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Egg description of three Mediterranean *Isoperla* species (Plecoptera, Perlodidae)

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ABSTRACT

The morphology and size of the egg of *I. carbonaria, I. insularis* and *I. tripartita* are described. Some differences in collar presence (and size), microphyle position, aerophyle size and distribution are pointed out. The differences among European *Isoperla* egg morphology is discussed.

Key words: Stonefly, *Isoperla, I. carbonaria, I. insularis, I. tripartita*, egg morphology, Italy, Greece.

RESUMEN

Descripción de los huevos de tres especies de *Isoperla* mediterráneas (Plecoptera, Perlodidae).

Se describe la morfología y tamaño del huevo de *I. carbonaria, I. insularis* e *I. tripartita.* Se destacan algunas diferencias en presencia de collar (y tamaño de éste), posición de los micropilos, y tamaño y distribución de los aeropilos. Por último, se discuten las diferencias existentes en morfología del huevo entre las especies europeas de *Isoperla.*

Palabras clave: plecóptero, *Isoperla, I. carbonaria, I. insularis, I. tripartita*, morfología de huevo, Italia, Grecia.

INTRODUCTION

Different aspects of the stonefly eggs have been often studied: morphology, cytology, hatching, development, etc., contributing a valuable information to the knowledge of the biology, phylogeny and taxonomy of this animal group (Frison, 1935; Hynes, 1941; Berthélemy, 1964; Zwick, 1973, Stark & Szczytko, 1982, 1988; Lillehammer & Okland, 1987; Nelson, 1988; Tierno de Figueroa & Sánchez-Ortega, 1999).

Particularly, in the genus *Isoperla* the studies on egg morphology represent a useful key for taxonomic differentiation (Lillehammer & Okland, 1987; Tierno de

Figueroa & Sánchez-Ortega, 1999) related to the fact that many species are only differentiable by the male peneal armature, and the only valid criteria for the female differentiation is sometimes the morphology of eggs that they mature. In fact, the form of the female subgenital plate (the only other character usually used) varies considerably among a same species populations (Lillehammer, 1974) and hence is not reliable. Nevertheless, the *Isoperla* egg studies are scarce in Southern Europe, and completely absent in Italy and Greece, where only the eggs of the species with wide European distribution are known. In relation with this, the aim of the present work is to describe the egg morphology of three species of *Isoperla* (*I. carbonaria*, *I. insularis* and *I. tripartita*) from Italy and Greece and compare these species egg morphology with those of other European *Isoperla*.

MATERIAL AND METHODS

Three species of *Isoperla* genus were studied: *I. carbonaria* Aubert 1953, collected in a spring in Mt. Pollino, Calabria (Italy), VII-5-1986; *I. insularis* (Morton, 1939) collected in river Temo, Villanova Monteleone, Sardinia (Italy), V-5-1993; and *I. tripartita* Illies 1954 collected in river Elisson, Davia, Tripoli, Peloponnes (Greece), V-26-1985, and Kato Lousi, Mt. Kelmos, Peloponnes (Greece), V-22-1985.

The eggs were obtained from female dissection and preserved in 70% alcohol in crystal vials. Then, the eggs were attached to stubbs, dried at 30° C for 24 hours, coated with gold in a Balzers Union MED 010 avaporator, and observed with a 5200 JEOL JMS electron microscopy. Picture were taken on a Kodak T-max film exposed at 200 ISO.

The measurements were obtained from SEM photographs. 10 eggs of each species (from at least three different females of each species) were measured following Tierno de Figueroa & Sánchez-Ortega (1999).

RESULTS

I. carbonaria

Egg size in µm (N = 10):
- Long axis: mean = 339; range = 311-389; SD = 23.74
- Short axis: mean = 243; range = 216-278; SD = 22.47

Egg colour = Brown, yellow.
Egg shape = oval form (some deformed eggs in the female oviduct).
Collar presence = yes (short).
Microphyles = equidistant, in the distal third. Without keels.
Aerophyles = 2.7-6 µm (small and big), irregularly distributed.
Chorionic cells= not clearly observed.

I. insularis

Egg size in µm (N = 10):
- Long axis: mean = 321; range = 310-340; SD = 9.94
- Short axis: mean = 247; range = 220-270; SD = 16.36

Egg colour = Brown, yellow.

Egg shape = oval form (some deformed eggs in the female oviduct)
Collar presence = not observed in more than 40 studied eggs.
Microphyles = not equidistant, in the distal third. Without keels.
Aerophyles = 2.3-3.8 µm (small), and regularly distributed.
Chorionic cells = pentagonal (some hexagonal) ones with 12-24 aerophyles.

I. tripartita

Egg size in µm (N= 10):
- Major diameter: mean = 327; range = 294-353; SD = 20.57
- Minor diameter: mean = 238; range = 221-250; SD = 9.28

Egg colour = Brown, yellow.
Egg shape = oval form (some deformed eggs in the female oviduct)
Collar presence = yes (long).
Microphyles = not equidistant, in the distal third. Without keels.
Aerophyles = 1.7-5.8 µm (very small and big), and very irregularly distributed.
Chorionic cells = present, but not clearly observed.

DISCUSSION

General characters shared with other *Isoperla* eggs are: brown or yellow colour, oval shape, size and presence-absence of collar and attachment disc, general characters in Perloidea stoneflies (Brinck, 1949; Khoo, 1964; Tierno de Figueroa & Sánchez-Ortega, 1999). Also, the presence of some deformed eggs in the female oviduct, possibly a mechanism of space saving (Khoo, 1964; Tierno de Figueroa *et al.*, 1998; Tierno de Figueroa & Sánchez-Ortega, 1999), has been observed.

The absence of collar and attachment disc in Perloidea egg can be a specific character or can appear in some eggs of a same female clutch but not in other, as has been pointed for *I. grammatica* by Khoo (1964) and for *I. grammatica* and *I. nevada* by Tierno de Figueroa and Sánchez-Ortega (1999). In our study, all the *I. insularis* eggs observed were without collar, while in the other two species eggs the collar was sometimes observed. The absence of collar and attachment disc in some Perloidea eggs has been associated with the need to enter interstitial environment to avoid exposure to the air when the stream become dry (Berthélemy, 1973). Nevertheless, *I.insularis* has never been collected in temporary streams. The opposite is true for *I. grammatica* (Aubert, 1963; own data).

Comparing with the other European *Isoperla* eggs, some differences can be pointed out. *I. curtata* Navás, 1921 is clearly different from all the other species by the lobed outline of the egg chorion cells (Tierno de Figueroa *et al.*, 2000). *I. grammatica* (Poda, 1761) and *I. nevada* Aubert, 1952, can be distinguished from the three studied species in this work by the punctuation (aerophyles) hexagonally arranged in clumps of 10-12 in the first one and less than 10 in the second one (Lillehammer & Okland, 1987; Tierno de Figueroa & Sánchez-Ortega, 1999). Probably this character shows a phylogenetic relation with *I. insularis* (with pentagonal, some hexagonal, chorion cells with 12-24 aerophyles) as has been also pointed for *I. difformis* (Klapálek, 1909) that can be distinguished from the other by the keels in the microphyles (Lillehammer & Okland, 1987). The *I. obscura* (Zetterstedt, 1840) egg can be distinguished from the others by the distinct egg ridges (Lillehammer & Okland, 1987).

Thus, this study confirms the importance of the egg morphology on the *Isoperla* species female identification as specific character. Future studies on other species egg morphology will be useful for the specific determination of these complex group.

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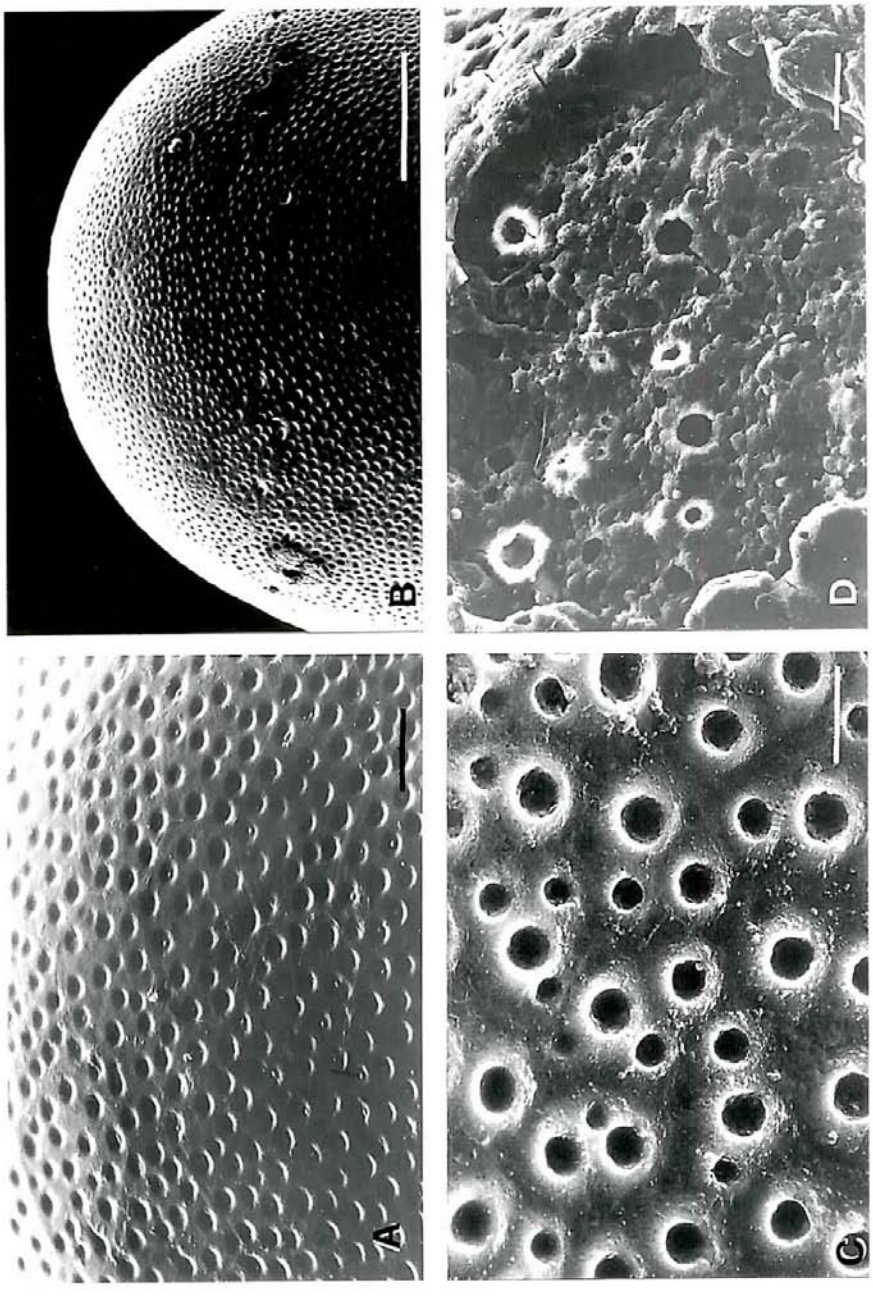

Fig. 1. Surface detail of *Isoperla insularis* egg showing the aerophyles (*A*) and aerophyles and microphyles (*B*). Surface detail of *Isoperla carbonaria* egg showing the aerophyles (*C*). Surface detail of *Isoperla tripartita* egg showing the aerophyles (*D*).

Scale for *A*, *C* and *D* 10 µm; for *B* 50 µm.