). Teil II: *rhodani-*, *vernus-* und *fuscatus*-Gruppe. *Spixiana* 4: 39-54.

Kopelke, J.P. und I. Müller-Liebenau. 1981b. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Ephemeroptera, Baetidae). Teil III: *buceratus-*, *atrebatinus-*, *niger-*, *gracilis-* und *muticus*-Gruppe. *Deutsche Entomol. Zeitschrift* 28: 1-6.

Kopelke, J.P. und I. Müller-Liebenau. 1982. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Ephemeroptera, Baetidae). Teil I: *alpinus-*, *lutheri-*, *pavidus-* und *lapponicus*-Gruppe. *Gewässer und Abwässer* 68/69: 7-25.

Koss, R.W. and G.F. Edmunds, Jr. 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. Ser. A*, 356: 1-15.

Mazzini, M. and E. Gaino. 1985. Fine structure of the egg shells of *Habrophlebia fusca* (Curtis) and *H. consiglioi* Biancheri (Ephemeroptera, Leptophlebiidae). *Internat. J. Insect. Morphol. Embryol.* 14: 327-334.

Studemann, D., P. Landolt et I. Tomka. 1987. Complément à la description de *Arthroplea congener* Bengtsson, 1908 (Ephemeroptera) et à son statut systématique. *Bull. Soc. Frib. Sc. Nat.* 76: 144-167.

Studemann, D., P. Landolt and I. Tomka. 1988. Morphology and taxonomy of imagines and eggs of Central and Northern European Siphlonuridae (Ephemeroptera). *Mitt. Schweiz. Entomol. Ges.* 61: 303-328.

Studemann, D. and I. Tomka. 1991. European Siphlonuridae (Ephemeroptera): a phylogenetic system for the four genera. P. 103-114 in J. Alba-Tercedor and A. Sanchez-Ortega (Eds.), *Overview and Strategies of Ephemeroptera and Plecoptera.* Gainesville: Sandhill Crane Press, Inc.

Studemann, D., I. Tomka and P. Landolt. 1992. Revision of the Iberian Siphlonuridae (Ephemeroptera). *Aquatic Insects* 14: 193-209.