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ARTICLE

External Morphology and Ultrastructure of Tegumental Glands of *Aegla platensis* (Crustacea, Anomura, Aeglidae) Pleopods: Might They Play A Role in Egg Attachment?

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ABSTRACT

Egg incubation on the female abdomen is the parental care behavior observed in aeglids, in which eggs are kept adhered to maternal pleopods and maintained, cleaned and aerated. In *A. platensis*, egg attachment occurs with the aid of pleopodal setae, which are twisted around their axis in the distal region, forming the funiculus, and pleopodal glands, which are responsible for the production of the adhesive substance that seems to be involved in egg fixation to pleopodal setae. Those glands are acini formed by secretory cells arranged concentrically around a central duct, giving them a rosette appearance. Two types of secretory cells were observed, those that produce electron-lucid vesicles and those having electron-dense ones. Both kinds of vesicles are released in a duct whose opening pore is located on the pleopodal surface and constitute the adhesive substance that coats eggs and pleopodal setae, ensuring egg fixation to the female body and maternal care maintenance. This study investigates the internal and external morphology of *Aegla platensis* pleopods, to understand the egg attachment process and identify the structures involved in this phenomenon. Three microscopy techniques are used: scanning electron microscopy (SEM), transmission electron microscopy (TEM), and optical microscopy (OM).

1. Introduction

Brood care is a widespread reproductive trait among decapod crustaceans, with egg and juvenile incubation being the most common manifestations of parental care [1-3]. Egg incubation is energetically demanding and might represent a great cost for females [4]. Nevertheless, it may guarantee the reproductive investment and enhance brood survivorship [5].

Among anomurans and brachyurans, eggs are kept under the female abdomen, fixed to abdominal appendages called pleopods. These structures are involved in the egg attachment process and, to better understand crustacean

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brood care, many researchers have studied the internal and external morphology of these appendages [6-16].

Aeglids are a peculiar group among anomurans, especially due to their morphology, ecology and biogeography [17]. The genus *Aegla* Leach, 1820 is endemic to the austral region of South America and comprises 69 described species, as well as others that are currently being described [18-20]. Together with *Clibanarius fonticula* Mclaughlin and Murray, 1990 and *Munidopsis polymorpha* Koelbel, 1892, they are the only anomurans that live in freshwater ecosystems [21-22], being found in rivers, streams and lakes, hidden under rocks, leaf litter or buried in sand [23-24].

Aeglid females have four pairs of pleopods that carry up to 60 eggs [25]. The entire embryonic development is completed under the female's abdomen, and the female is responsible for egg aeration, cleansing, removal of non-viable eggs, and protection against predation [19,26-28]. In aeglid males, the abdominal appendages are absent or vestigial[29], highly suggesting the role of pleopods in egg incubation and brood care.

Despite the observation of some behaviors related to the egg attachment process in aeglids [30], the internal and external morphology of pleopods are unknown. Thus, the aim of this work was to describe the internal and external morphology of these appendages in *Aegla platensis* Schmitt, 1942.

2. Methods

2.1 Sampling

Ovigerous females of *A. platensis* were collected in July 2010 in Minero Creek, Taquara, Rio Grande do Sul (29º 46' S - 50° 53' W) and transported to the laboratory for further analyses. Specimens were anesthetized with ice and pleopods of six ovigerous females were dissected. Some of the eggs adhering to pleopods were removed to maintain an average of four eggs per appendage.

2.2 Optical Microscopy (OM)

The appendages were fixed in Bouin's solution for 24 h, decalcified with 10% EDTA for 7 days, dehydrated in an ascending series of alcohol solutions (70, 95 and 100%) and diaphanized in xylol. Then, the material was infiltrated and embedded in paraffin, and 10 µm thick histological sections were obtained using a microtome (RM2145; Leica, Austria). The sections were processed, stained stained with hematoxylin and eosin (Manual 1960) and photographed using the software Axiolab under an optical microscope (Carl Zeiss, Germany).

2.3 Scanning Electron Microscopy (SEM)

The material was fixed in 10% buffered formalin, post-fixed with 2% osmium tetroxide phosphate solution for 2 h and dehydrated in increasing concentrations of alcohol (to 100%) and alcohol/acetone solution (1:1). Dehydrated samples were dried through critical point, mounted onto stubs with double-sided tape, and sputter-coated with gold. SEM photographs were taken using a JSM 5800 scanning electron microscope (JEOL, Japan).

2.4 Transmission Electron Microscopy (TEM)

Immediately following dissection, appendages were fixed with Karnovsky (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer), washed with buffer, and post-fixed with 1% osmium tetroxide for 1 h. Dehydration was conducted using an ascending alcohol series (30, 50, 70, 95 and 100%) followed by pure acetone. After this preparation, the material was infiltrated in Durcupan ACM resin (araldite) and acetone (1:1) and then embedded in resin without dilution for 24 h. Polymerization followed at 60 °C for 48-72 h. Semithin sections (1 µm) were obtained using an ultramicrotome (Ultracut UCT 2.0; Leica) and stained with 1% toluidine blue (Merck, Germany) diluted in 1% sodium tetraborate (Ecibra, Brazil). Area selection was performed and ultrathin sections (70 nm) were obtained using the same ultramicrotome and mounted on copper grids (200 mesh). These sections were contrasted with 2% uranyl acetate (Merck) and 1% lead citrate (Merck) [31]. The ultrathin sections were examined using a transmission electron microscope (JEM 1200 EX II; JEOL).

2.5 Setal Classification

Setal classification is based on Martin and Felgenhauer, Jacques, Watling and Teodósio and Masunari [32-35].

3. Results

3.1 External Morphology: Setae and Egg Attachment

Two types of simple seta (without cuticular projections) were found along the appendages: long seta (Figure 1A) and stout seta (Figure 1B). Long setae were grouped in tufts distributed throughout the entire pleopod (Figure 1A). Stout setae had shorter and thickened stems, and were distributed along the appendage without a clear pattern(Figure 1B).

Analysis of the external morphology of pleopods revealed that eggs are attached individually to a long setae, also known in the literature as pleopodal setae or oosetae

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(Figure 1C). During the egg attachment process, a group of setae that form the same tuft twist around their own axis in the distal region, forming the funiculus (or stalk), to which the egg is fixed (Figure 1D). Moreover, the egg adhesion process also includes an adhesive substance that seems to cover not only the setae, but also the egg, as a single structure (Figure 1D).

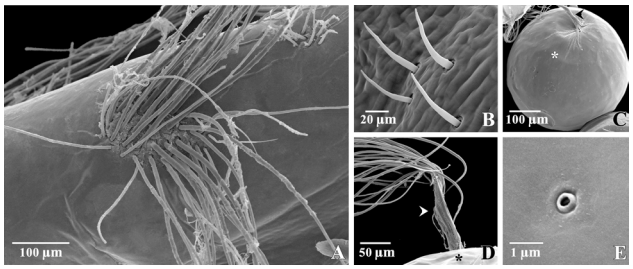

Figure 1. Scanning electron micrographs of *Aegla platensis* pleopods.

Note: A. Details of a tuft of long setae. B. Details of four stout setae. C. Details of the insertion region of oosetae on egg (white asterisk) and funiculus (black arrow). D. Details of egg (black asterisk) and funiculus (white arrow), evidencing the group of twisted pleopodal setae. E. Details of a pore.

3.2 Internal Morphology: Pleopodal Glands

Female pleopods have numerous pores along their surface (Figure 1E). These pores seem to be connected to the tegumental glands (pleopodal glands). The pleopodal glands are rosette-like structures formed by the agglomeration of acini (Figure 2A). Each acinus has numerous secretory cells (Figure 2B) and one duct cell (Figure 2C). Secretory cells are arranged concentrically around the duct cell where they release their products, giving the gland a rosette shape.

The secretory cells are pyramidal in shape, with spherical or oval nucleus. They have a wide basal surface toward the basal lamina and a smaller apical surface facing the lumen of the acinus. Two types of secretory cells can be distinguished: 1) mucous-secreting cells (Figure 2B) with nucleus located at the base of the cell and containing numerous granules or vesicles filled with electron-lucid material, and 2) serous secretory cells (Figure 2B), which have electron-dense granules in the cytoplasm and nucleus is located in the basal third. In both types of secretory cells, granules appear to merge as they approach the apical region of the cell, through where they presumably release their contents into the central duct.

The duct cell has less developed rough endoplasmic reticulum and golgi complex than secretory cells, an ovoid or spherical nucleus, and a duct that passes through its entire length and opens into the channel with the pleopodal pore cited above as external aperture (Figure 2C).

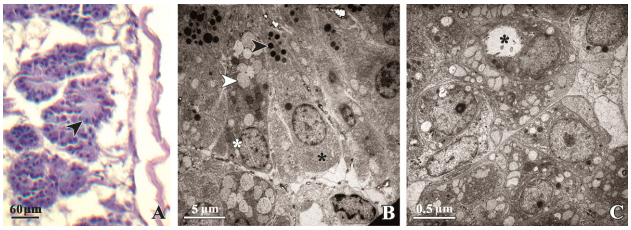

Figure 2. Photomicrography of an *Aegla platensis* pleopod histological section and electron micrograph of pleopodal glands.

Note: A. Groups of pleopodal glands (longitudinal section). Note the lumen of the acinus (arrowhead). B. Details of two types of secretory cells: mucus secreting cells with electron-lucid vesicles (white arrowhead); nucleus localized on the base of the cell (white asterisk); serous-secreting cells with electron-dense granules (black arrowhead); nucleus is located in the basal third (black asterisk). C. Details of a duct cell and the channel (black asterisk).

4. Discussion

In many decapods, the main function of pleopods is related to egg attachment, grooming and juvenile fixation [29,36-37]. Furthermore, Rabalais, Scholtz and Vogt [38-40] suggested that freshwater habitats, especially lotic ones, require specific adaptations in individuals that are able to colonize them. Among these adaptations is increased brood care in comparison to related marine groups, a feature that aims to reduce brood mortality, thus preventing offspring from being carried by the water flow or avoiding predation [41-42]. Considering that the genus *Aegla* is one of the three genera of anomurans that colonized freshwater habitats, the presence of specialized structures in its pleopods might be an important adaption to guarantee high reproductive fitness in this environment.

Like other aeglid species, *A. platensis* occurs in brooks and rivers with a considerable current [19]. During copulation (female under male), we observed that females release the oocytes through the genital pores inside the abdominal chamber where they are immersed in kind of 'soup' with a spermatophore-like structure [30]. Once copulation is finished, females close their abdominal chamber, pressing the telson and uropods against the last thoracic sternites. At this time, clearly, there is a movement of pleopods inside the female abdominal chamber, probably adding oocyte fertilization and also fixing eggs to oosetae. A few minutes later, the eggs are already attached to pleopods [30]. Interestingly, all of these events happen within a few minutes. Thus, we could hypothesize that, for aeglid species living in environments with a current, fast and efficient egg attachment is an excellent adaptation.

SEM analysis revealed the presence of two morphologically distinct types of setae in the pleopod cuticle of *A. platensis*. Both types, long and stout setae, were pre-

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viously described by Martin and Felgenhauer [32] and are commonly found on different decapods appendages, being usually related to crustacean grooming [43]. Furthermore, both kinds of setae have been found on other appendages of *A. platensis* such as pereiopods and maxillipods [32]. Stout setae are less numerous than long setae and they are apparently not directly involved in egg attachment. On the other hand, long setae are related to egg fixation and embryo incubation. The function of long setae in egg attachment has also been observed in other decapod crustaceans, such as crayfish (*Austropotamobius pallipes* Lereboullet, 1858 [16] and *Cherax cainii* Austin and Ryan, 2002[4]), lobster (*Homarus americanus* Milne-Edwards, 1837 [14]), shrimp (*Palaemon macrodactylus* Rathbun, 1902 [36]) and crab (*Sesarma haematocheir* de Haan, 1833 [44-46]).

The presence of a funiculus, as observed in this study, has also been identified in other decapod species[4][37][46] and its origin has often been discussed. In some species, the funiculus is formed within the abdominal cavity with the aid of long setae[9][36][46] or simply by deposition of the substance excreted by pleopodal glands[37]. However, the funiculus is derived from the outer layer of the egg in other species [48-52] or has a dual origin (long setae and egg outer layer)[16]. In *A. platensis*, it is known that the funiculus formation has no contribution from the egg layer since Lizardo-Daudt and Bond-Buckup[25] did not find any structure that could be involved in this process. Moreover, as shown here, the funiculus seems to be formed by addition of an adhesive substance to pleopodal setae.

Tegumental glands are characteristic of decapod crustacean integument, being found in variable quantities and in different parts of the body [53-54] such as statocysts, gonopods, gills, pereiopods, pleopods and uropods [55-59]. The structural components of these glands also vary according to the species and function they present [53-55].

The tegumental glands observed in the pleopods of *A. platensis* are morphologically very similar (rosette-like acini) to those found in the pleopods of other decapod species [11,13,56], as well as those found in other appendages of different decapod species [46,59-61], including the pereiopods of *A. platensis* [30].

Pleopodal glands of *A. platensis* are composed of one duct cell surrounded by many secretory cells that have well-developed rough endoplasmic reticulum and golgi complex, which are generally related to secretory activity in other organisms [62]. In mucous secretory cells, electron-lucid granules are observed in the cytoplasm, suggesting that the material they secrete contains mucopolysaccharides. In contrast, serous secretory cells present electron-dense granules, indicating that the material they secrete contains protein [62].

Several functions have been attributed to tegumental glands, such as carapace hardening in specific structures like mouthparts and esophagus [15,63], chemical signaling [64], and mucus production in mouthparts to assist ingestion [65-66]. When found in pleopods, such glands are related to the production of an adhesive substance responsible for egg attachment [13,56]. Thus, we suggest that pleopodal glands are related to egg attachment in *A. platensis*.

This study investigated, for the first time, the egg attachment mechanism in aeglids by examining the internal and external morphology of female pleopods. Nevertheless, further investigations are needed, focusing on the importance of the funiculus and egg layer composition, as well as the histochemical analysis of compounds from the tegumental glands.

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