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SCANNING ELECTRON MICROSCOPY OF THE EGGS OF *PALINGENIA LONGICAUDA* (OLIVIER) (EPHEMEROPTERA : PALINGENIIDAE)

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Abstract – The eggs of *Palingenia longicauda* (Ephemeroptera : Palingeniidae) were studied by scanning electron microscopy (SEM), and showed a biconvex shape with the 2 aspects joined along a thick peripheral border. The eggs were completely wrapped by an exochorion that differs in thickness and organization according to the region of the chorionic surface. The thickest part of the exochorion formed a plaque, which also covered part of the peripheral border, and was constituted of a network of filaments. The remaining part of the exochorion was composed of a thin wrinkled sheet. The micropyle, hitherto unknown in Palingeniidae, appeared as a round opening penetrating into the plaque. The fibrillar network surrounding the micropyle dove-tailed with the egg chorion, forming a differentiated raised process. This peculiar interconnection facilitates egg anchoring to the substratum, and is an adaptation of the fibrous coat to the aquatic environment.

Index descriptors (in addition to those in title): Mayflies, ootaxonomy, egg chorion, adhesive exochorion, micropyle.

INTRODUCTION

THE EGG organization in mayflies has been described in several studies using scanning electron microscopy (SEM). The fine morphology of the chorionic surface, which includes micropyles and peculiar projections for egg adhesion after deposition in water, has been elucidated earlier (Gaino and Mazzini, 1987, 1988). These studies have included previous light microscopic data (Degrange, 1960; Koss, 1968; Koss and Edmunds, 1974), and have been useful for ascertaining the systematic status of several species (Malzacher, 1982; Gaino *et al.*, 1987, 1989).

The eggs of *Palingenia longicauda* differ from the classical model in Ephemeroptera (see review in Koss and Edmunds (1974)) in their ellipsoidal shape, a feature resembling that of the eggs of the Indian species, *Anagenesia minor*, which also lacks attachment structures on the egg surface (Chopra (1927) in Koss and Edmunds (1974)). Recently, Russev (1987) has illustrated the egg shell of *Palingenia longicauda* by SEM and described the egg shape as lenticular. Apart from this description, no investigation has shown micropylar devices. Their number, position, and organization are unknown in Palingeniidae, one of the ancient families of Ephemeroptera.

The aim of this work was to investigate, by SEM, the fine organization of the egg chorion in *P. longicauda* and to describe the structural feature of the chorion.

MATERIAL AND METHODS

Eggs removed from alcohol-preserved specimens of *Palingenia longicauda*, collected by Dr M. Sartori (Natural Museum, Lausanne) during a field trip in Hungary (26–29 June 1990), were measured using light microscopy. Length and width were determined from a sample of 30 randomly selected eggs. The measurements were given as means (± standard deviation, SD). For SEM examination, 28 eggs were dehydrated in an ethyl alcohol series, critical-point dried using CO_2 in a Bomar apparatus, mounted with double-sided tape on SEM stubs, and sputter-coated with gold–palladium in a Balzer Union evaporator. They were observed with a Philips EM 515 electron microscope at 24 kV.

RESULTS

The egg of *P. longicauda* is nearly ellipsoidal and measures about 418 μm (± 9.5) along the major axis and about 375 μm (± 8.6) along the minor axis. At SEM level, it is completely wrapped by an exochorion, that forms a continuous envelope, a part of which constitutes a sort of plaque restricted to one side of the egg and protruding on the chorionic surface (Fig. 1). Some micrographs of selected sectors of the envelope, taken after transverse sectioning, show that the plaque is the region where the envelope is thickest and least adherent to the chorion (Fig. 2). In the remainder of the egg shell, the envelope is thin and forms a laminar sheet (Fig. 2). The plaque forms multiple ridges and its surface is smooth, which contrasts with the wrinkled appearance of the surface of the laminar sheet (Fig. 3). This sheet is closely juxtaposed to the egg shell. Its removal discloses the underlying surface of the chorion, which at higher magnification, reveals a pattern of densely packed granules (inset in Fig. 2). In eggs with mechanically broken shell, the chorion consists of an 8.5-μm-thick layer composed of vertically disposed lamellae arranged in a squamous-shaped organization (Fig. 4). Inside the oviducts the exochorion enhances egg-to-egg adhesion, thus facilitating egg clustering in linear sequence (Fig. 5). The lenticular shape of the eggs is best appreciated from a point of view showing both dorsal and ventral surfaces by SEM (Fig. 6) that reveals each egg having a biconvex shape with a thick peripheral border (Fig. 7). The exochorion is similarly organized on both aspects and the plaques are continuous along the peripheral border of the egg (Fig. 7). Only one micropyle is present (Fig. 8). It consists of a round opening of 8 μm in diameter that interrupts the uniform envelope and leads to the sperm duct (Fig. 9), a slender canal of 1 μm in diameter, that penetrates inwards in regard to the minor axis of the egg and perpendicularly to the peripheral border (Fig. 10). These features suggest that the micropyle belongs to the funnelform type. The lumen of the micropyle may vary in diameter, depending on the state of folding of the plaque in the micropylar area.

FIG. 1. A SEM view of discoidal egg of *Palingenia longicauda* with prominent plaque (PL) on one side.

FIG. 2. A mechanically broken sector of egg envelope showing thick plaque (PL) detached from chorion and surrounding laminar sheet (LS). Detail of chorionic surface with densely packed granules (inset).

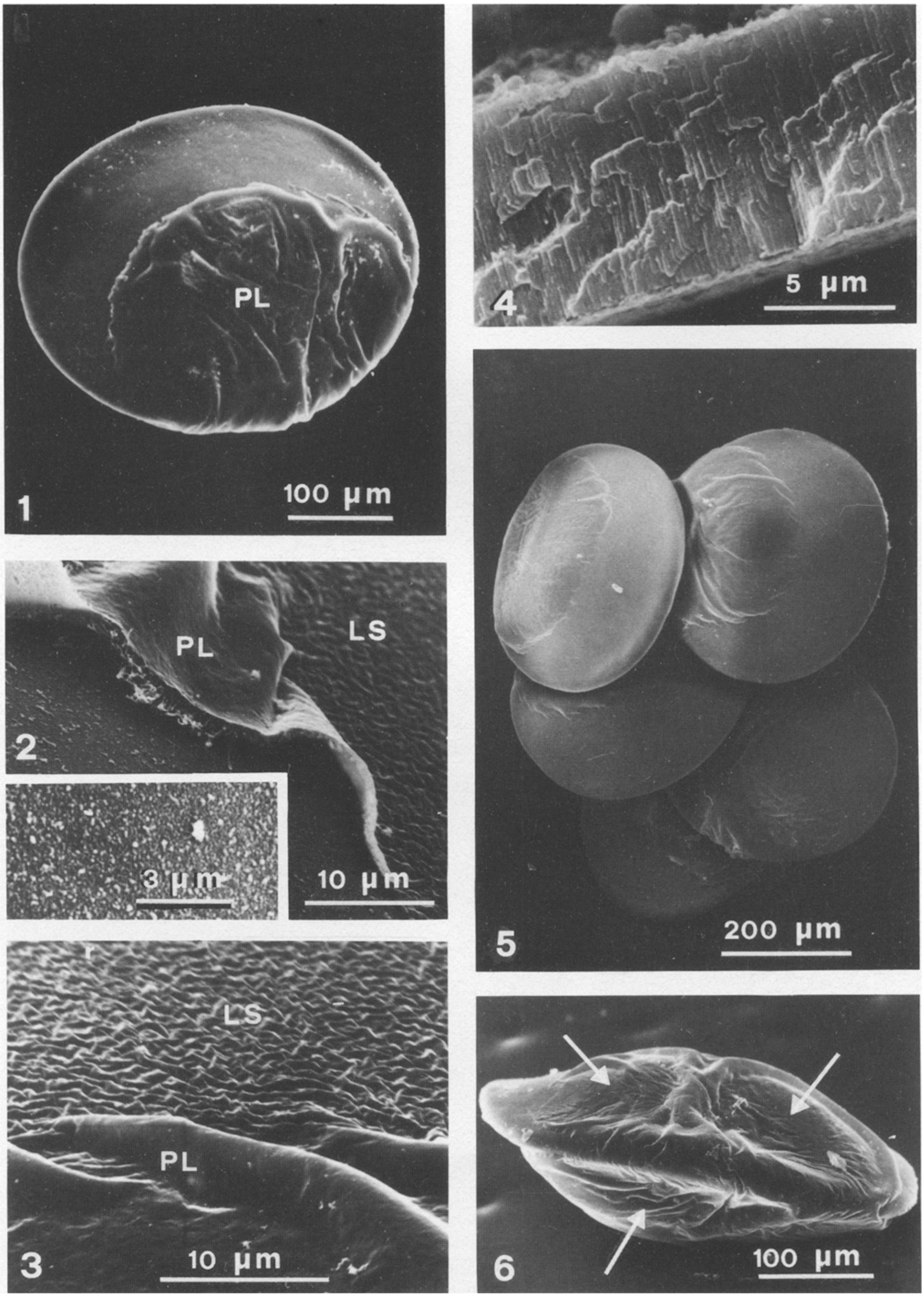
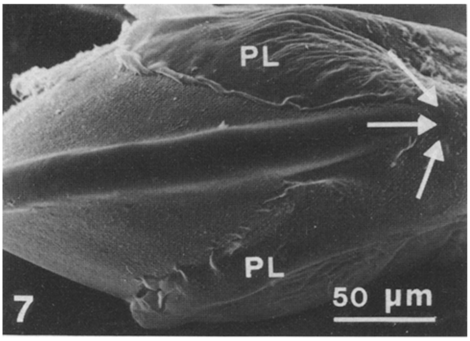


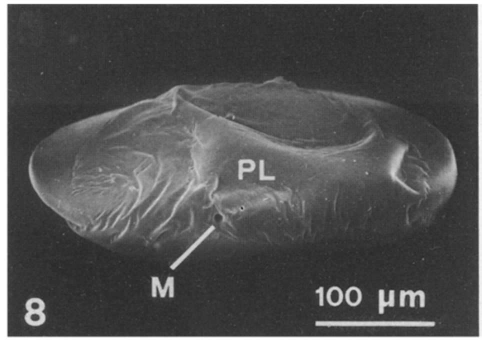
FIG. 3. Another view of egg envelope showing ridges of plaque (PL) and wrinkled surface of laminar sheet (LS).

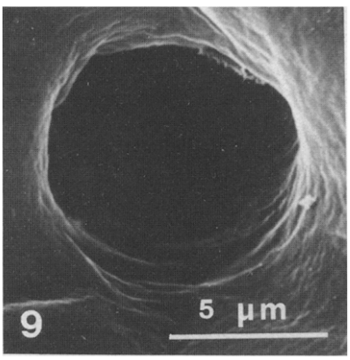
FIG. 4. A section of chorion with its squamous organization due to vertically arranged lamellae.

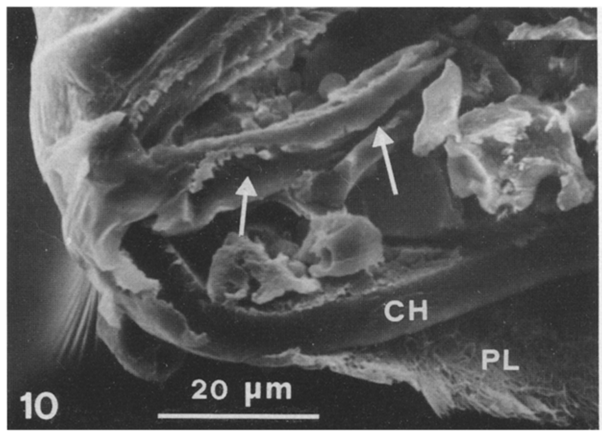
FIG. 5. A cluster of eggs attached by their exochorionic envelope.

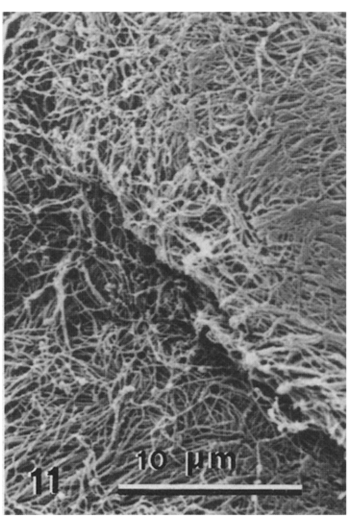
FIG. 6. Both biconvex aspects of egg are wrapped by a continuous plaque (arrows).











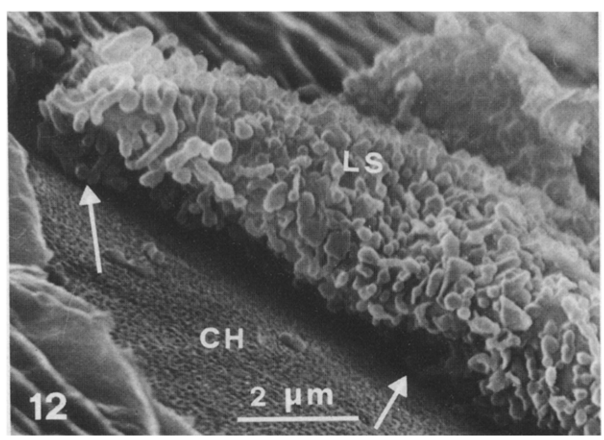


FIG. 7. A lateral view of egg with its typical biconvex shape. Plaques (PL) are continuous along one side of peripheral border (arrows).

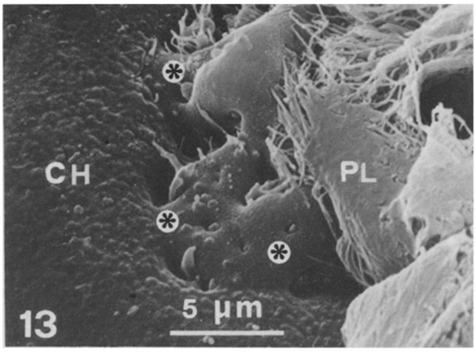
FIG. 8. A single micropyle (M) is visible at peripheral border covered by thick plaque (PL).

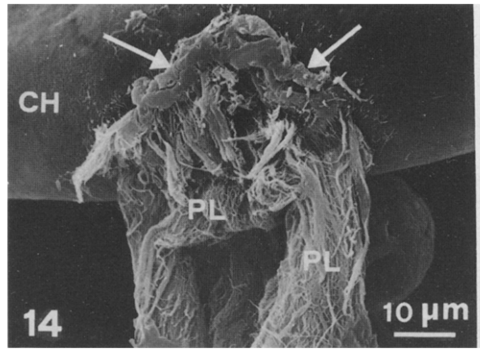
FIG. 9. Round micropyle opening leading to sperm duct.

FIG. 10. SEM view of a cross-section of egg at the level of sperm duct (arrows) showing chorion thickness (CH) and surface of plaque (PL) facing chorion.

FIG. 11. Inner part of envelope facing chorion, composed of a network of fibrils.

FIG. 12. Inner part of laminar sheet (LS) showing irregularly arranged protrusions (arrows) for adhesion to chorion (CH).





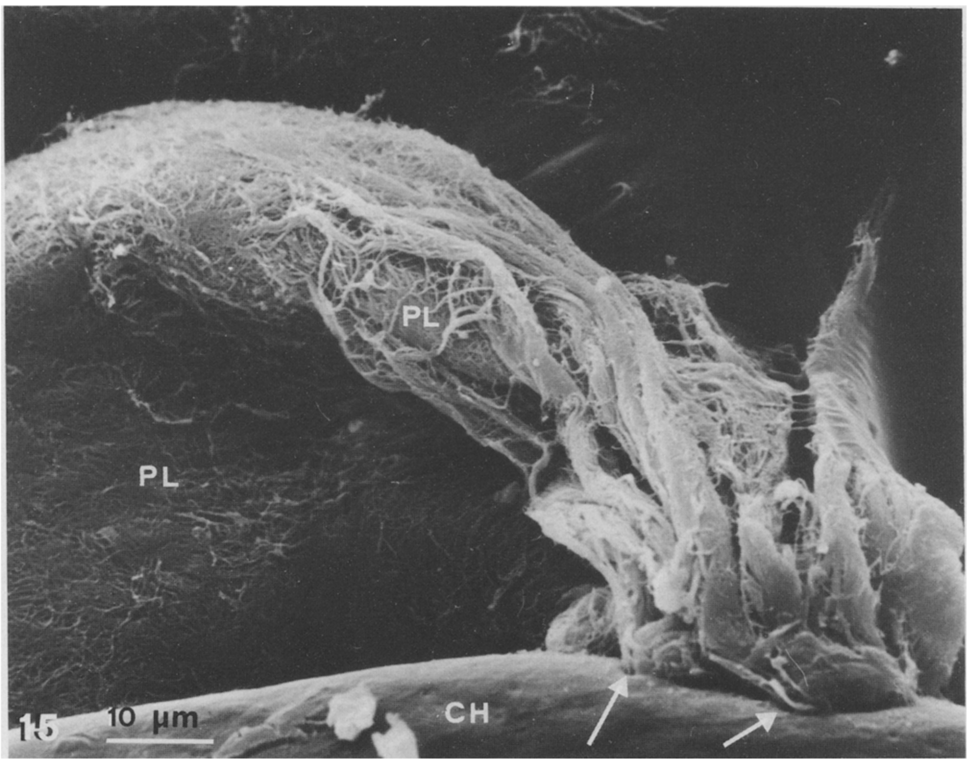


FIG. 13. Interconnection (asterisks) between fibrous matrix of plaque (PL) and chorion (CH) is visible (a lateral view).

FIG. 14. After partial mechanical detachment of plaque (PL), a narrow band (arrows) connecting plaque to the chorion (CH) is visible at micropylar area.

FIG. 15. Mechanical folding of plaque (PL) results in strands of fibrous structures strongly connected (arrows) to chorion (CH).

The varying thickness of the egg envelope depends on the organization of this covering both in the plaque and in the laminar sheet. The plaque consists of a dense reticulation of filaments variously arranged and oriented, producing a fibrous mat (Fig. 11). This organization is revealed by raising and folding the plaque without disrupting its integrity. Such a structure is not detectable from the outermost surface of the plaque because the filaments are embedded in an amorphous matrix, which determines the smooth appearance of the plaque surface (Fig. 3). By contrast, the thin laminar coat strongly adheres to the shell, which cannot be raised without breaking. Experimental breaks show a rough sheath composed of irregularly arranged projections apposed to the chorion (Fig. 12). When the plaque is mechanically detached, a peculiar interconnection between the fibrous matrix and the chorion is revealed around the micropyle. Indeed, the plaque is strongly attached in this area to the egg shell (Fig. 13) by a narrow band that constitutes a specific device (Fig. 14). This feature assures the adhesion of the plaque to the chorion. When the plaque is mechanically raised and folded, it constitutes a stalk connected to the egg at the micropylar area (Fig. 15).

DISCUSSION

The present study revealed 2 unusual features in mayfly eggs: the biconvex shape of the eggs and the organization of the exochorion.

Adhesive structures are generally involved in egg-to-egg cohesion and in anchorage to the substratum. In Diptera, they enable egg–carrier relationships (Cogley and Cogley, 1989); in a representative of Collembola, they may also be involved in protection of the eggs against microorganisms (Larink and Biliński, 1989).

Several types of chorionic attachment structures have been shown in mayflies and categorized in 2 main groups, according to their fibrous or non-fibrous components (Koss and Edmunds, 1974). The fibrous structures, which include the adhesive layers, are the most diversified models with an adhesive function. The adhesive layers consist of a relatively uniform suprachorionic compact mass of fibres enveloping the eggs.

The eggs of *P. longicauda* show specific adhesive layers. These surface structures may perform 2 main roles: (1) as a protective layer for the stored eggs, they prevent superficial injury during accumulation within the oviducts; and (2) as anchoring devices, they allow egg adhesion to the substratum after deposition in water. Because anchoring chorionic projections are lacking in this species, the massive fibrillar network of the plaques may serve as the adhesive system. In some Plecoptera, this function is performed by an attachment disc whose elastic protein component allows egg adhesion to the substratum (Rościszewska, 1991). Even though SEM cannot explain the mechanisms that transform the fibrous covering into a permanent adhesive device, it can suggest a working hypothesis on the sequence of events following egg/water interaction. The close relationship of the fibrous layer with the chorion at the micropylar area is highly suggestive of a stable connection of the envelope to the egg at deposition. It is known that the eggs of this species are dragged for a long distance, up to 3 km (Russev, 1973), and that only eggs settled on adequate clay substrata develop normally (Russev, 1987). Thus, the anchoring of the eggs represents an important step to allow further development.

Apart from a lepidopteran species showing a viscous club-like structure produced by egg exudation (Chauvin and Chauvin, 1980), adhesive material is generally secreted inside the reproductive system. A recent study carried out on the mayfly *Habrophlebia eldae*, whose eggs have no differentiated anchoring devices (Gaino and Mazzini, 1984), showed mucus-like material, characterized by a fibrillar component, within the ribs that run on the egg surface (Mazzini and Gaino, 1985). It was documented that follicle cells are engaged in the synthesis of such material in the panoistic ovarioles (Gaino and Mazzini, 1990a). Furthermore, during transit in the oviducts, eggs are covered by other mucous material produced by the synthetic activity of the epithelial cells delimiting the luminal wall (Gaino and Mazzini, 1990b). According to Brinck (1957), mayflies lack accessory glands that in other insects are usually involved in adhesive layer secretion. This peculiarity supports the view that the fibrous plaques of *P. longicauda* are also synthesized inside the reproductive apparatus. Because the follicular epithelium of insects is the source of egg envelope deposition (King and Koch, 1963; Beams and Kessel, 1969; Favard-Séréno, 1971; Mathew and Rai, 1975; Norton and Vinson, 1982), chorion/fibrous layer interconnection at the micropylar area is probably established in ovarioles during choriogenesis. This intimate relationship is peculiar of mayflies and could be a new acquisition. This hypothesis is supported by the position of the sole micropyle in the plaque, the target of egg/sperm interaction, which becomes competent for egg adhesion after settlement in water. The location of the micropyle and the common folding of the fibrous envelope surrounding its opening, explain why it escaped notice.

Palingeniidae are supposed to be a direct derivation from the Ephemeridae, the latter already present in the Lower Cretaceous (McCafferty, 1990), owing to some plesiomorphic characters shared by primitive genera of these burrowing families (Edmunds, 1973; Landa, 1973). By contrast, under an ootaxonomic point of view, Palingeniidae express through *P. longicauda*, a very specialized representative. This feature has to be taken into account for comparison in evaluating hypotheses on the evolutionary trends of mayflies.

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