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Ultrastructure of the Attachment Disc in *Perla sp.* (Plecoptera) Egg

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Synopsis

The attachment disc of eggs of *Perla sp.* (Plecoptera) is a specialized structure. It serves to fix the eggs to stones under water. The preliminary data, basing on scanning and transmission electron microscopic observations, concerning the morphology of attachment disc are reported.

Introduction

Plecoptera are insects which are distributed all over the world, common in submontane regions of the temperate climate zone. They can be found in the neighbourhood of streams and small rivers with a distinct water current, because clean water high in oxygen, is indispensable for their development.

One of many features adaptive to the aquatic habitat is that newly laid eggs of Plecoptera stick strongly to stones, to gravel or to sand grains in the river bed. Because of this they remain in place in spite of water movement. The specialized surface structre responsible for this permanent attachment of an egg is the so called attachment disc (Hinton, 1981). In his book Hinton (1981) made only a few remarks about this interesting structure.

The presentation of preliminary investigation data on the attachment disc in *Perla sp.* is the aim of the present paper.

Materials and Methods

Adult females of *Perla sp.* were collected in the Bieszczady mountains (south-east part of Poland) close to stream banks during their egg laying period. The insects were decapitated, then the dissected ovaries and egg clusters were fixed. The material was examined under the phase-contrast light microscope, with a scanning electron

microscope (SEM) and with a transmission electron microscope (TEM).

1. For phase-contrast light microscope studies, egg clusters were fixed in 2.5% glutaraldehyde on 0.1 M phosphate buffer, pH 7.4 for 72 hr. Then the eggs were isolated from clusters, rinsed in distilled water, placed in a droplet of insect Hoyle solution and examined under a Peraval Interphaco Carl Zeiss Jena phase-contrast microscope.

2. For SEM studies egg clusters were fixed in glutaraldehyde (for details see point no. 1), then eggs were isolated from clusters, dehydrated with acetone, air dried and coated with gold. After this they were examined with a Tesla BS 300 scanning electron microscope.

3. For TEM studies after preliminary fixation in glutaraldehyde (for details see point no. 1) ovaries were rinsed several times in phosphate buffer, pH 7.4 with the addition of sucrose, and fixed for 2 hr with 2% OsO_4 in 0.1 phosphate buffer, pH 7.4. The specimens were then rinsed several times in distilled water, dehydrated in a graded series of alcohol and acetone, and embedded in Epon 812. After this they were vacuum dried for 2 hr. Blocks were cut on the Tesla BS 490 A ultramicrotome.

Semithin epon sections were stained with solution of metylene blue in 1% borax. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined in Tesla BS 500 electron microscope.

Results

The yellowish-brown oval egg of *Perla sp.* is 0.4 mm long and 0.2 mm wide. The surface of the chorion obtained by SEM shows a regular hexagonal sculpture similar in appearance to a honey-comb. In the centre of each hexagon there is a semispherical structure of unknown composition and origin (Figs. 1, 2, 4).

The posterior end of the egg (in an ovariole oriented towards the opening of the oviduct) is covered by a cap like attachment disc (Figs. 1, 2, 3) with similar hexagonal sculpture. Each of the hexagons, however, displays a granular structure (Fig. 4) and contains not one but from three to six of the semispherical structures mentioned above (Figs. 2, 4).

The phase-contrast microscope reveals the existence of a stem, sunk in the chorion, which anchors the attachment disc. The second finding is that semispherical structures are in turn supported and/or connected to tiny rod like structures (Fig. 3).

The attachment disc development was investigated as well. While examining semithin sections it was found that immediately above the already formed chorion, at the egg end oriented towards the opening of the oviduct, there is cap like layer of a formless substance which in turn is covered by a layer of follicular cells. Then at the border between follicular cells and the attachment disc and in the attachment disc itself, some dense stained spots appear (Fig. 5 a), *i. e.*, droplets of unknown origin and composition.

From the TEM studies it appears, that during formation the attachment disc is made of medium electron density spongy material (Fig. 5). The space between the follicular cells and the attachment disc is filled by similar material, which however is not quite so condensed. This space is confined on one side, by the outer part of the

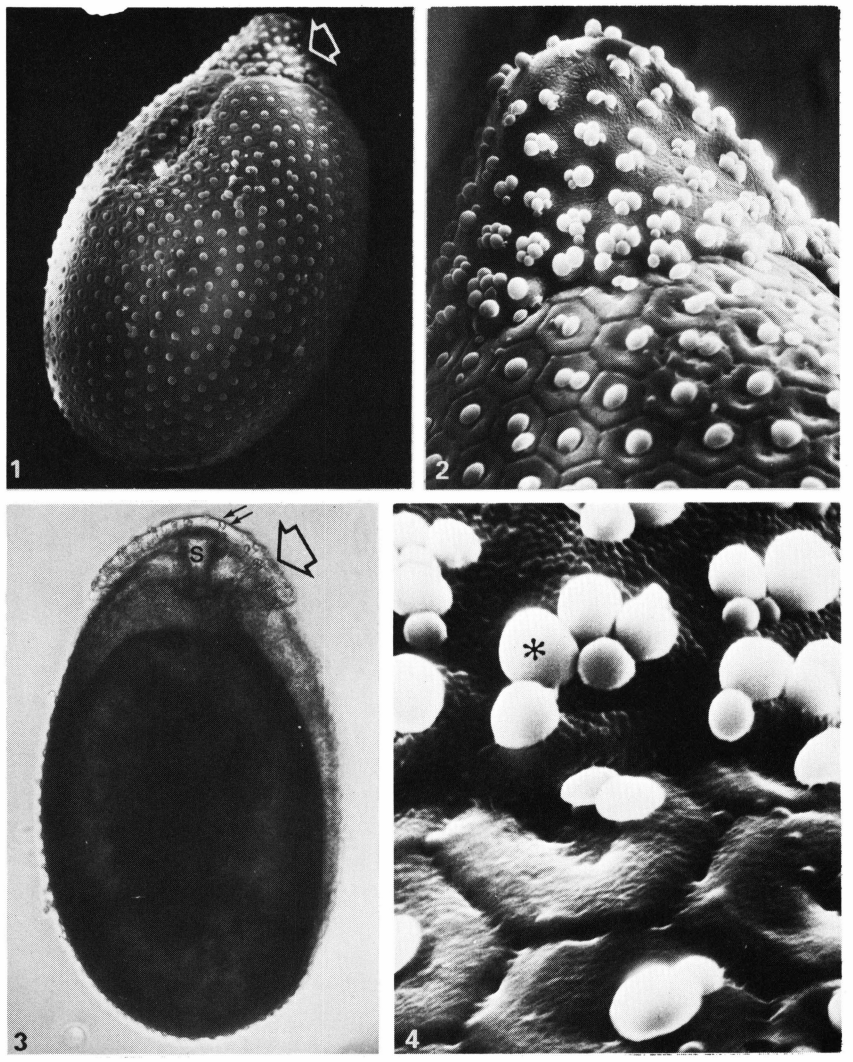

Fig. 1. SEM micrograph of the egg of *Perla sp.* × 160.
Fig. 2. The attachment disc (enlarged fragment of Fig. 1). × 570.
Fig. 3. Phase-contrast micrograph of an egg. × 170.
Fig. 4. Enlarged fragment of Fig. 2. × 5,500.
s, stem; asterisk, semispherical structure; double arrow, rod like structures; single arrow, attachment disc.

attachment disc, which is a layer of much heavily condensed material, and on the other side by the follicular cell's apical zone furnished with numerous microvilli (Figs. 5, 6). In the space described one can also notice regularly distributed fields composed of material similar to that of the attachment disc.

The densely stained spots (droplets) observed in semithin sections were found to be composed of a substance similar to that of the attachment disc but much more

heavily condensed. The droplets on the side closest to the attachment disc are covered with a thin electron dense layer (Fig. 5).

Discussion

Most species of Plecoptera lay between 100 and 500 eggs, usually in several batches (Hinton, 1981). The females of *Perla sp.* carry clusters of eggs on their abdomens. After being laid the clusters disperse in the water, and single eggs cling onto surface of stones or grains of gravel, fixed on by the attachment disc. This structure, according to Miller (1939, cited after Hinton, 1981) is sticky. It is also elastic and can be stretched even 10 times of its normal height before it tears apart.

From the preliminary investigation in *Perla sp.* it appears that the attachment disc develops from a modified chorion. The shape is that of a cap which is anchored to the egg by a stem. The stem is elastic and can undergo much elongation (Rościszewska, unpublished) which is in agreement with Miller's observations.

In the SEM studies it was found that the surface of the chorion and the attachment disc itself display a honey-comb like sculpture which according to the data of Margaritis and collaborators (1980), seems to be follicular cells imprints.

Each hexagon is decorated by one (rarely one or three) and in the case of the attachment disc by three to six semispherical structures of unknown composition (see Results). A reasonable assumption is that the structures are directly responsible for "glueing" the attachment disc (to a smaller extent the other parts of the egg surface also) to the stone under water. Certainly, this could be considered as an adaptive feature in Plecoptera.

The process of development of the attachment disc has not yet been described in literature. From the present investigation on *Perla sp.* it appears that the structure develops on the chorion (Fig. 5 a) with the evident involvement of follicular cells. Facts supporting this supposition are as follows: numerous ribosomes and elements of rough endoplasmic reticulum occur in their cytoplasm and numerous follicular cell microvilli project towards the attachment disc. Furthermore, the substance contained within the space between follicular cells and the attachment disc seems to represent the precursor of the material building up the attachment disc.

Regularly distributed fields, which can be seen in this space, probably represent a surface layer of the chorion possesing a characteristic honey-comb sculpture.

At the present stage of the investigation it is difficult to elucidate the role of the densely stained droplets within the attachment disc (and within the space under follicular cells), however, it seems probable that they are connected with the formation of the semispherical structures occuring on the hexagones. The possibility that the droplets are local reservoirs of a sticky (polymer ?) substance forming these structures on the egg surface, can not be excluded. Furthermore, it might be suggested that this sticky substance could be transported to the surface of the chorion through tiny rod-like structures observed within the disc in phase-contrast microscope.

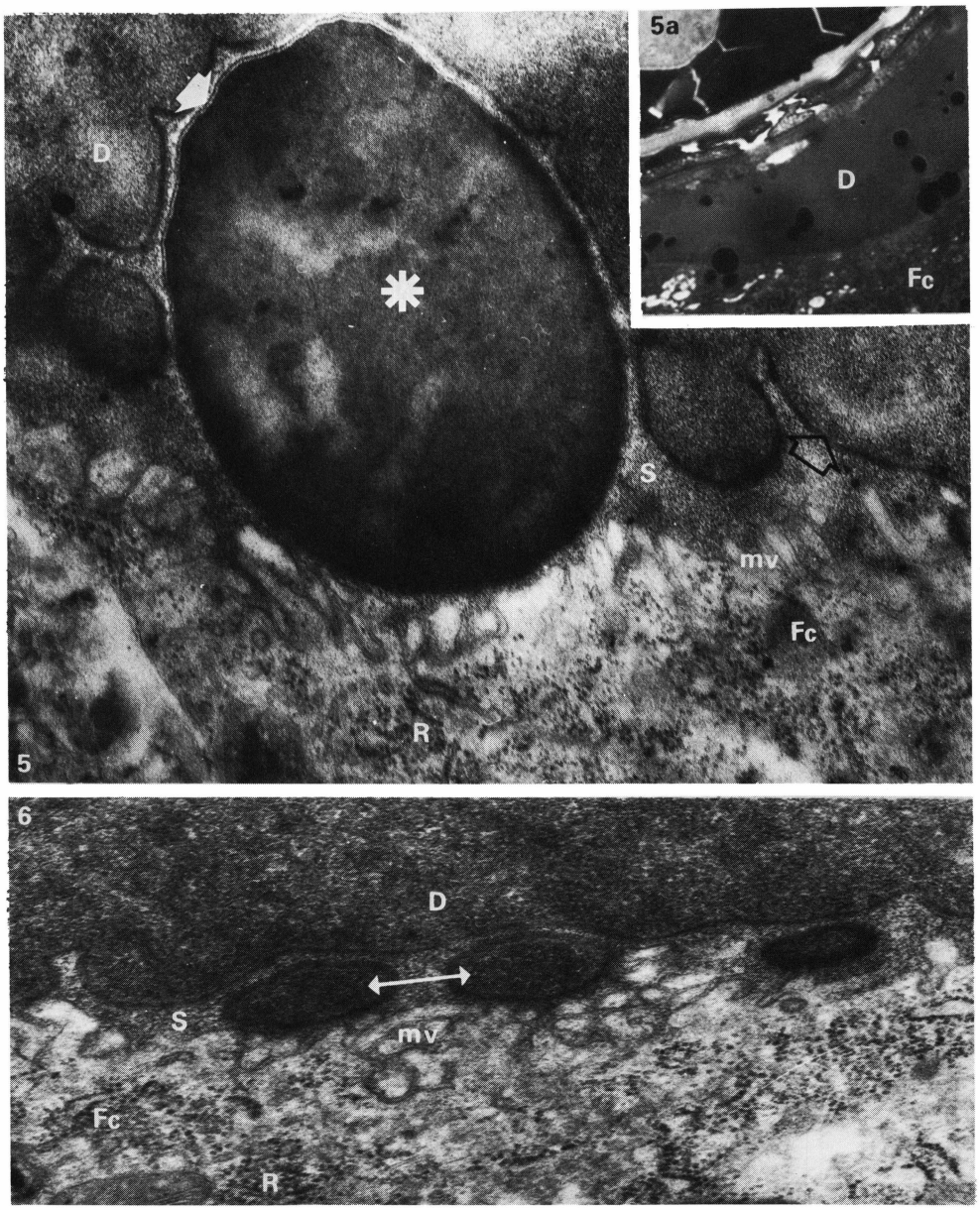

Fig. 5. Electron micrograph ot a fragment on the attachment disc. × 30,000.
Fig. 5 a. Semithin section of not fully developed attachment disc. × 960.
Fig. 6. Electron micrograph of regularly placed fields on the developing attachment disc. × 25,000.
D, fragment of attachment disc; Fc, follicular cell; mv, microvilli; R, ribosomes; S, space between attachment disc and follicular cells; asterisk, droplet of unknown composition; black arrow, outer part of attachment disc; bold white arrow, layer covering droplet; white arrow, regularly placed fields.

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Addendum

Since this paper was submitted for publication, the author found an excellent paper of Kishimoto and Ando (1985) on the embryology of a plecopteran *Kamimuria tibialis*, including descriptions of the attachment disc. The shape and structure of the attachment disc of *K. tibialis* are in good agreement with those of *Perla sp.*

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