

# EGGS OF EPHEMERELLIDAE (EPHEMEROPTERA)

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The eggs of thirty one-species of Palaearctic and Nearctic Ephemerellidae are investigated by scanning electron microscopy. The absence of a polar cap in *Eurylophella* and the presence of two polar caps in *Timpanoga* are confirmed. All the other species studied present one polar cap. The chorionic pattern can not be used to solve generic problems in the taxonomy of Ephemerellidae.

## INTRODUCTION

The taxonomic and phylogenetic problems inherent in the family of Ephemerellidae have been exposed e.g. by MCCAFFERTY (1991) and STUEDEMANN *et al.* (1995). The *Timpanoga* complex was clarified by MCCAFFERTY & WANG (1994) and the fourteen species of Nearctic *Eurylophella* were revised by FUNK & SWEENEY (1994). JACOB (1993) suggested a new classification for twelve European species.

The eggs structures have been used in many taxonomic works, in most cases for other families than Ephemerellidae (e.g. KOSS & EDMUNDS, 1974; KOPELKE, 1980; KOPELKE & MÜLLER-LIEBENAU, 1981a, 1981b, 1982; MALZACHER, 1982; GAINO & MAZZINI, 1984; MAZZINI & GAINO, 1985; GAINO *et al.*, 1987, 1989; STUEDEMANN *et al.*, 1988; KLUGE *et al.*, 1995). During the VIIth Conference on Ephemeroptera 1992 in Maine, we presented the first series of Ephemerellidae eggs (STUEDEMANN *et al.*, 1995). Later on, colleagues sent us many other specimens, from Nearctic and Transpalaearctic areas. The aim of the present study is to complete our knowledge of Ephemerellidae eggs in order to use egg structures for further taxonomic or phylogenetic work.

## MATERIAL AND METHODS

The thirty one species studied are listed in Table 1. The material is deposited in the Entomological Department (cf. address of the authors). The eggs were extracted from female imagines, except those in Figs 23-24 taken from *Ephemerella mesoleuca* larva and subimago respectively. They were prepared for the scanning electron microscopy (SEM) as described by STUEDEMANN *et al.* (1987).

## RESULTS AND DISCUSSION

### *Egg structure and variability*

The thirty one species investigated are shown on Figs 1-54. According to KOSS & EDMUNDS (1974), the polar cap of Ephemerellidae eggs is composed of compressed, non-coiled threads (visible in Fig. 24). Like the knob-terminated-coiled threads (=KCT, e.g. Fig. 29), they are probably used for the egg attachment on the substrate. The absence of polar cap and KCT on the eggs of *Eurylophella* species (Figs 1-8) could be linked to their habitat, rivers with a relatively low current speed. GAINO & BONGIOVANNI (1992) also mentioned the possibility of adaptation of the adhesive structures for survival.

Adhesive structures in one species can sometimes be present in different arrangements: a row of KCT basically around the polar cap of *Serratella albai* (Fig. 28) and *S. deficiens* are present on some eggs but not on others in the same female. Specimens of different populations showed this phenomenon.

The form and the length of the polar cap can differ from an egg to another for *Serratella ignita*, as explained by GAINO & BONGIOVANNI (1992). We observed these variations also in *Drunella cornutella*, *D. lepnevae*, *D. submontana*, *Ephemerella ikononovi*, *E. mesoleuca*, *E. nuda*, *E. setigera*, *E. zapekinae*, *Ephemerella* sp. 1, *Ephemerella* sp. 2, and *Serratella hispanica*.

Eggs extracted from larvae, subimagines or imagines are identical in general shape and chorionic structure for *Ephemerella mesoleuca* (Figs 23-25), *Torleya major* and *Serratella ignita*. This situation appears for *Dolania* and *Ephemerella* (Soldan, personal communication).

On the other hand, the eggs of *Ameletus* (KANG & YANG, 1994) and *Paraleptophlebia* (SOLDAN, 1979) change their shape in subimaginal and imaginal stages. The regular shape is achieved after the oviposition, due to the hydration (Soldan, personal communication).

The eggs can be covered in the oviduct with a thick epithelium whose fragments are shown in Fig. 26. The chorion of investigated eggs is composed of two layers (Figs 32, 34, 44). The outer one is built up by rod-like sticks (Figs 27, 31, 36) and covered with a thin epithelium (Fig. 34). The

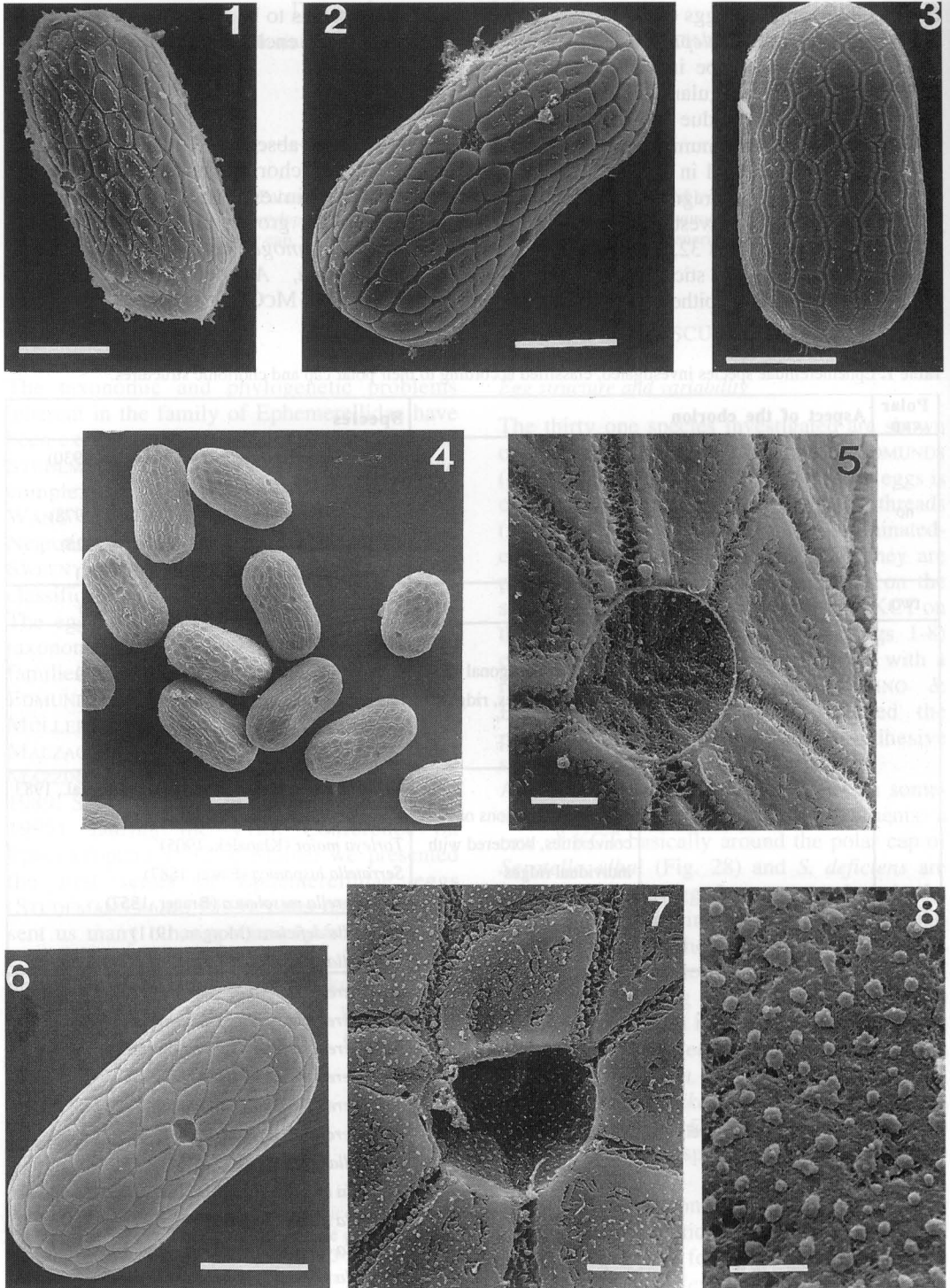
inner layer seems to be formed by a homogeneous material and encloses the yolk substances.

#### Classification

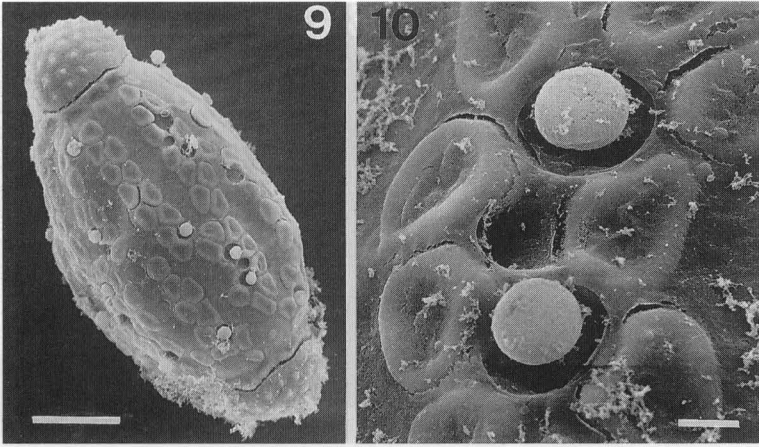
The presence or absence of polar cap(s) and the aspect of the chorion surface allow us to classify the investigated Ephemerellidae species into six groups of species (Table 1). For the *Timpanoga* complex (*Eurylophella*, *Timpanoga*, *Attenella*), we use the nomenclature of McCAFFERTY & WANG (1994).

**Table 1.** Ephemerellidae species investigated, classified according to their polar cap and chorionic structures.

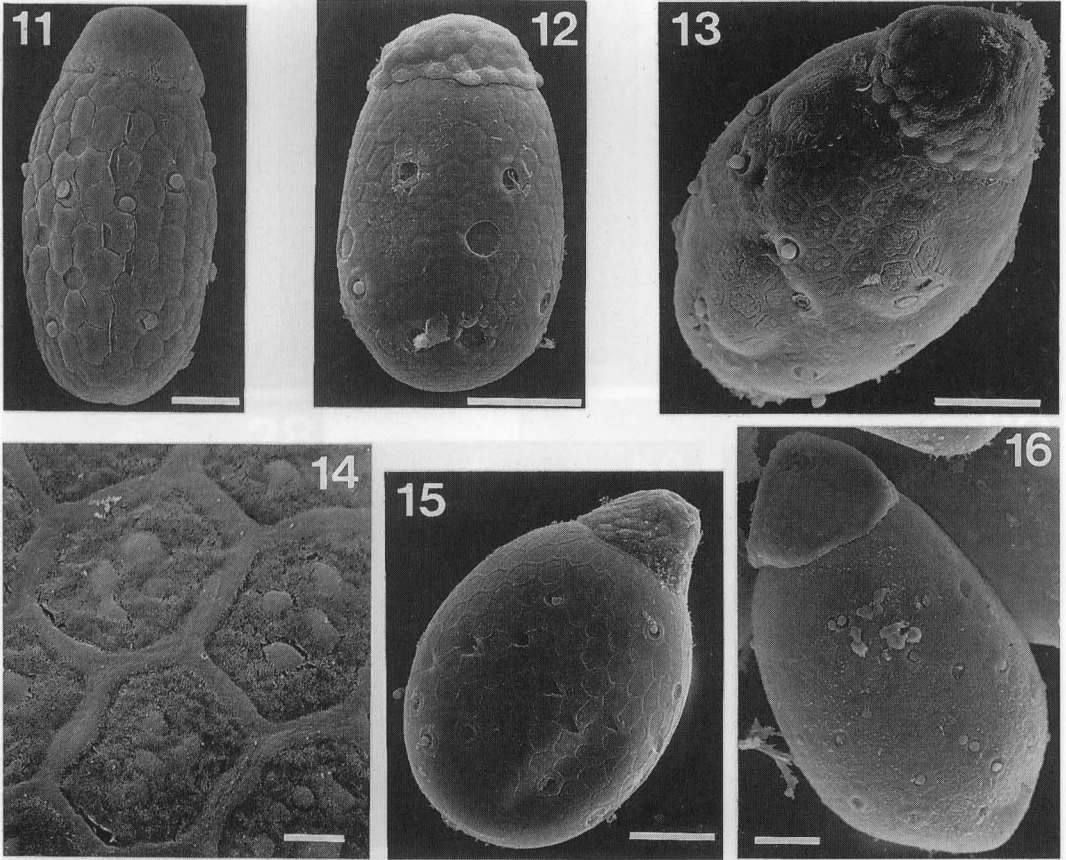
Polar cap	Aspect of the chorion		Species
no	polygonal plates, bordered or not		<i>Eurylophella verisimilis</i> (McDunnough, 1930) <i>Eurylophella karelica</i> Tiensuu, 1935 <i>Eurylophella iberica</i> (Keff. & Da Terra, 1978) <i>Eurylophella funeralis</i> (McDunnough, 1925) <i>Eurylophella aestiva</i> (McDunnough, 1931)
two	longitudinal rows of scales		<i>Timpanoga simplex</i> (McDunnough, 1925)
one	geometrical macrorelief covering the chorion (visible by light microscopy)	juxtaposed hexagonal structures (scales, ridges, depressions)	<i>Ephemerella notata</i> Eaton, 1887 <i>Ephemerella mucronata</i> (Bengtsson, 1909) <i>Attenella attenuata</i> (McDunnough, 1925) <i>Attenella margarita</i> (Needham, 1927) <i>Ephemerella nuda</i> Tschernova, 1952
		circular depressions or convexities, bordered with individual ridges	<i>Drunella paradinasi</i> Gonz. Tan. & Gar. Jal., 1983 <i>Ephemerella ikononovi</i> Puthz, 1971 <i>Torleya major</i> (Klapalek, 1905) <i>Serratella hispanica</i> (Eaton, 1887) <i>Ephemerella mesoleuca</i> (Brauer, 1857)
		net like ridges all over the surface	<i>Serratella deficiens</i> (Morgan, 1911) <i>Serratella albai</i> Gonz. Tan. & Gar. Jal., 1984
one	no geometrical macrorelief, chorion uniform		<i>Ephemerella zapkinae</i> Bajkova, 1967 <i>Ephemerella</i> sp. 2, from Kazakhstan <i>Ephemerella setigera</i> Bajkova, 1965 <i>Ephemerella kozhovi</i> Bajkova, 1967 <i>Ephemerella aurivillii</i> Bengtsson, 1908 <i>Ephemerella</i> sp. 1, from Pamyr <i>Serratella ignita</i> (Poda, 1761) <i>Drunella lata</i> (Morgan, 1919) <i>Drunella lepnevae</i> (Tschernova, 1949) <i>Drunella cornutella</i> (McDunnough, 1931) <i>Drunella submontana</i> (Brodsky, 1930) <i>Drunella coloradensis</i> (Dodds, 1923) <i>Drunella doddsi</i> (Needham, 1927)



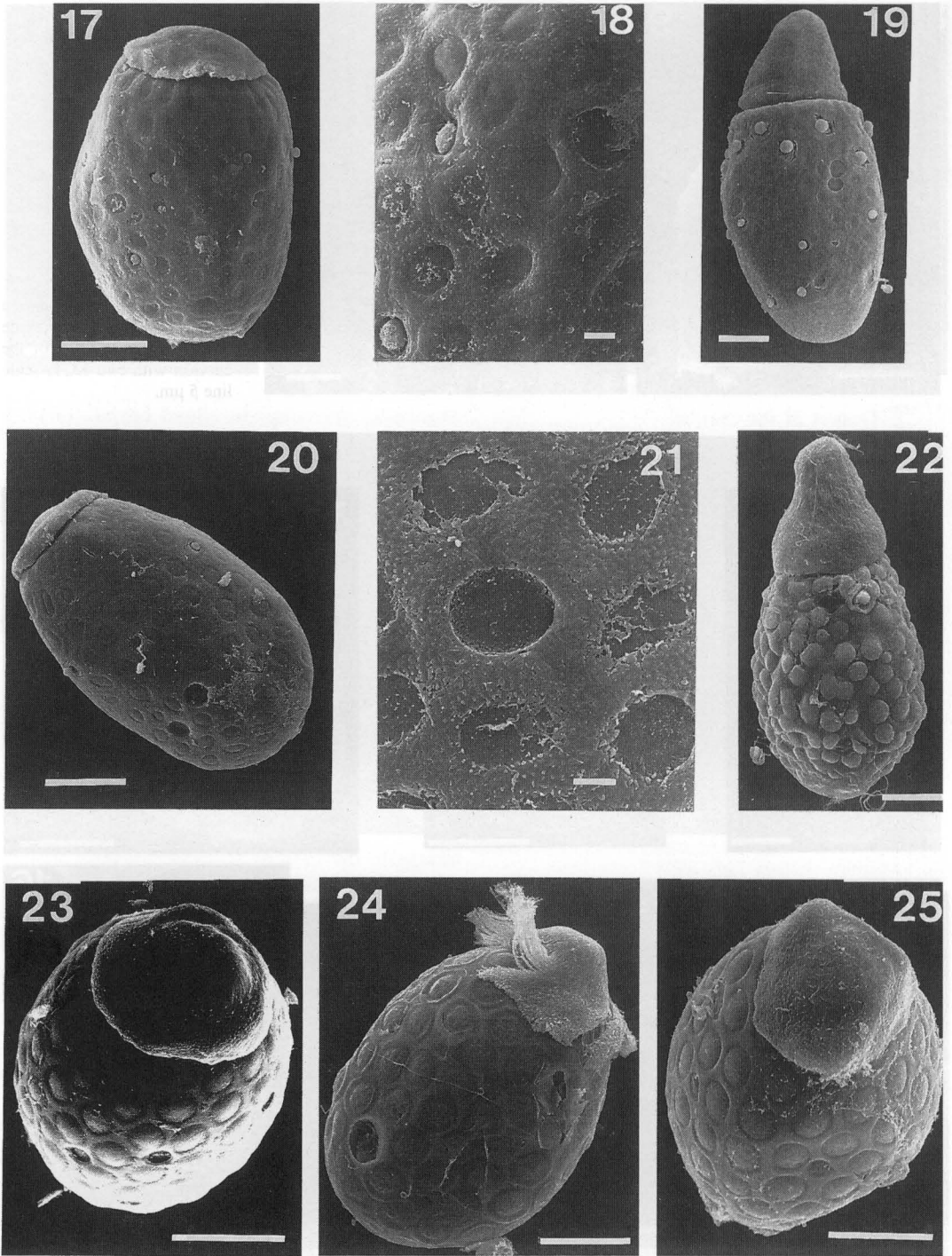
**Figs 1-8.** Eggs of *Eurylophella*. 1: *E. verisimilis*; 2: *E. karelica*; 3: *E. iberica*; 4-5: *E. funeralis*; 6-8: *E. aestiva*. 1, 2, 3, 4, 6: general view, scale line 50  $\mu\text{m}$ ; 5, 7: detail of chorion with micropyle, scale line 5  $\mu\text{m}$ ; 8: ultrastructure of the chorion, scale line 1  $\mu\text{m}$ .



**Figs 9-10.** Egg of *Timpanoga simplex*. 9: general view, scale line 50  $\mu$ m; 10: detail of the chorion with two KCT, scale line 5  $\mu$ m.



**Figs 11-16.** Eggs. 11: *Ephemerella notata*; 12: *E. mucronata*; 13-14: *Attenella attenuata*; 15: *A. margarita*; 16: *Ephemerella nuda*. 11, 12, 13, 15, 16: general view, scale line 50  $\mu$ m, 14: detail of the chorion, scale line 5  $\mu$ m.



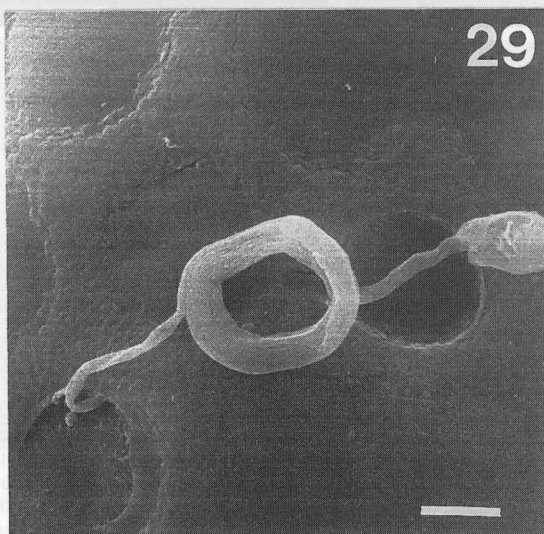
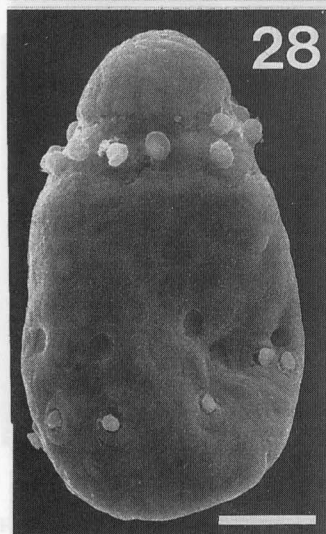
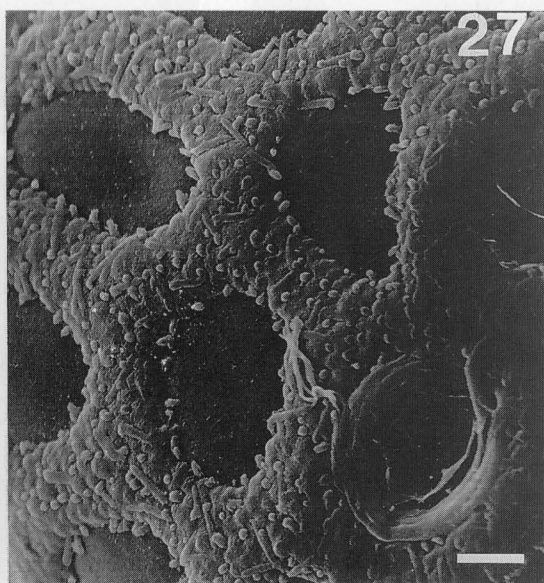
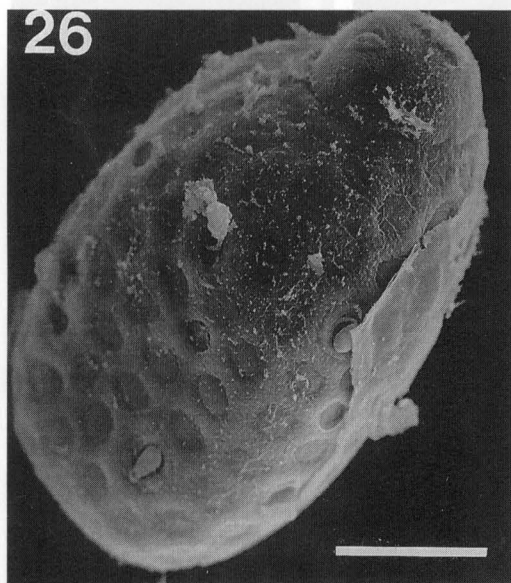
**Figs 17-25.** Eggs. 17-18: *Drunella paradinasi*; 19: *Ephemerella ikonomovi*; 20-21: *Torleya major*; 22: *Serratella hispanica*; 23-25: *Ephemerella mesoleuca*, egg extracted from 23: larva, 24: subimago, 25: imago. 17, 19, 20, 22, 23, 24, 25: general view, scale line 50  $\mu$ m; 18, 21: detail of the chorion, scale line 5  $\mu$ m.



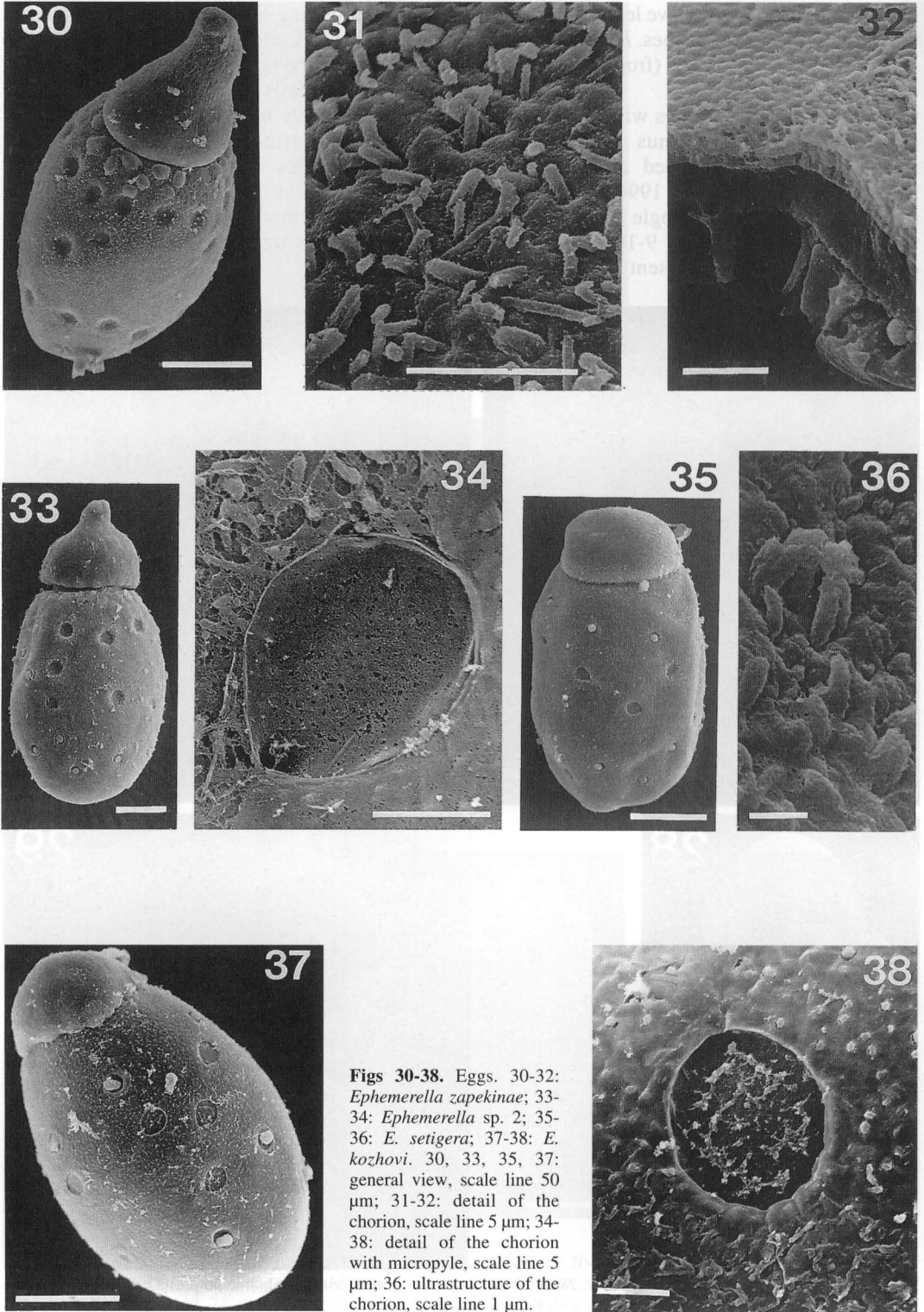
For the other species, we have left the current or the most recent generic names. *Ephemerella* sp. 1 (from Pamyр) and sp. 2 (from Kazakhstan) are undescribed now.

Only the first group squares with a taxonomic existing group: the genus *Eurylophella*, relatively easily recognized at the generic level (FUNK & SWEENEY, 1994). *Timpanoga* (*Danella*) *simplex* is the single studied species with two polar caps (Figs 9-10). In the four other groups, the eggs present one polar cap.

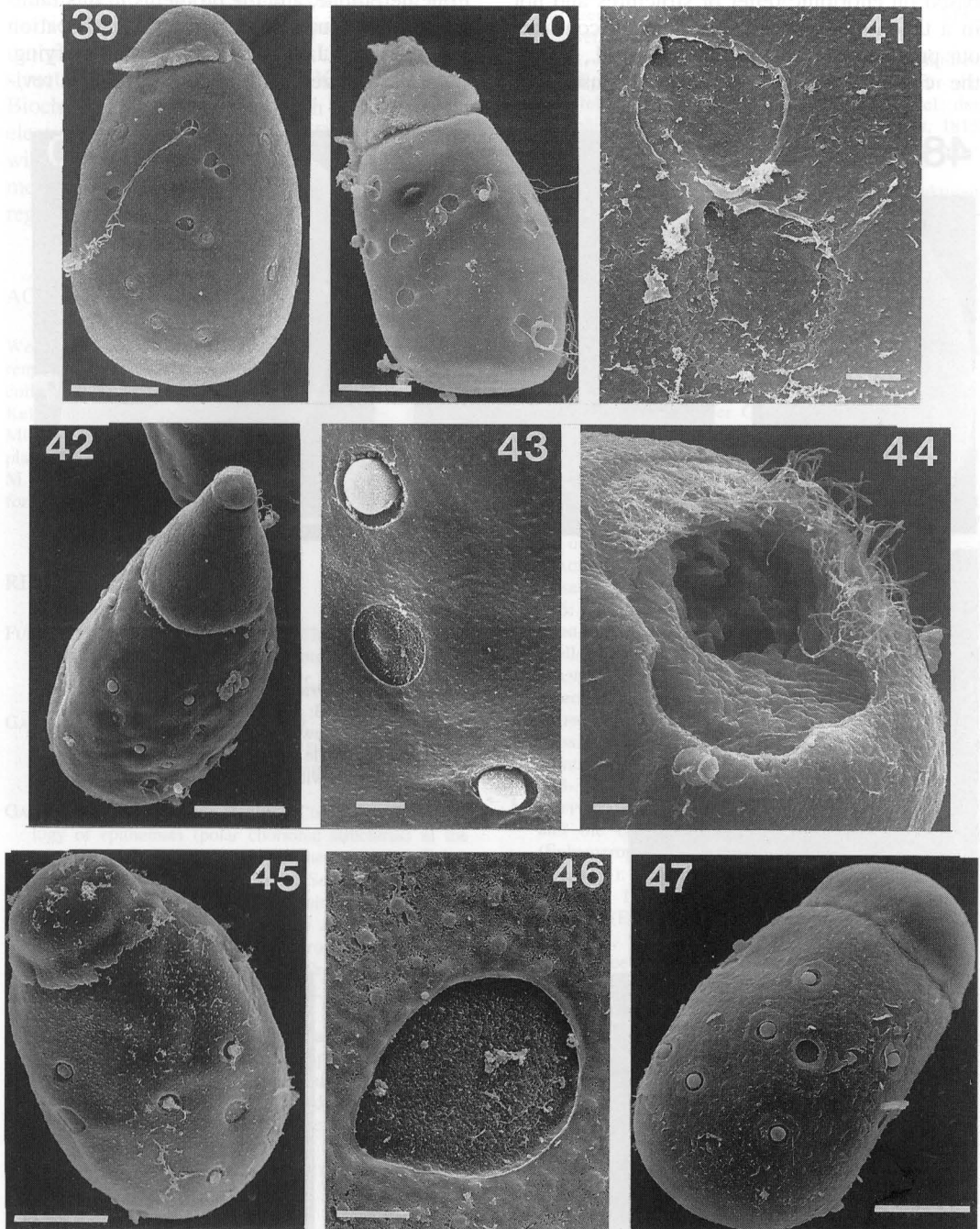
This is surprising only for *Attenella attenuata* (Fig. 13) and *A. margarita* (Fig. 15), because in this genus, the eggs are supposed to lack the polar cap (MCCAFFERTY & WANG, 1994). The *Drunella* species investigated present an uniform chorion surface without any geometrical macrorelief (Figs 45-54). No wonder that *D. paradinasi* (Figs 17-18) is different! The application of this generic name is probably not correct (Kluge, personal communication).



Figs 26-29. Eggs. 26-27: *Serratella deficiens*; 28-29: *S. albai*. 26, 28: general view, scale line 50  $\mu$ m; 27: detail of the chorion, scale line 5  $\mu$ m. 28: detail of the chorion with KCT, scale line 5  $\mu$ m.



**Figs 30-38.** Eggs. 30-32: *Ephemerella zapekinae*; 33-34: *Ephemerella* sp. 2; 35-36: *E. setigera*; 37-38: *E. kozhovi*. 30, 33, 35, 37: general view, scale line 50  $\mu$ m; 31-32: detail of the chorion, scale line 5  $\mu$ m; 34-38: detail of the chorion with micropyle, scale line 5  $\mu$ m; 36: ultrastructure of the chorion, scale line 1  $\mu$ m.

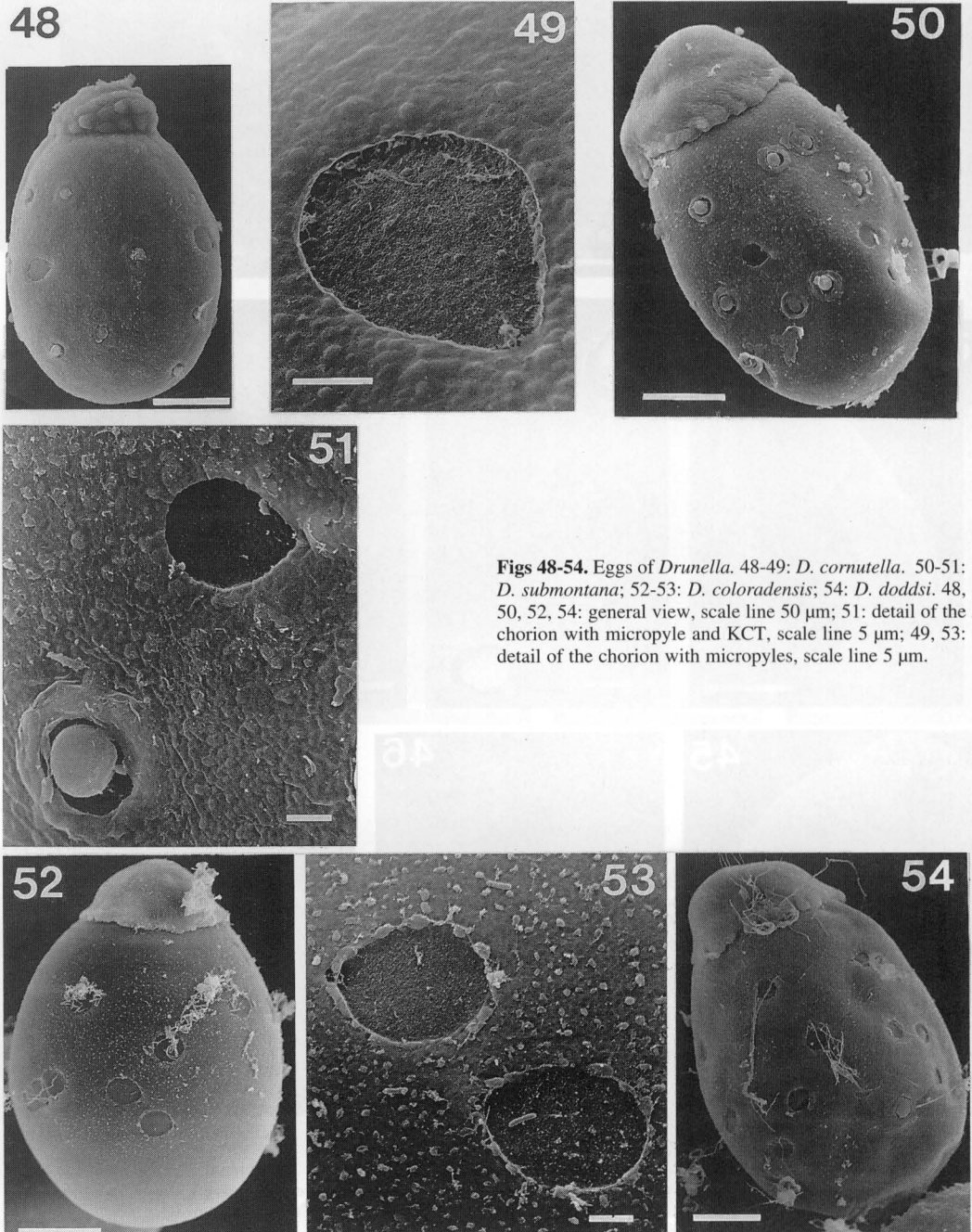


**Figs 39-47.** Eggs. 39: *Ephemerella aurivillii*; 40-41: *Ephemerella* sp. 1; 42-44: *Serratella ignita*. 45, 46: *Drunella lata*; 47: *D. lepnevae*. 39, 40, 42, 45 47: general view, scale line 50  $\mu\text{m}$ ; 41: detail of the chorion with micropyles, scale line 5  $\mu\text{m}$ ; 43: detail of the chorion with micropyle and KCT, scale line 5  $\mu\text{m}$ ; 46: detail of the chorion with micropyle, scale line 5  $\mu\text{m}$ .



For the eggs with one polar cap, the groups are based on chorionic relief or structures and not in a taxonomic meaning. This study confirms our previous results (STUEMANN *et al.*, 1995): the chorionic pattern can not be used for

taxonomic purposes at generic level in the Ephemerellidae, and the problems in this family remain unsolved. The classification suggested by JACOB (1993) is not satisfying. Even if focalized on European species, a revi-



**Figs 48-54.** Eggs of *Drunella*. 48-49: *D. cornutella*. 50-51: *D. submontana*; 52-53: *D. coloradensis*; 54: *D. doddsi*. 48, 50, 52, 54: general view, scale line 50  $\mu$ m; 51: detail of the chorion with micropyle and KCT, scale line 5  $\mu$ m; 49, 53: detail of the chorion with micropyles, scale line 5  $\mu$ m.

sion of the Ephemerellidae should include the Transpalaeartic species and comparisons with the Nearctic species. We have to investigate more morphological characters, including internal structures and pterothorax (KLUGE, 1994). Biochemical investigations such as isoenzyme electrophoreses and analysis of DNA sequences will help to solve the problematics in Ephemerellidae. Moreover, the characters should be regarded from a phylogenetic point of view.

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#### REFERENCES

- FUNK, D.H. & SWEENEY, B.W. 1994. The larvae of Eastern North American *Euryophella* TIENSUU (Ephemeroptera: Ephemerellidae). Trans. Amer. Ent. Soc. 120(3): 209-286.
- GAINO, E., BELFIORE, C. & MAZZINI, M. 1987. Ootaxonomic investigation of the Italian species of the genus *Electrogena* (Ephemeroptera, Heptageniidae). Boll. Zool. 54: 169-175.
- GAINO, E. & BONGIOVANNI, E. 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. & MAZZINI, M. 1984. Scanning electron microscope study of the eggs of some *Habrophlebia* and *Habroleptoides* species (Ephemeroptera, Leptophlebiidae). In: LANDA, V. et al. (Eds), Proc. IVth Int. Conf. Ephemeroptera. CSAV, pp. 193-202.
- GAINO, E., MAZZINI, M., DEGRANGE, C. & SOWA, R. 1989. Etude en microscopie à balayage des œufs de quelques espèces de *Rhithrogena* EATON groupe *alpestris* (Ephemeroptera, Heptageniidae). Vie Milieu 39 (3/4): 219-229.
- JACOB, U. 1993. Zur Systematik und Verbreitung der europäischen Ephemerellidae (Ephemeroptera). Verh. Westd. Ent. Tag. 1992: 101-110.
- KANG, S.C. & YANG, C.T. 1994. Three new species of the genus *Ameletus* from Taiwan (Ephemeroptera: Siphonuridae). Chinese J. Entomol. 14: 261-269.
- KLUGE, N.J. 1994. Pterothorax structure of mayflies (Ephemeroptera) and its use in systematics. Bull. Soc. Ent. France 99(1): 41-61.
- KLUGE, N.J., STUEDEMANN, D., LANDOLT, P. & GONSER, T. 1995. A reclassification of Siphonuroidea (Ephemeroptera). Mitt. Schweiz. Ent. Ges. 68: 103-132.
- KOPELKE, J.P. 1980. Morphologische Studien an den Eiern der Eintagsfliegen (Ephemeroptera) aus der Emergenz der zentralafrikanischen Bergbaches Kalengo. Mitt. Schweiz. Ent. Ges. 53: 297-311.
- KOPELKE, J.P. & MÜLLER-LIEBENAU, I. 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. & MÜLLER-LIEBENAU, I. 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: *bucерatus*-, *atrebatinus*-, *niger*-, *gracilis*- und *muticus*-Gruppe. Deutsche Entomol. Zeitschrift 28: 1-6.
- KOPELKE, J.P. & MÜLLER-LIEBENAU, I. 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. & EDMUNDS, G.F. 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. & GAINO E. 1985. Fine structure of the egg shells of *Habrophlebia fusca* (CURTIS) and *H. consiglioi* BIANCHERI (Ephemeroptera, Leptophlebiidae). Int. J. Insect. Morphol. Embryol. 14: 327-334.
- MCCAFFERTY, W.P. 1991. Toward a phylogenetic classification of the Ephemeroptera (insecta): a commentary on systematics. Ann. Ent. Soc. Amer. 84(4): 343-360.
- MCCAFFERTY, W.P. & WANG, T.Q. 1994. Phylogenetics and the classification of the *Timpanoga* complex (Ephemeroptera: Ephemerellidae). J. N. Am. Benthol. Soc. 13(4): 569-579.
- SOLDAN, T. 1979. Spermatogenesis and oogenesis in mayflies (Ephemeroptera). In: PASTERNAK, K. & SOWA, R. (Eds), Proceedings of the second International Conference on Ephemeroptera. Warszawa-Krakow, pp. 267-272.
- STUEDEMANN, D., LANDOLT, P. & TOMKA, I. 1987. Complément à la description de *Arthroplea congener* BENTGSSON, 1908 (Ephemeroptera) et à son statut systématique. Bull. Soc. Frib. Sc. Nat. 76: 144-167.
- STUEDEMANN, D., LANDOLT, P. & TOMKA, I. 1988. Morphology and taxonomy of imagines and eggs of Central and Northern European Siphonuridae (Ephemeroptera). Mitt. Schweiz. Ent. Ges. 61: 303-328.
- STUEDEMANN, D., LANDOLT, P. & TOMKA, I. 1995. Eggs of European Ephemerellidae (Ephemeroptera). In: CORNUM, L.D. & CIBOROWSKI, J.H. Current directions in research on Ephemeroptera, pp. 407-422. Canadian Scholars' Press Inc., Toronto.