Proc. IVth Intern.Confer.Ephemeroptera
V.Landa et al.(eds.),193-202,ČSAV,1984

SCANNING ELECTRON MICROSCOPE STUDY OF THE EGGS OF SOME HABROPHLEBIA AND HABROLEPTOIDES SPECIES (EPHEMEROPTERA, LEPTOPHLEBIIDAE)

E. Gaino[1] and M. Mazzini[2]

[1]Instituto di Zoologia dell'Università, Via Balbi, 16126 Genova, Italy
[2]Instituto di Zoologia dell'Universià, Via Mattioli, 53100 Siena, Italy

Abstract. The eggs of some species belonging to the genus Habrophlebia and Habroleptoides have been investigated by a scanning electron microscope /SEM/. SEM investigation carried out on species of Habrophlebia, namely H. fusca, H. lauta, H. consiglioi and on species of Habroleptoides, namely H. modesta, H. auberti, H. umbratilis, allowed to point out some differences in the structural organisation of eggs in both genera. Number, dimension and lokalisation of the micropylar orifices appear to be similar in Habrophlebia and Habroleptoides, while the external chorionic pattern indicates that the sculpturing of the eggs seems to be species-specific. The longitudinal organisation of cristae - thin in Habroleptoides and wider in Habrophlebia - is typical for all the examined species, except Habroleptoides auberti which shows polygonal structures coating the egg. Egg chorion characteristics appear to be of high taxonomic value in Ephemeroptera as in many other insect orders.

Chorion, micropyle, taxonomic value, 8 spp.

Studies carried out on the eggs of Ephemeroptera pointed out that they present morphological characteristics which can be utilized in taxonomic investigations. Moreover, these female gametes can help to identify imagoes or still immature stages in which the commonly used features do not allow a specific screening to be realized (Koss 1968).

Observations in light microscopy on the morphology of the eggs were formerly carried out by Bengtsson (1913), Morgan (1913) and Smith (1935), and more recently by Degrange (1960), Koss (1968) and Koss and Edmunds (1974). Such investigations also made it possible to draw up taxonomic keys based upon the organisation of the chorion and to put forward some phylogenetic hypotheses.

The scanning electron microscope (SEM) contributed to a better morphological definition of the shell sculpture, giving detailed information on micropylar apparatus, polar caps, accessory structures and adhesive layers. All this allowed to shed light on the fine organisation of eggs in a high number of species belonging to many insect orders (for a cfr. see Hinton, 1981).

SEM was used with Ephemeroptera to point out the fine egg morphology in many species of Baetidae (Kopelke and Müller-Liebenau 1981a, b, 1982) and Caenidae (Malzacher 1982), and in 14 other species belonging to four different families collected in Central Africa (Kopelke 1980). It should be noted that scanning electron micrographs of eggs have been frequently included in many works of systematics over the last few years (Flowers 1980, Pescador and Peters 1982, Landa and Soldán 1982), further confirming that also the exochorion organisation is taken into consideration along with the other taxonomic features.

As Leptophlebiidae eggs show a remarkable differentiation in exochorionic sculpturing and in attachment structures (Koss and Edmunds 1974), this investigation refers to 6 species belonging to the genera Habrophlebia and Habroleptoides in order to point out the characteristics which can be utilized in specific discrimination.

MATERIAL AND METHODS

Source of eggs

Eggs were taken by dissecting mature nymphs and imagoes collected in Italy or coming from various European localities.

Habrophlebia fusca (Curtis) - coll. of C. Belfiore, Lazio; coll. of E. Gaino, Piemonte; coll. of U. Jacob, Dresden.

Habrophlebia lauta Eaton - coll. of U. Jacob, ČSSR; coll. of R. Sowa, Poland.

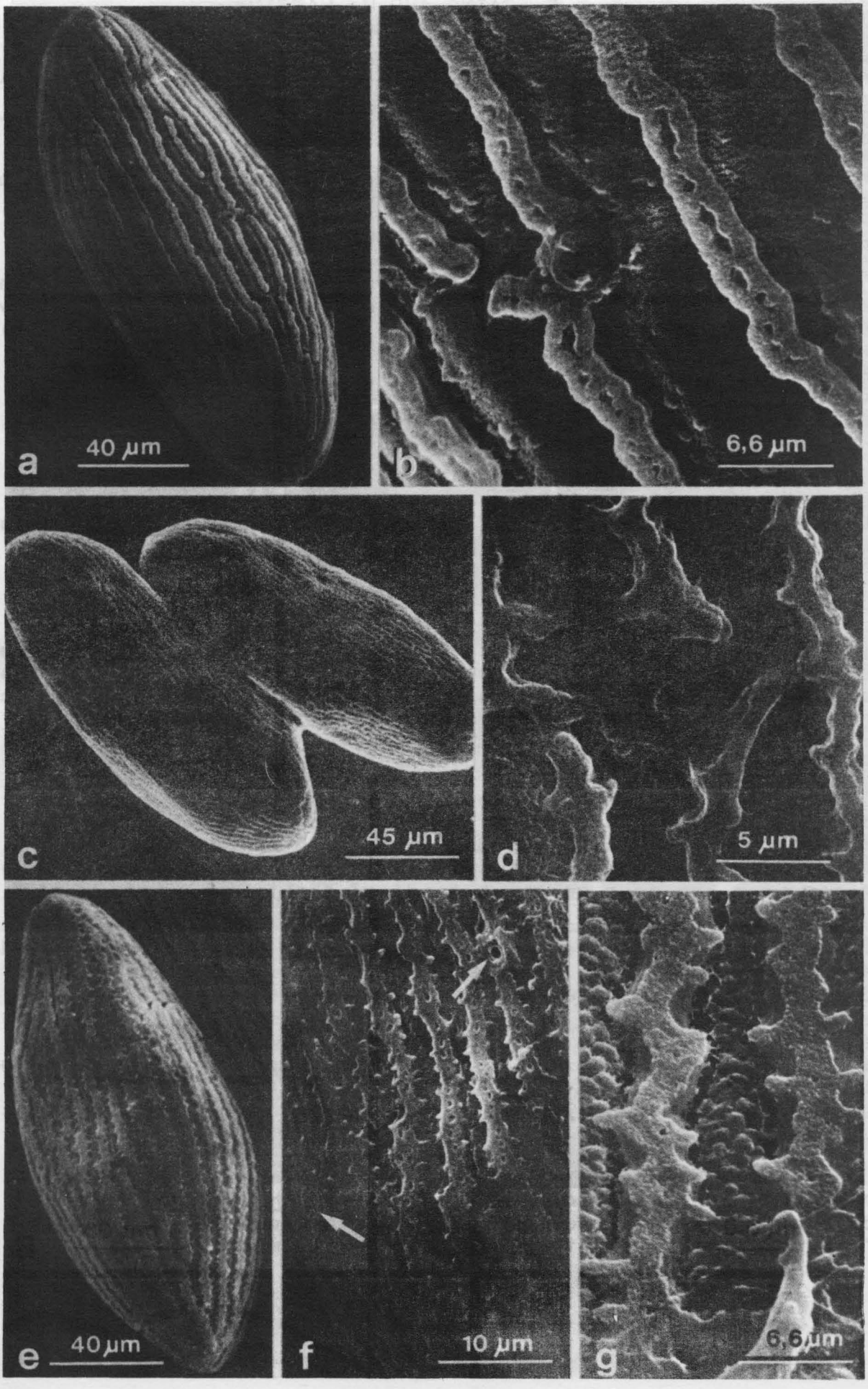
Habrophlebia consiglioi Biancheri - coll. of E. Biancheri, Sardegna.

Habrophlebia sp. - coll. of E. Gaino, Sardegna.

Habroleptoides modesta (Hagen) - coll. of E. Gaino, Piemonte; coll. of C. Belfiore, Lazio.

Habroleptoides umbratilis (Eaton) - coll. of E. Biancheri, Liguria. The systematic position of this species has been recently defined by Belfiore (1983).

Fig. 1: Habrophlebia. H. fusca /a-b/ a, egg; b, chorion and micropyle; H. lauta /c-d/ c, egg; d, chorion and micropyle; H. consiglioi /e-f/ e, egg; f, chorion and micropyles /arrows/; H. sp. /Sardegna/ /g/ g, chorion.


a
40 µm
b
6,6 µm
c
45 µm
d
5 µm
e
40 µm
f
10 µm
g
6,6 µm

Habroleptoides gr. umbratilis (Eaton) - coll. of C. Belfiore, Lazio; coll. of E. Gaino, Ligurian Alps.

Habroleptoides auberti (Biancheri) - coll. of E. Biancheri, Switzerland.

For each species the average egg size was calculated from about 10 measurements.

Preparation of the eggs

For scanning electron microscopy, eggs conserved in alcohol or fixed for 2 hr in 4 % glutaraldehyde and 5 % paraformaldehyde in 0.1 M cacodilate buffer, pH 7.2 at 4°C (Karnovsky 1965), were dried by the critical point method using liquid CO_2 in a Bomar apparatus, attached to specimen holders by silver conducting paint, coated with gold in a Balzers Union evaporator and observed with a JEOL JSM 2. Some specimens were observed without critical point treatment.

Terminology

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

OBSERVATIONS

It is firstly important to note that all of the Leptophlebiidae eggs examined in this research have an ovoidal shape and appear lacking in polar caps and in accessory attachment structures.

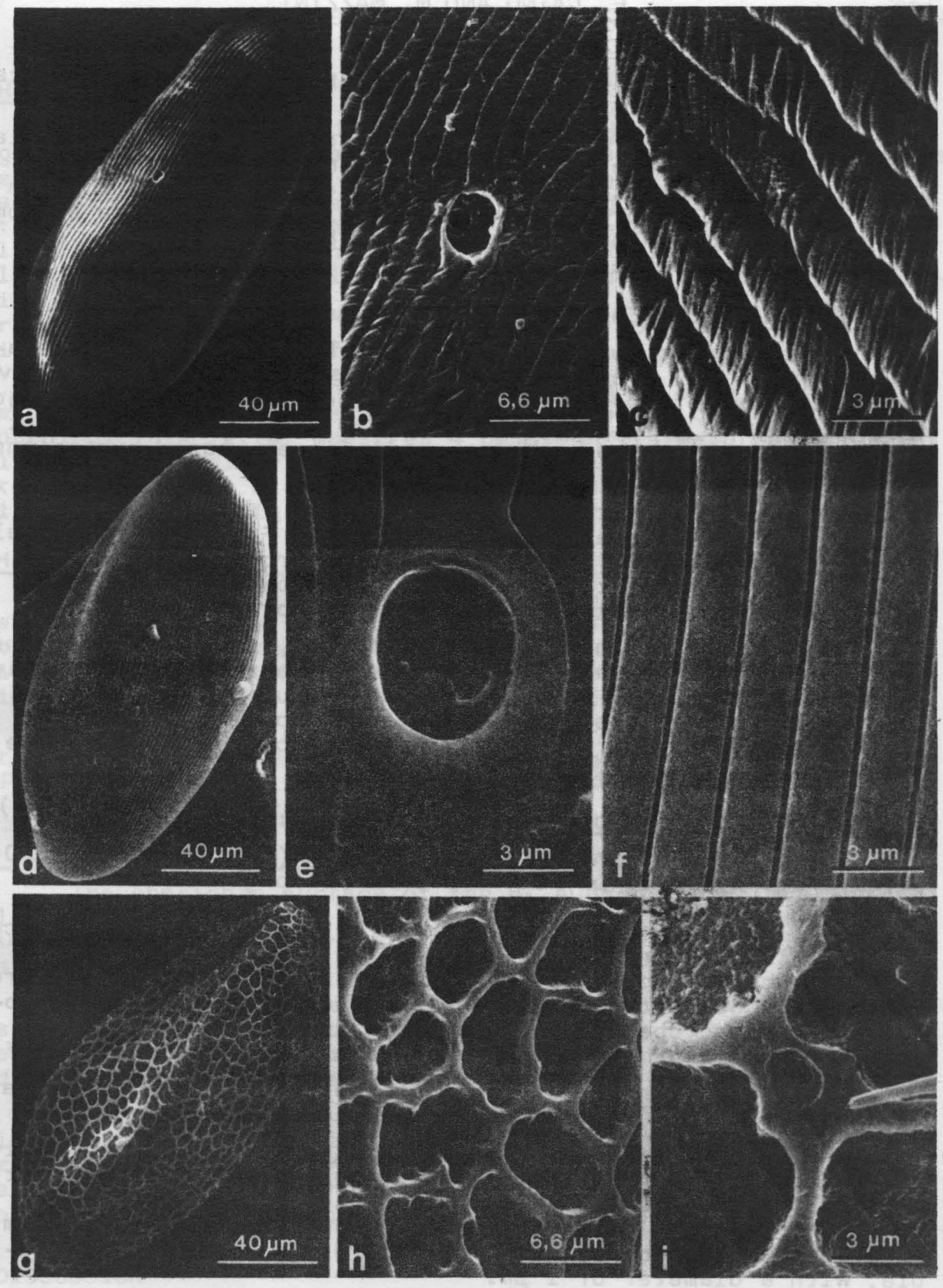
Habrophlebia fusca (Curtis)(Table I: Figs. a, b)

When examined under the SEM, H. fusca eggs (average length 185 µm, average width 75 µm) present a pattern consisting of numerous longitudinal raised costae which can sometimes converge (Table I: Fig. d). Each costa, about 2.7 µm wide is separated from the next one by a space of about 6 - 7 µm.

The length of each costa is variable: some of them are as long as the egg, while others stretch over more or less extended tracts. Costae are not straight but have a sinuous pattern due to irregular projections and indentations along their outside margin (Table I: Fig. b). The dorsal surface of the costae shows many orifices with a diameter of 0.3 µm, interspaced by about 1 µm, but sometimes united to make up a sort of canaliculus (Table I: Fig. b). The spaces between the raised costae show a finely granular matrix from which rounded protuberances

---

Fig. 2: Habroleptoides. H. modesta /a-c/ a, egg; b, chorion and micropyle; c, chorionic detail; H. umbratilis /d-f/ d, egg; e, micropyle detail; f, chorionic detail; H. auberti /g-i/ g, egg; h, chorion sculpturing; i, micropyle detail.


a
40 µm
b
6,6 µm
c
3 µm
d
40 µm
e
3 µm
f
3 µm
g
40 µm
h
6,6 µm
i
3 µm

having a diameter of 1 µm stand out, especially near the costae (Table I; Fig. b).

The micropyle (Table I: Fig. b), located in the equatorial or subequatorial zone, consists of a ring with a diameter of 3 - 4 µm, the central part of which directly leads to a sperm guide (chorionic depression without sculptures). At the bottom of such depression a micropylar opening having a diameter of about 1 µm is visible.

Habrophlebia lauta Eaton (Table I: Figs. c, d)

The chorionic outside surface of the H. lauta egg (average length 190 µm, average width 72 µm) shows raised costae having a diameter of 1.7 µm arranged in the direction of the major egg axis (Table I: Fig. c). Such costae, interspaced by about 4 - 5 µm, may converge and show small finger-shaped expansions along their margins (Table I: Fig. d).

The chorionic outside surface shows a finely granulated aspect in the intercostal spaces (Table I: Fig. d). The dorsal surface of the costa (Table I: Fig. d) is rough and the fine sculpturing, similar to that of the intercostal spaces, does not presents the openings observed in the previous species.

The micropyle, in the equatorial zone, is generally found in the intercostal space or in a zone where the costal line is interrupted (Table I: Fig. d); the apparatus consists of a ring having diameter of 4 - 5 µm with an opening of 1.5 µm leading to a funnelform depression at the bottom of which the micropylar orifice is visible.

Habrophlebia consiglioi Biancheri (Table I: Figs. e, f)

The chorion of the H. consiglioi egg (average length 230 µm, average width 97 µm) shows 4 µm wide longitudinal costae coverging in some areas and interspaced by up to about 5 µm (Table I: Fig. e). Each costa is characterized by the presence of lateral spiniform expansions protruding over about 2 µm into intercostal space. In areas where such formations are present, distances between costae are reduced to 1 - 2 µm. In zones without protuberances the surface is uniformly granular with elements having a diameter of about 1 µm (Table I:Fig. f).

The dorsal surface of the costae shows openings having a diameter of 1 µm and interspaced by 2 µm.

One or more subequatorial micropyles can be found usually in the zone of costal line interruption (Table I: Fig. f) but also in the intercostal space. Micropyles appear constituted by a ring having a diameter of 5 µm leading into a funnelform cavity at the bottom of which the micropylar opening is visible, with a diameter of 1 µm.

SEM investigations conducted on eggs taken from Habrophlebia sp. nymphs from Sardegna, allowed us to attribute these specimens to H. consiglioi on the base of the chorionic fine structure (Table I: Fig. g). The aquatic stage of the species was still unknown (Gaino, in prep.).

Habroleptoides modesta (Hagen)(Table II: Figs. a, b, c)

The chorionic outside surface of the H. modesta egg (average length 180 µm, average width 76 µm) is completely run across slightly raised costae which sometimes converge (Tabl II: Fig. a). Each costa, which is about 1.8 µm long, runs very close to the adjacent ones so that the interspaces are very small (Table II: Fig. b). Each formation is slightly twisted along its longitudinal axis (Table II: Fig. b) and, at great magnification, such organisation appears as a thin banding. The banding results oblique to the major axis of the costae and repeating itself with a period of 0.4 µm (Table II: Fig. c).

Most of the examined eggs of H. modesta show at least two equatorial micropyles each of them consisting of an elliptical opening. This latter measures 4 µm along its major axis and 1.5 along the minor one, and leads to a depression at the bottom of which the micropylar orifice opens with a diameter of 1 µm (Table II: Fig. b).

Habroleptoides umbratilis (Eaton)(Table II: Figs. d, e, f)

The H. umbratilis egg (average length 190 µm, average width 92 µm) shows slightly raised costae arranged very close to each other on the chorion (Table II: Fig. d). Each costa is 1.6 µm wide and, unlike the previous species, shows no banding (Table II: Fig. f).

Most examined eggs present two micropyles placed in equatorial position. The edgeless aperture leading to the micropylar opening has an elliptic shape and measures 5 x 3 µm (Table II: Fig. e).

Habroleptoides auberti (Biancheri)(Table II: Figs. g, h, i)

The chorionic surface of the H. auberti eggs (average length 178 µm, average width 82 µm) shows a reticulation made up of irregular polygons the sides of which vary in length but are about 1 µm wide and thick (Table II: Fig. g).

The matrix of the areas defined by polygonal formations shows a fine granulation (Table II: Fig. h). Along the inside edges of the polygons it is possible to see openings with the diameter of 1 µm interspaced by about 2 µm (Table II: Fig. h).

The micropyle is situated in the equatorial zone at the intersection of several polygons (Table II: Fig. i). In this zone the rise is higher and reaches a width of 2.5 µm. The edgeless micropyle has a diameter of 1.5 µm while the diameter of the micropylar opening is 0.8 µm.

DISCUSSION

The present SEM investigation carried out on the eggs of 6 species of Leptophlebiidae belonging to the genera Habrophlebia and Habroleptoides, allowed to point out some morphological characteristics of the chorion which can be considered as specific. In particular, the Habrophlebia species are very

alike in the general organisation of the egg and in chorionic sculpturing. Egg sculpture is made up, in this case, of a system of raised costae running across the egg in the direction of its major axis. The fine structure of costae, their pattern, the morphology of lateral expansions and the presence or absence of small openings in their dorsal surface, however, allow a sure diagnosis of species.

This happens also in the genus Habroleptoides in which H. modesta and H. umbratilis are very similar in the organisation of longitudinal costae but differ in the oblique banding presented exclusively by H. modesta. The H. auberti egg, instead, is sharply different from those of the previous species as its chorionic structure shows a network of irregular polygons.

Egg sculpturing observed in the examined species not only confirm that Habrophlebia and Habroleptoides are well distinct genera (Peters and Edmunds 1970, Peters 1979), but also allow them to be discriminated among the other Italian Leptophlebiidae genera, which have been studied to date only by light microscopy (cfr. Hinton 1981).

Submicroscopical examinations of chorionic structures, however, not only aim at working out taxonomic keys as a support to specialists of systematics, but they can also give, in agreement with Koss (1968), very important diagnostic data particularly useful for the "critical" species not sufficiently discriminated by traditional morphological methods. In our specific case, for example, the SEM investigation carried out on H. consiglioi eggs allowed us to recognize the nymphal stastages of the species which were still unknown.

The present SEM study was not confined to the examination of chorionic sculptures but was extended in each species to the fine structure of the micropyle. In fact, the characterisation of such structures can also contribute to a better knowledge of the fertilisation mechanisms occurring in this group of insects.

The micropyle of the examined species consists of a funnelform depression deepening in the chorion and is sometimes surrounded by a sort of slightly raised ring which conveys sperms into the underlying micropylar opening. Equatorial or subequatorial location of micropyles observed in Habrophlebia and Habroleptoides is peculiar if compared with the other insect orders. Such result is in agreement with SEM studies carried out on the eggs of new Ephemeroptera species (Malzacher 1982, Kopelke 1980, Landa and Soldán 1982, Pescador and Peters 1982), whereas no micropyles were observed in the eggs of Baetis species by Kopelke and Müller-Liebenau (1981a, b, 1982).

In Habrophlebia and Habroleptoides eggs the presence of a funnelform micropyle, the absence of attachment structures and of polar caps are characteristics which seem to be typical of the Leptophlebiidae eggs. The morphology of such female gametes seems then in agreement with the position occupied by Leptophlebiidae within the group of Ephemeroptera as shown by the

diagrammatic representation of phylogenetic relationships proposed by Koss and Edmunds (1974).

ACKNOWLEDGEMENTS

We wish to express our gratitude to Dr. C. Belfiore (Roma), Dr. U. Jacob (Dresden), Dr. R. Sowa (Kraków) for sending specimens and to Dr. R. Poggi (Genova) for allowing us to examine E. Biancheri's collection kept in the Museum of Natural History "A. Doria" (Genova).

REFERENCES

Belfiore, C. 1983 - Note su alcune specie del genere Habroleptoides Schoenemund, con segnalazione per l'Italia di H. auberti (Biancheri, 1954). Boll. Soc. ent. ital., Genova, 115: 5 - 6.

Bengtsson, S. 1913. Undersökningar öfver äggen hos Ephemeriderna. Ent. Tidskr., 34: 271 - 320.

Degrange, Ch. 1960: Recherches sur la réproduction des Ephéméroptères. Trav. Lab. Hydrobiol. Piscic. Univ. Grenoble, 51: 7 - 193.

Flowers, R.W. 1980. A review of the Nearctic Heptagenia (Heptageniidae, Ephemeroptera). In: Flannagan J.F. and Marshall, K.E. (eds.): Advances in Ephemeroptera biology. p. 93 - to 102, Plenum, N. York, London.

Hinton,H.E. 1981. Ephemeroptera. In: Biology of insect eggs. 2, p. 475 - 485. Pergamon Press, Oxford.

Karnowsky, M.S. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol., 27: 137 A.

Kopelke, J.P. 1980. Morphologische Studien an den Eiern der Eintagsfliegen (Ephemeroptera) aus der Emergenz des zentralafrikanischen Bergbaches Kalengo. Bull. Soc. ent. Suisse, 53: 297 - 311.

Kopelke, J.P. and 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 (Insecta: Baetidae). Teil II: rhodani-, vernus und fuscatus-Gruppe. Spixiana, 4: 39 - 54.

Kopelke, J.P. and 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: buceratus-, atrebatinus-, niger-, gracilis- und muticus-Gruppe. Dtsch. Ent. Z., 28 (1 - 3): 1 - 6.

Kopelke, J.P. and 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 (Insecta: Baetidae). Teil 1: alpinus-lutheri-, pavidus- und lapponicus-Gruppe. Gewass., Abwass., 68/69: 7 - 25.

Koss, R.W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. Ann. ent. Soc. Am., 61: 696 - 721.

Koss, R.W. and Edmunds, G.F. 1974. Ephemeroptera eggs and their contribution to phylogenetic studies of the order. Zool. J. Linn. Soc., 55: 267 - 349.

Landa, V. and 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). Stuttgarter Beitr. Naturk., 15: 1 - 15.

Morgan, A.H. 1913. A contribution to the biology of mayflies. Ann. ent. Soc. Am., 6: 371 - 441.

Pescador, M.L. and Peters, W.L. 1982. Four new genera of Leptophlebiidae (Ephemeroptera: Atalophlebiinae) from southern South America. Aquatic Insects, 4: 1 - 19.

Peters, W.L. 1979. Taxonomic status and phylogeny of Habrophlebia and Habroleptoides (Leptophlebiidae: Ephemeroptera). In: Pasternak K. and Sowa R. (eds.): Proc. 2nd Intern. Confer. Ephemeroptera, Kraków, 1975, p. 51 - 55.

Peters, W.L. and Edmunds G.F. 1970 Revision of the generic classification of the Eastern Hemisphere Leptophlebiidae (Ephemeroptera). Pac. Insects, 12: 157 - 240.

Smith, O.R. 1935. The eggs and egg-laying habits of North American mayflies. In: Needham, J.G., Traver, J.R. and Hsu, Y. Ch. (eds.): The biology of mayflies, p. 67 - 89, Comstock, Ithaca, N.York.