THE USE OF EGG MORPHOLOGY IN THE TAXONOMY OF SOME SPECIES OF THE GENUS *RHITHROGENA* (EPHEMEROPTERA, HEPTAGENIIDAE)

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Mayfly eggs have morphological features which can be used for taxonomic purposes: 1 - chorionic sculpture, 2 - attachment structures on the surface of the chorion and the poles of the egg, 3 - micropyle structure. These characteristics are species-specific and useful for identification of closely related species or groups of species from the same genus. The possibilities of identifying some *Rhithrogena* species from Southern Poland are presented.

INTRODUCTION

The various attachment structures at the surface of mayfly eggs are closely related to their reproductive biology. All species lay their eggs in fresh waters of various kinds, mainly in flowing water. Stable and rapid attachment of the eggs to the substratum immediately after they are laid prevents them from being carried downstream and affects the reproductive success of the species. Mayfly eggs vary greatly in both their shapes and chorionic structures. The latter hold the eggs to the substratum or drifting objects. These structures are species-specific and can be used for taxonomic purposes (KOSS, 1968; KOSS & EDMUNDS, 1974).

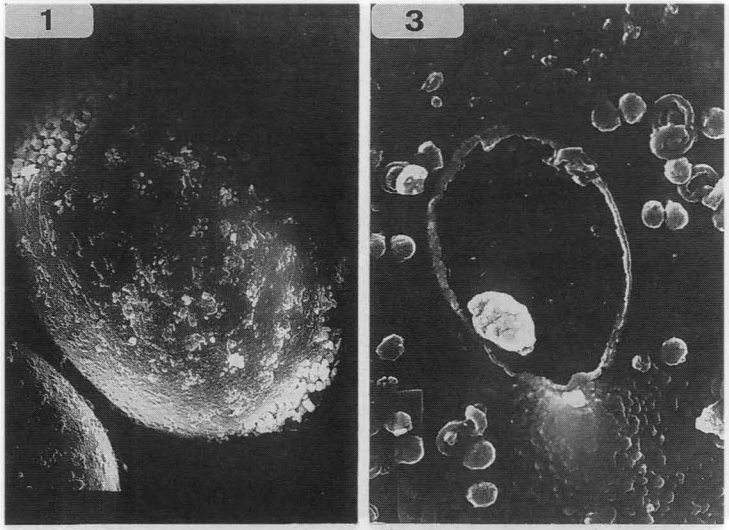
In Poland the family Heptageniidae is represented by six genera (*Arthroplea, Epeorus, Rhithrogena, Ecdyonurus, Electrogena* and *Heptagenia*) covering 34 species. The egg morphology of eight *Rhithrogena* species of the groups *hybrida, loyolaea* and *semicolorata* is described.

MATERIALS AND METHODS

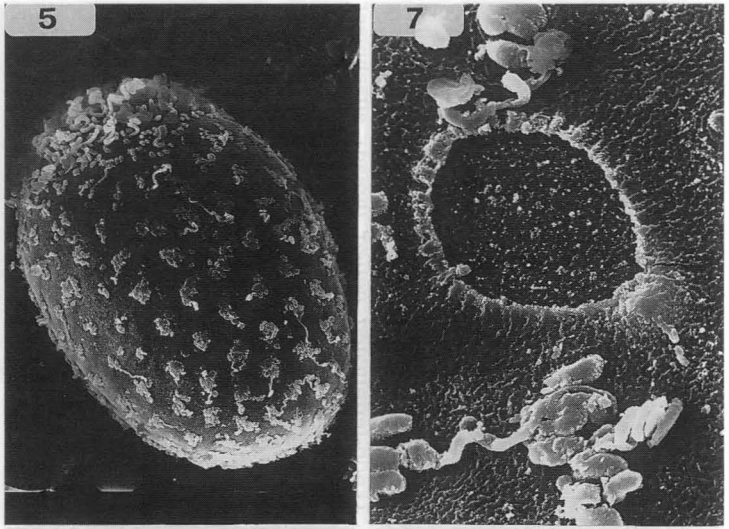
The eggs for scanning electron microscope (SEM) observation were taken from mature nymphs, subimagines and imagines. The stations where the material was collected are presented in Table 1. The insects were fixed with 75% alcohol. The eggs were removed from several individuals (or even several stages) of each species to avoid developmental aberrations. The standard method (KARNOWSKY,

Table 1. Station and developmental stage of the *Rhithrogena* species used: fn - female nymph, fs - female subimagines, fi - female imagines.

Species	Region	Water course	Altitude (m)	Date	Stage
R. carpatoalpina	Babia Góra	Potok Markowy	650-700	1986-06-06	fn
R. circumtatrica	Tatry	Potok Chochołowski	1000-1100	1986-06-11	fi
R. iridina	Beskid Sadecki	Potok Mała Roztoka	450-500	1985-07-25	fn
R. loyolaea	Babia Góra	Potok Czarna Woda	900-1000	1986-07-17	fn
R. podhalensis	Tatry	Potok Chochołowski	1000-1100	1986-06-11	fi
R. puytoraci	Beskid Sadecki	Potok Mała Roztoka	600-700	1985-07-25	fn
R. semicolorata	Pieniny	Dunajec	450	1986-05-07	fs
R. wolosatkae	Bieszczady	Potok Terebowiec	1000	1986 -06-10	fn



Figs 1-4. *Rhithrogena circumtatrica*. 1: general outline of the egg (X 245); 2: egg pole with concentration of large KCT attachment structures (X 660); 3: micropyle (X 2340); 4A: chorionic surface with groupings of macrogranules and KCT threads between them (X 2130); 4B: chorion with KCT partly extended (X 1500).



Figs 5-8. *Rhithrogena podhalensis*. 5: general outline of the egg (X 255); 6: egg pole with concentration of large KCT attachment structures (X 515); 7: micropyle (X 1520); 8: chorionic surface with peglike macrogranules and small KCT attachment structures (X 1390).

1965; KAPOOR & ZACHARIAH, 1984; ZURWERRA *et al.*, 1984) was used to prepare the eggs for SEM. Fixation in 4% glutaraldehyde was omitted (eggs were fixed in 75% alcohol) (GAINO *et al.*, 1987; GAINO & MAZZINI, 1987, 1988). The material was first treated in alcohol and acetone and then critical-point dried using CO_2, and finally placed on holders and coated with carbon and gold. All the observations were done in a JSM - 35 JEOL 25kV scanning microscope.

Morphological details possibly useful for taxonomic purposes were examined. Egg size was determined, as well as the range and means for the populations used. The means of egg sizes for all populations (when available) of the species are in parentheses next to the corresponding egg sizes from the population represented in the SEM images. Means of size ranges are in square brackets. For other measurements (micropyle, granules, egg pole) the means of ten measurements of a given characteristic are given. All sizes are in µm.

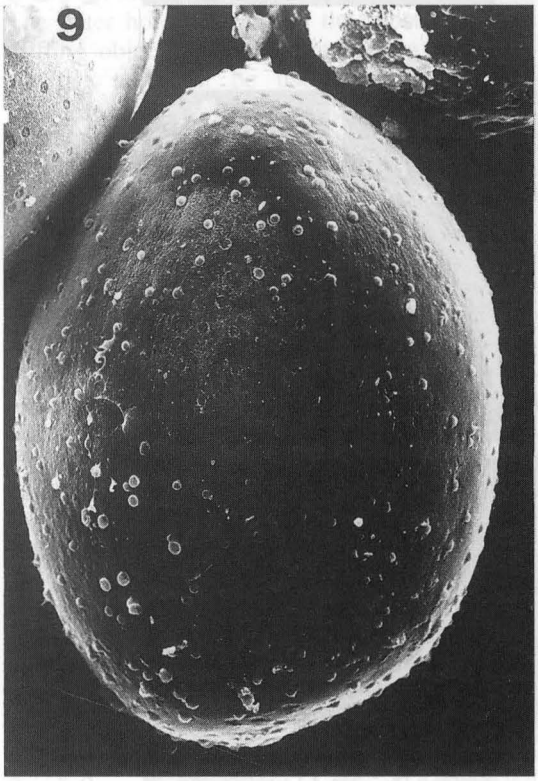
The basic classification and terminology of morphological structures are according to KOSS (1968) and KOSS & EDMUNDS (1974) with slight modifications.

The SEM images are of eight *Rhithrogena* species from Poland belonging to the groups *hybrida (R. circumtatrica, R. podhalensis, R. wolosatkae), loyolaea (R. loyolaea)* and *semicolorata (R. carpatoalpina, R. iridina, R. puytoraci, R. semicolorata).*

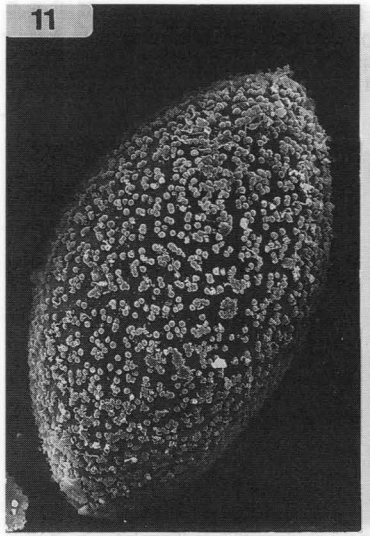
RESULTS

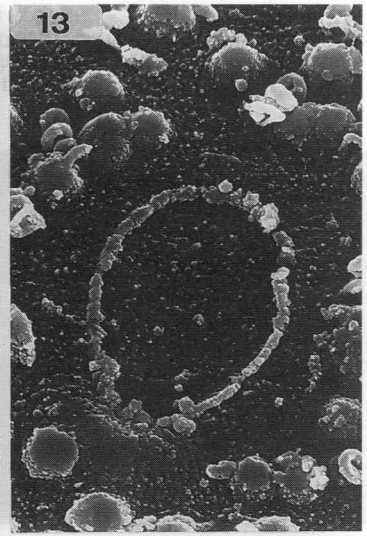
Rhithrogena circumtatrica SOWA & SOLDÁN, 1986

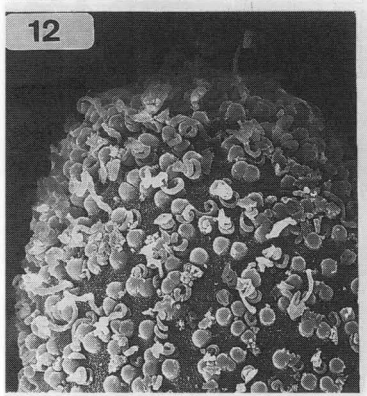
The egg has been briefly described by SOWA & SOLDÁN (1986). The egg is oval (Fig. 1). Dimensions: 189.0-210.0 µm in length [200.2 µm] and 105.0-133.0 µm in width [118.7 µm]. The surface of the chorion is rugose with indistinct tubercular structure. The whole surface is covered with groups of round macrogranules (1.6 µm in diameter), with one or two KCT (knob-terminated coiled thread) attachment structures between them (Figs 4A, 4B). There are concentrations of large KCT attachment structures at both poles with scattered round macrogranules which are a bit larger than those from the chorionic surface (Fig. 2). There are one or two oval micropyles in the equatorial or subequatorial area, 17.6 µm long and 11.1 µm wide, with the micropylar canal 19.1 µm long. The micropylar rim is narrow, with no specific structure (Fig. 3).

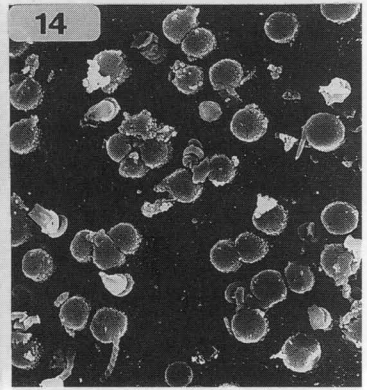


Figs 9-10. General outline of the egg. 9: *Rhithrogena wolosatkae* (X 465); 10: *Rhithrogena carpatoalpina* (X 455).

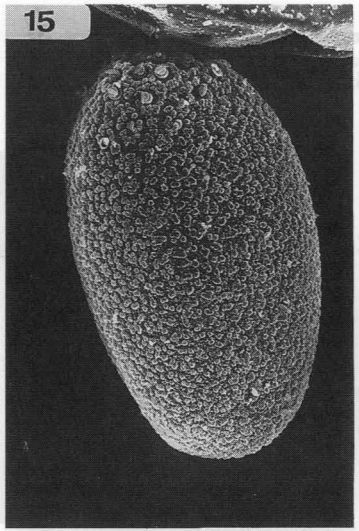


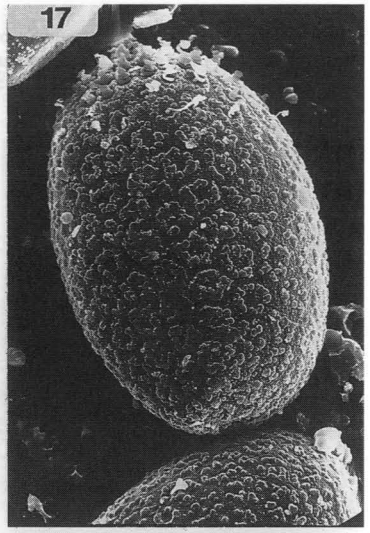


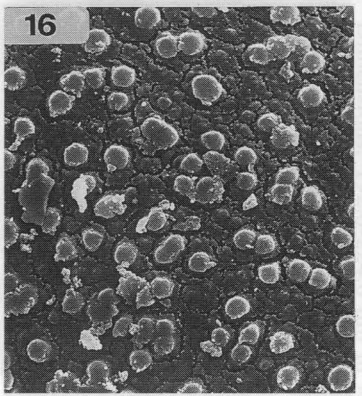


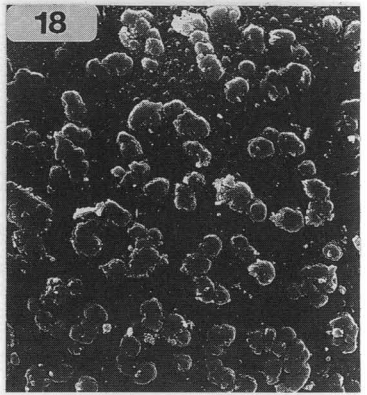


Figs 11-14. *Rhithrogena loyolaea.* 11: general outline of the egg (X 243); 12: egg pole with concentration of large KCT attachment structures (X 515); 13: mycropyle (X 1520); 14: chorionic surface of the egg with macrogranules and small KCT attachment structures (X 1400).

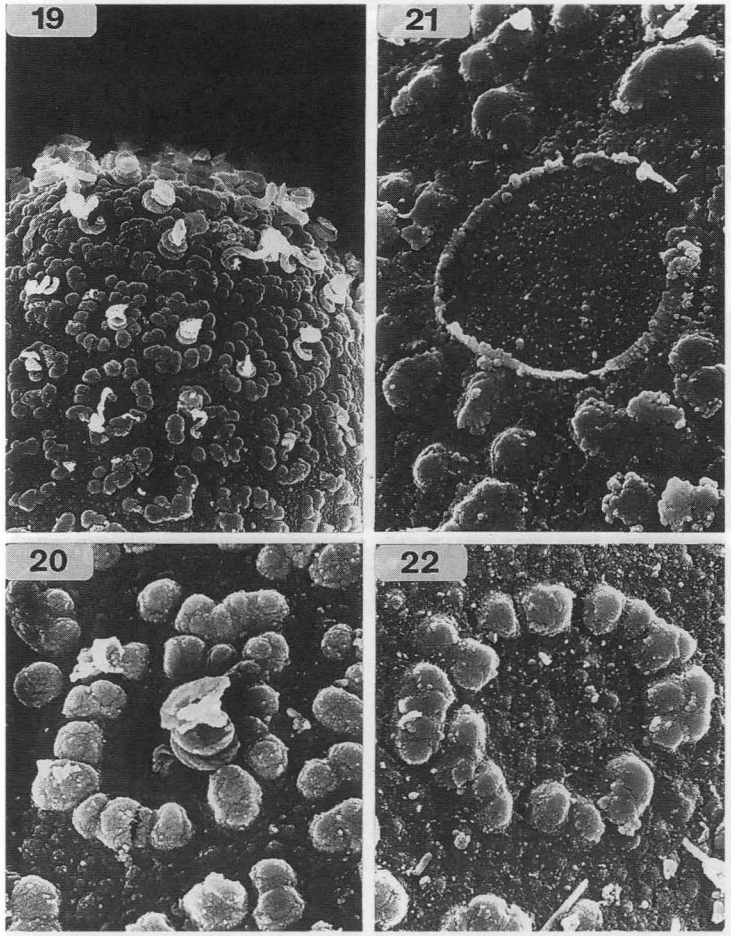




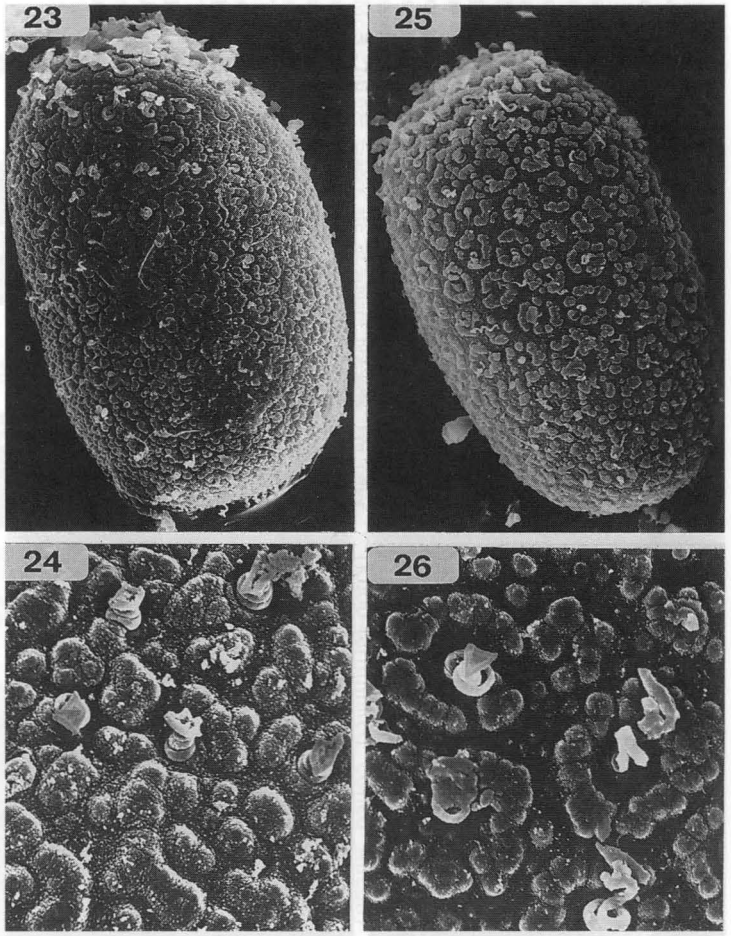




Figs 15-18. *Rhithrogena iridina.* 15: general outline of the egg (X295); 16: chorionic surface with scattered macrogranules (X 1210); 17: general outline of the egg (X 275); 18: chorionic surface with macrogranules grouped into rings (X 830).



Figs 19-22. *Rhithrogena iridina.* 19: egg pole with groupings of large KCT attachment structures (X 490); 20: KCT attachment element from the egg pole in the middle of a ring of macrogranules (X 2200); 21: micropyle (X 2430); 22: rings of macrogranules from the chorionic surface (X 2200).



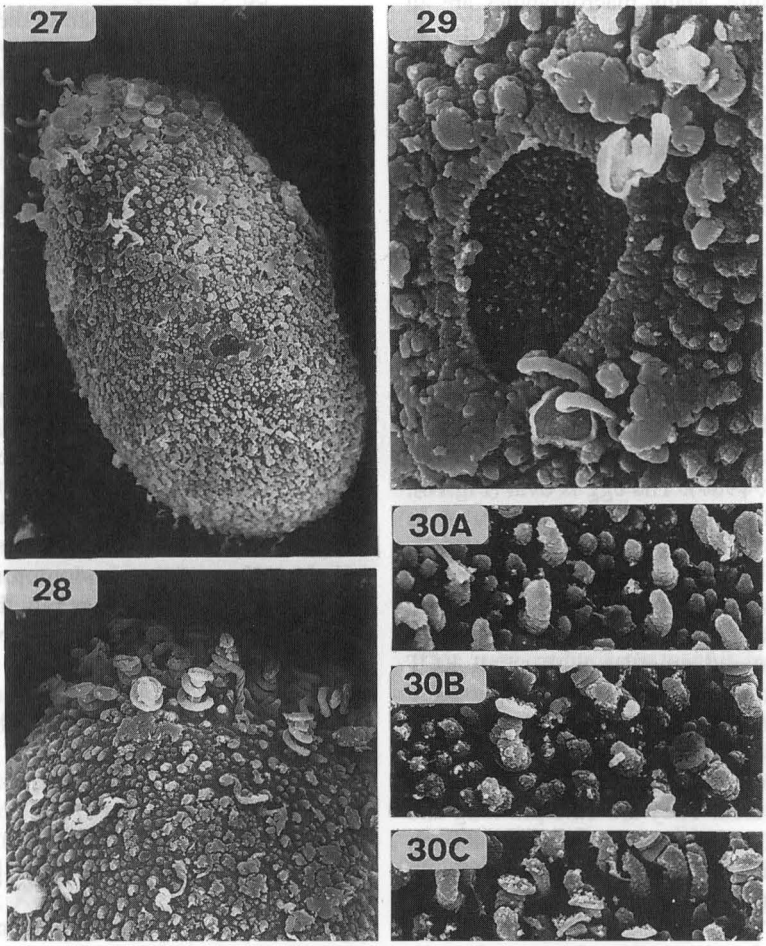
Figs 23-26. *Rhithrogena puytoraci.* 23: general outline of the egg (X 300); 24: chorionic surface of the egg (X 1400); 25: general outline of the egg (X 290); 26: chorionic surface of the egg, with rings of macrogranules (X 1470).

Rhithrogena podhalensis SOWA & SOLDÁN, 1986

The egg has been described by SOWA & SOLDÁN (1986). The egg is oval (Fig. 5). Dimensions: 189.0-203.0 µm in length [194.0 µm] and 126.0-153.0 µm in width [132.1 µm]. The surface of the chorion is rugose with delicate granular structure. There are concentrations of peglike macrogranules evenly scattered all over the surface (3.0-3.5 µm long and 1.3 µm wide). In each concentration there are one to three KCT attachment structures located on the peglike macrogranules that support them (Fig. 8). There is a concentration of large KCT attachment structures at one egg pole, with numerous peglike macrogranules between them (Fig. 6). There are one or two oval micropyles, usually in the subequatorial area (closer to the pole with the KCT elements), 17.2 µm long and 11.7 µm wide, with the micropylar canal 15.3 µm long. The micropylar rim is quite wide and denticulate (Fig. 7).

Rhithrogena wolosatkae KŁONOWSKA, 1987

The egg has been partially described (KŁONOWSKA, 1987). The egg is oval (Fig. 9). Dimensions: 192.0-224.0 µm in length [198.5 µm] and 126.0-154.0 µm in width [142.0 µm]. There are sparse, round microgranules (0.7-1.1



Figs 27-30. *Rhithrogena semicolorata.* 27: general outline of the egg (X 330); 28: egg pole with concentration of large KCT attachment structures (X 570); 29: micropyle (X 2470); 30A: chorionic surface in the equatorial area (X 1720); 30B: chorionic surface in the area of the pole with large KCT attachment structures (X 2000); 30C: chorionic surface in the area of the posterior pole (X 1855).

μm in diameter) and more frequent small KCT attachment structures in groups of two or three elements. There are between one and three oval micropyles in the equatorial area (rarely in the subequatorial area), 7.8 μm long and 7.0 μm wide, with the micropylar canal 18.3 μm long. The micropylar rim is moderately wide and slightly denticulate.

Rhithrogena loyolaea NAVÁS, 1922

The egg is oval or spindle-shaped (Fig. 11). Dimensions: 224.0 (227.0)-252.0 (259.0) μm in length [240.3 (243.6) μm] and 133.0 (126.0)-154.0 (154.0) μm in width [143.7 (140.7) μm]. The surface of the egg is rugose with clear granular structure. Many macrogranules are all over the surface of the chorion (3.3-3.5 μm in diameter), sometimes in groups of two or three elements. There are also small KCT attachment structures emerging from the surface all over the chorion. There are fewer of them in the equatorial area (Fig. 14). There is a concentration of KCT structures at one egg pole (Fig. 12). There are one or two oval micropyles in the equatorial or subequatorial area, 13.6 μm long and 9.8 μm wide, with the micropylar canal 15.4 μm long. The micropylar rim is thin and slightly denticulate (Fig. 13).

Rhithrogena carpatoalpina KŁONOWSKA *et al.* 1987

The egg has been partially described (KŁONOWSKA *et al.*, 1987). The egg is oval (Fig.10). Dimensions: 182.0 (175.0)-203.0 (203.0) μm in length [188.5 (188.8) μm] and 105.0 (105.0)-112.0 (119.0) μm in width [110.1 (109.8) μm]. The surface of the chorion is rugose, with granular structure. It is composed of numerous randomly scattered granules with small KCT attachment structures. A group of larger KCT attachment structures emerging from the chorion can be seen at one egg pole. One or two oval micropyles are located in the equatorial or subequatorial area. They are 12.8 μm long and 10.6 μm wide, with the micropylar canal 8.6 μm long. The micropylar rim is wide, with no evident pattern.

Rhithrogena iridina KOLENATI, 1895

The egg is oval (Figs 15, 17). Dimensions: 175.0 (175.0)-210.0 (210.0) μm in length [192.1 (195.6) μm] and 102.0 (98.0)-122.0 (133.0) μm in width [111.0 (117.2) μm]. The surface of the chorion is rugose, with a slightly tubercular pattern. Many macrogranules (2.2-2.6 μm in diameter) are scattered all over the surface of the chorion (Fig. 16), sometimes grouped into more or less distinct circles (Fig. 18). There is a concentration of large KCT attachment structures at one pole (Fig. 19). The attachment structures at the pole are surrounded by a ring of macrogranules (Fig. 20). Smaller KCT attachment structures extend one-third of the egg's length (beginning from the pole with the large KCT structures). Attachment structures were never observed in the lower part of the egg (Fig. 22). Between one and three oval micropyles are located in the equatorial or subequatorial area, 13.9 μm long and 10.3 μm wide, with the micropylar canal 12.5 μm long. The micropylar rim is narrow, with no specific structure (Fig. 21).

Rhithrogena puytoraci SOWA & DEGRANGE, 1987

A detailed description of the egg can be found in SOWA & DEGRANGE (1987c). The egg is oval (Figs 23, 25). Dimensions: 175.0 (168.0)-203.0 (210.0) μm in length [196.9 (194.2) μm] and 105.0 (98.0)-133.0 (133.0) μm in width [120.9 (119.0) μm]. Over the whole chorion surface there are many concentrations of macrogranules, either randomly scattered (Fig. 24) or tending to group into circles or to form some wreath-like groupings (Fig. 26). Small KCT attachment structures can be found over the whole surface of the chorion or, in the case of the wreath-like groupings, in the middle of them. The further from the pole with the large KCT attachment structures, the smaller they are. There are one to three oval micropyles in the subequatorial area, 15.2 μm long, 11.3 μm wide, with the micropylar canal 8.5 μm long. The micropylar rim is narrow and slightly denticulate.

Rhithrogena semicolorata (CURTIS, 1834)

The egg is oval (Fig. 27). Dimensions: 168.0-189.0 μm in length [175.8 μm] and 98.0-113.0 μm in width [106.0 μm]. The surface of the chorion is rugose, with delicate granular structure and numerous round microgranules

(0.4-0.9 μm in diameter) all over the surface. There are also peglike macrogranules (2.5-3.0 μm in length and 1.2 μm in width). They are sparse and rather small in the equatorial area (Fig. 30A), more numerous and larger at the pole region with the large KCT elements (Fig. 30B), and the most frequent and largest in the anterior pole of the egg (Fig. 30C). From the equatorial area to the anterior pole (with no groupings of attachment elements) there are small, randomly located small KCT attachment elements on the peglike macrogranules. They are most frequent at the anterior pole region (with no groupings of large KCT attachment structures). In the equatorial area their number declines, with only a few elements at the pole region where the large KCT are located. There is a grouping of large KCT attachment structures with peglike macrogranules between them at one egg pole (Fig. 28). There are one or two oval micropyles in the equatorial area (they are less frequent in the subequatorial area), 16.3 μm long and 9.8 μm wide, with the micropylar canal 7.0 μm long. The micropylar rim is wide and denticulate (Fig. 29).

DISCUSSION

According to Koss (1968) and Koss & Edmunds (1974) there are three morphological characteristics of eggs useful for taxonomic purposes: 1 - the sculpturing of the chorion surface; 2 - various attachment structures (a - adhesive layers; b - attachment structures on the chorion surface; c - «polar cap» or other attachment structures located at the poles); and 3 - micropyle structures.

In Poland six genera from the family Heptageniidae can be found: *Arthroplea, Epeorus, Rhithrogena, Ecdyonurus, Electrogena* and *Heptagenia.* Their eggs (except *Epeorus*; personal observations) are quite similar and could be categorized as «typical heptageniid eggs» with KCT attachment structures, a tuberculate chorion and entirely chorionic micropyles (Koss, 1970; Koss & Edmunds, 1974). There are no satisfactory descriptions of the egg morphology of the genus *Ecdyonurus* so far. Identifing its species based on egg morphology is more difficult and requires practice with the material. Very detailed SEM descriptions of the egg morphology of five European species of the genus *Electrogena* are available (Gaino & Mazzini, 1987; Gaino *et al.*, 1987). Substantial differences in egg morphology are observed within this genus, especially in regard to the appearance of both the attachment structures and the chorion surface. For the genus *Heptagenia* only Degrange (1960) gives preliminary descriptions of two European species; there are no descriptions using SEM. In recent literature concerning the genus *Rhithrogena*, egg morphology greatly helped identify new species (Sowa & Soldán, 1986; Alba-Tercedor & Sowa, 1987; Belfiore, 1987; Kłonowska, 1987; Kłonowska *et al.*, 1987; Sowa & Degrange, 1987a, 1987b, 1987c; Gaino & Mazzini, 1988; Sartori & Oswald, 1988; Sartori & Sowa, 1988; Sowa *et al.*, 1988; Gaino *et al.*, 1989; Kłonowska, 1989, Mazzini & Gaino, 1990).

In contrast to the statement of Koss (1968) and Koss & Edmunds (1974) that it is possible to identify only genera within the family Heptageniidae, the present investigation suggests that species-specific discrimination is possible. In *Rhithrogena,* distinctive egg morphology exhibiting no substantial variability of structures (some variability was observed only in *R. iridina* and *R. puytoraci*) was evident.

The chorion surface of the examined *Rhithrogena* species is rugose, with distinct granules. Their size, form and arrangement varied substantially between all examined species from the groups *Rhithrogena hybrida, loyolaea* and *semicolorata.* In the family Heptageniidae, *Rhithrogena* species seem to have the most distinctive chorionic sculpture. This may prove very useful in species identification.

In the examined species there were no typical adhesive layers. European Heptageniidae lack the typical adhesive layers (unpublished data). Exceptions are *Epeorus sylvicola,* with a smooth chorion covered by very small, round tubercles (Degrange, 1960; personal observations), and *Electrogena gridellii* and *Electrogena fallax*, with the chorion covered by irregular granules (Gaino *et al.*, 1987). These structures are probably a granular adhesive layer on the chorion surface of the eggs of these species.

The attachment structures on the chorion surface of *Rhithrogena* are of two kinds:
1. Knob-terminated coiled thread (KCT) composed of many spiraled fibers; the thread is attached to the centre of the knob. The attachment structure emerges directly from the chorionic surface (e.g. *R. wolosatkae, R. loyolaea, R. iridina, R. puytoraci*) (SOWA & SOLDÁN, 1986; ALBA-TERCEDOR & SOWA, 1987; SOWA & DEGRANGE, 1987c; SARTORI & OSWALD, 1988; SARTORI & SOWA, 1988; GAINO *et al.*, 1989).
2. KCT attachment structure located on granules of various types which are basal excrescences emerging from the chorion surface (e. g. *R. podhalensis, R. carpatoalpina, R. semicolorata*) (KŁONOWSKA *et al.*, 1987; GAINO *et al.*, 1989).
Some of the large KCT structures in examined *Rhithrogena* are concentrated at one or two egg poles and constitute large adhesive layers. This holds true for the majority of *Rhithrogena* species (SOWA & SOLDÁN, 1986; ALBA-TERCEDOR & SOWA, 1987; GAINO *et al.*, 1989)
Although KCT attachment structures are characteristic of Heptageniidae eggs (KOSS, 1968, 1973; KOSS & EDMUNDS, 1974), their specific features such as size, location on the chorion surface, concentration at the egg poles, and granules surrounding or supporting KCT structures, are useful in identifying the species.
The micropyle is the structure that enables penetration of the sperm into the egg. There is an oval micropyle in Heptageniidae: the micropyle opening is in the form of a large rounded depression of the chorion surface with various sculpturings on its rim (KOSS, 1968, 1970; KOSS & EDMUNDS, 1974). The micropyle in *Rhithrogena* as well as in the whole family Heptageniidae is typical (suprachorionic; two or more micropyles, usually equatorial). Apart from being useful in identifying the family, this structure can be examined along with other morphological features of the egg to identify the genus and species: the structures of the examined *Rhithrogena* eggs are clearly apomorphic (KCTs, concentration of KCTs at egg poles, various kinds of tubercles, micropyle structure).
It would be useful to develop keys for egg morphology features to help identify difficult groups and closely related species.

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