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# THE EGG OF *PSEUDOCENTROPTILUM PENNULATUM*: A SCANNING ELECTRON MICROSCOPY (SEM) STUDY (*)

(*Ephemeroptera Baetidae*)

KEFFERMÜLLER & SOWA (1984) have described two groups within the European species of the genus *Centroptilum* Eaton, *C. luteolum* and a number of related species including *C. pennulatum*. These authors have proposed to refer the species of the latter group to the genus *Pseudocentroptilum* created by BOGOESCU (1947) for *P. motasi*. Among the morphological characteristic employed to specify the taxonomic status of the genera *Centroptilum* and *Pseudocentroptilum*, the egg chorion structure has been studied in particular using the traditional optical microscopy techniques (KEFFERMÜLLER & SOWA, 1975, 1984). In the genus *Centroptilum*, chorionic decoration patterns have been described as species-specific by DEGRANGE (1960) and evidenced at SEM level by LOWEN & FLANNAGAN (1990) in *C. infrequens*, a species belonging to the *C. pennulatum* group. However, no detailed investigation has been hitherto carried out.

## *Materials and Methods*

Adults females engaged in egg deposition were collected in late spring (June, E. Gaino & E. Bongiovanni leg. and det.) along the Berlino stream (close to the village of Rossiglione, Genova - Italy, at about 300 m on sea level). For scanning electron microscopy (SEM) investigation, eggs adhering to the abdomen of the specimens were fixed in Karnovsky medium (1965) in cacodilate buffer for 1 h, rinsed in the same buffer and dehydrated in a graded series of ethanol. Selected material was critical-point dried using $CO_2$ in a Bomar apparatus, attached to specimen holders by silver conducting paint and coated with gold-palladium in a Balzer Union evaporator. Specimens were observed with a Philips EM 505 electron microscope.

## *Results*

Mated females of *P. pennulatum* (Eaton) lay aggregates of eggs that adhere together in clusters to the last abdominal segments before deposition in water. According to DEGRANGE (1960), each female lays down about 1300-2400 eggs per season.

The eggs measured approximately 170 µm in length and 110 µm in width, in a sample of 12 randomly selected eggs. Both polar regions had a rounded shape. The chorionic surface was completely covered by large-mesh reticulation of ridges

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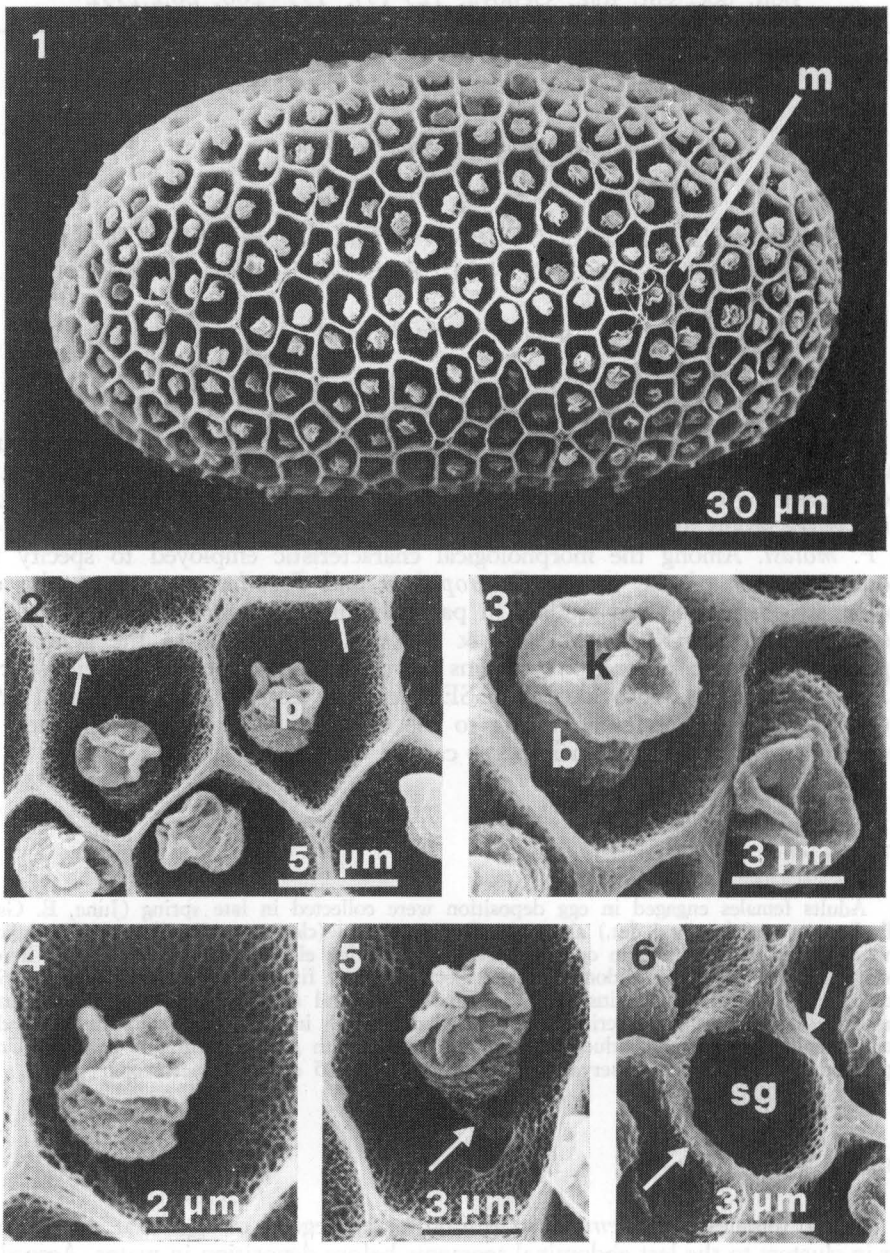


Fig. 1 – Overview of the egg of *P. pennulatum*; m, micropyle. Fig. 2 – Alveolar pattern of the chorion consisting in a network of large-mesh reticulation of ridges (arrows) delimiting irregular polygons. Each polygon includes a papilla (p). Egg shell shows an alveolar organization due to the infoldings of the chorion. Fig. 3 – Papillae displaying their peculiar "mushroom" configuration with a basal part (b) and a terminal knob (k). Fig. 4 – Network of fibrils that covers each chorionic infolding. Fig. 5 – A papilla supported by a thin stalk (arrow). Fig. 6 – Micropyle delimited by ridges (arrows) showing its sperm guide (sg) covered by a homogeneous fibrillary network.

that gave rise to irregular polygons, each including a short inner projection (Fig. 1). These projections correspond to papillae according to the terminology of KEFFERMÜLLER & SOWA (1984). About 26 polygons were seen on the longitudinal axis and about 13 on the transverse axis on one side of the egg. Polygons presented a varying number of sides (from 4 to 7) of different length (from 2.5 μm to 8 μm). The chorionic surface bounded by the ridges showed deep infoldings giving an alveolar pattern (Fig. 2). The emerging papillae displayed a "mushroom" configuration. They consisted of two parts: a massive basal region with a spinge-like texture and a smooth part resulting in a folded laminar knob (Fig. 3). Alveoles were characterized by a network of fibrils that often converged to form a complex system of variably packed lines (Fig. 4). Some micrographs revealed that papillae were supported at the base by a thin stalk (Fig. 5). The micropyle was evident in one micrograph at the junction of a few polygons. It was located approximately midway between the equator and one pole and consisted of a nearly circular sperm guide (about 4 μm in diameter), delimited by the same ridges composing the chorionic reticulation, and covered by a homogeneous fibrillary network (Fig. 6).

Papillae usually were located eccentrically inside the polygons. The uniformly fibrillar covering of the polygons presented a pocket from which the stalks of the papillae emerged (Fig. 7). The visible portion of the stalks was short (1-2 μm), very thin (0.5 μm) and displayed a surface similar to that of the basal region of the papillae but with a more compact aspect (Fig. 7). The spongy texture of the basal region of the papillae consisted of irregularly arranged grooves (Fig. 7). This feature was common to the whole chorionic projections. By contrast, the terminal knob displayed either a conical (Fig. 8) or a variably flattened configuration (Figs 9, 10) due to the different folding of its laminar component. According to their aspect, the papillae had a variable height, ranging from 2.5 μm in the most flattened forms to 3.5 μm in the most folded ones.

## *Discussion*

Mayfly eggs have been employed for phylogenetic purposes in several studies (DEGRANGE, 1960; KOSS, 1968; KOSS & EDMUNDS, 1974; MALZACHER, 1982, 1986; GAINO *et al.*, 1987, 1989). The adhesive properties of chorionic sculpturings have been investigated for the role of egg attachment devices in preventing eggs from drifting after deposition in water (GAINO & MAZZINI, 1987, 1988).

Among Baetidae, the egg shells of some species of *Centroptilum* and *Pseudocentroptilum* present peculiar decorations. Studies on several species of the genus *Baetis* have emphasized that egg configuration is not always useful for taxonomic purposes (KOPELKE & MÜLLER - LIEBENAU, 1981 a, 1981 b, 1982). By contrast, the meshwork reticulation pattern and the configuration of the papillae, have provided a set of characteristics that have been employed to investigate the taxonomic position of several species of the above genera (KEFFERMÜLLER & SOWA, 1975, 1984).

Our SEM investigation has established the morphological details on the egg shell decoration and has elucidated morphologic features of the papillae that may be relevant for the adhesive function. According to DEGRANGE (1960), eggs laid in water strongly adhere with the papillae to the substrate. A possible sequence of events postulates that the flexible stalks can extend outwards the papillae after egg deposition in water. As a consequence, eggs gest in touch with the substrate

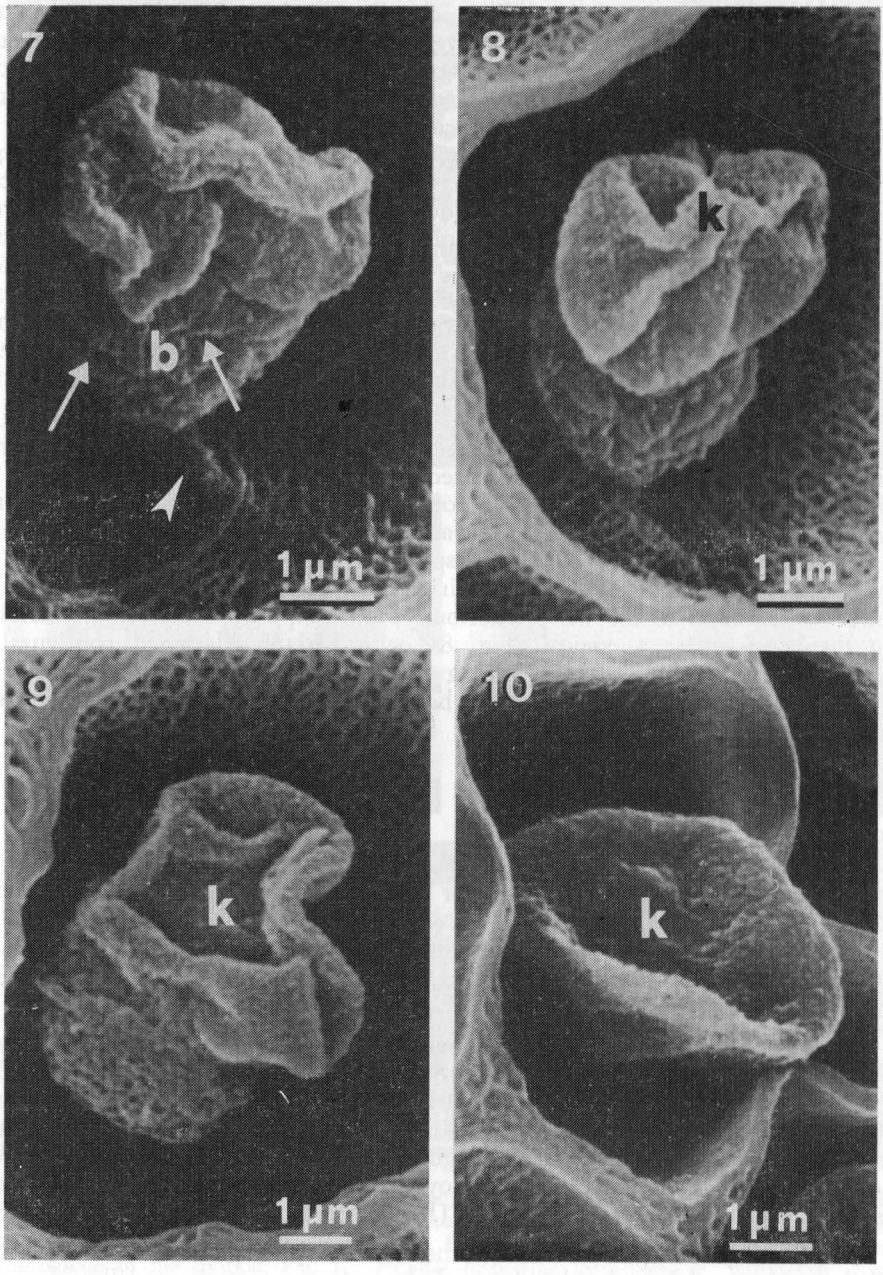


Fig. 7 – The egg of *P. pennulatum*; enlarged view of a papilla whose stalk (arrowhead) infolds within a chorionic pocket. Note the spongy basal region (b) with irregularly arranged grooves (arrows) and the more compact texture of the stalk. Figs 8, 9, 10 – Three views of papillae showing different aspects of their terminal knob (k) which is completely folded (8), partially folded (9) and unfolded (10).

and the apical region of the papillae enlarges, anchoring the eggs to the substrate. This sequence is similar to that postulated in several species of Heptageniidae for the terminal knob of the coiled threads (GAINO & MAZZINI, 1987, 1988). Adhesion could be improved by liquid absorption by the sponge-like matrix of the basal part of each papilla that replaces the coiled threads usually supporting the terminal knob in Ephemeroptera (KOSS, 1968). Therefore, the papillae represent peculiar chorionic projections and this finding arises questions on their origin and evolution. Considering that in mayflies the knob-terminated coiled threads represent the most apomorphic state among attachment structures (KOSS & EDMUNDS, 1974), the papillae of *P. pennulatum* could be tentatively included in the category of non fibrous attachment structures, the most plesiomorphic ones. Yet, at present, on the basis of SEM studies alone, there are difficulties in defining the structure of the basal part of the papillae and further investigation by transmission electron microscopy is needed in order these chorionic projections being conveniently categorized.

The schematic drawings of the eggs in several species of *Pseudocentroptilum* show species-specific differences in structure and size of the papillae. Additional SEM data on other species of this genus could be useful to ascertain 1) if the architecture of the papillae evidenced for *P. pennulatum* is a common characteristic, 2) the species-specific organization of the papillae and 3) the relationship between the structure of the papillae and their adhesive properties.

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## RIASSUNTO

*L'uovo di* Pseudocentroptilum pennulatum *visto al microscopio elettronico a scansione* (*SEM*) (Ephemeroptera Baetidae).

Le uova di *P. pennulatum* osservate al SEM presentano un reticolo in rilievo sul corion che scompartisce la superficie in poligoni irregolari. Questi si approfondano verso l'interno e realizzano una struttura ad alveoli ricoperti da una matrice fibrillare. Ogni alveolo contiene una caratteristica propaggine a forma di fungo, la papilla, sorretta da un sottile peduncolo che si approfonda nel corion. Ogni papilla presenta una parte basale a tessitura spugnosa, in cui confluisce il peduncolo, ed una apicale costituita da una lamina distesa o variamente ripiegata. Il micropilo, a forma quasi circolare, è localizzato verso uno dei poli. L'organizzazione delle papille è in armonia con la loro funzione adesiva.

## ABSTRACT

The eggs of *P. pennulatum* were investigated by scanning electron microscopy (SEM). They showed a meshwork reticulation arising on the surface and forming irregular polygons. These showed deep infoldings realizing an alveolar pattern covered by a fibrillary network. The papillae, a series of "mushroom" projections, emerged inside the alveoles. Each papilla penetrated the chorion by a stalk and consisted of a basal region with sponge-like texture and a superimposed folded or flattened laminar knob. The nearly circular micropyle was located towards one egg pole. The organization of the papillae was in agreement with the adhesive function performed by these projections.

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