ULTRASTRUCTURE OF EPIDERMAL CILIA AND CILIARY ROOTLETS IN SCAPHOPODA

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ABSTRACT

Ciliary structure in Scaphopoda is hitherto unknown and may provide information useful for phylogenetic analyses. Here we describe the ultrastructure of the ciliary apparatus of multiciliated epidermal cells of four species of Scaphopoda: Antalis entalis, Antalis occidentalis, Entalina tetragona and Cadulus propinquus, revealed by transmission electron microscopy. In all studied species the cilia have long whip-like distal ends. The rootlet apparatus consists of a basal foot, a short anterior ciliary rootlet and a long vertical rootlet. In other molluscan classes, the presence of an anterior rootlet has previously only been shown in species of the Neomeniomorpha, Chaetodermomorpha and Polyplacophora, while such a rootlet is absent in Gastropoda, Bivalvia and Cephalopoda. Twin rootlets, such as present in species of lamellibranch Bivalvia and postembryonic Cephalopoda probably represent a split vertical rootlet. The discovery of an anterior rootlet in Scaphopoda shows that the presence of paired ciliary rootlets is not a synapomorphy of a clade comprising the aplacophoran Neomeniomorpha and Chaetodermomorpha and the Polyplacophora, but that it represents a plesiomorphy of the Mollusca.

INTRODUCTION

The Scaphopoda are a taxon of molluscs comprising slightly more than 500 recent species (Steiner & Kabat, 2001). Its phylogenetic position in relation to other molluscan classes has long been a matter of discussion. Runnegar & Pojeta (1974) introduced the so-called Diasoma concept, uniting scaphopods and bivalves based on palaeontological data. This view seemed to be well supported by similarities in the nervous system, the burrowing foot and a seemingly twofold shell formation (see Haszprunar, 2000). Based on further morphological data Waller (1998), in contrast, placed the scaphopods as close relatives of gastropods and cephalopods. Wanninger & Haszprunar (2001) also showed that the assumed two-fold shell formation in the Scaphopoda is a misinterpretation, and that scaphopods in fact only have one larval shell. Moreover, the Diasoma-concept has been challenged based on molecular data (Steiner & Dreyer, 2003; Halanych, 2004; Passamanec, Schander & Halanych, 2004) that place Scaphopoda as the sister group to Cephalopoda.

Ultrastructural characters of the ciliary apparatus of multiciliated epidermal (ectodermal) cells are useful in evaluations of phylogeny, because there is a high probability of homology when comparing ciliary structures from different taxa (cf. Tyler, 1979). Ciliary rootlets have also previously been shown to be phylogenetically informative in molluscs (Lundin & Schander, 1999, 2001a, b, c) and related taxa (Lundin, 1997, 1998; Lundin & Schander, 2003). There are for example clear differences between the Conchifera, the Polyplacophora and the aplacophoran taxa Neomeniomorpha and Chaetodermomorpha.

In the scaphopod mantle cavity, anterior to the anal bulb, there is a series of transverse ciliary bands ranging in number from 1 to 30 depending on species and body size (for details see Steiner, 1991). These circulate water through the mantle cavity from the posterior opening to the anterior one. Water can also be expelled from the mantle cavity through the posterior opening by withdrawal of the foot (Reynolds, 2006).

In this paper we describe the ultrastructure of mantle cavity cilia of scaphopods with special focus on the ciliary rootlet structure and discuss the phylogenetic implications of our findings.

MATERIAL AND METHODS

Specimens of Antalis entalis (Linnaeus, 1758), Antalis occidentalis (Stimpson, 1851), Entalina tetragona (Brocchi, 1814) and Cadulus propinquus (G.O. Sars, 1878) were collected at Espenegrund Marine Station outside of Bergen, Norway in March 2004. Additional specimens of A. entalis were collected at Tjärnö Marine Biological Station outside Strömstad, Sweden, in February 2004. The material was fixed for transmission electron microscopy (TEM) in 2.5% glutaraldehyde in sodium cacodylate buffer, followed by postfixation in 1% osmium tetroxide in sodium cacodylate buffer and transfer to storage in 70% ethanol. The specimens were embedded in Agar 100 Epoxy resin in March 2004. Sections were given different orientations to obtain views of the ciliary apparatus from diverse angles. Semi-thin sections of the embedded specimens were put on slides and examined by light microscopy to locate ciliated areas. Standard methods for TEM were used for ultrathin microtome sectioning, mounting of sections on grids, and contrast staining with lead citrate and uranyl acetate (see Lundin & Schander, 1999).

For examinations we used a JEOL JEM-1230 located at the molecular imaging centre (MIC) at the Medical Department of the University of Bergen, Norway.

RESULTS

The epidermal ciliary structure is identical in the four examined species. The cilia have the typical $9 \times 2 + 2$ microtubule pattern. The cilia are long, with slender whip-like distal ends.
Epidermal cilia and brush border of Entalina tetragona. In the middle is a cross-section of a cilium through its slender distal part, close to the tip (arrow). TEM. Scale bar = 1.0 μm.

The microvilli among the epidermal cilia are slender, often more or less beaded and occasionally branched. In sparsely ciliated areas of the epidermis the microvilli often form a dense brush border (Fig. 4A). In Entalina tetragona (Fig. 4A) and Cadulus propinquus (Fig. 4B) the microvilli are distinctly beaded. In the latter species, the villi are relatively short compared to other studied species. In all studied species the glycocalyx is relatively thin and weakly layered (Fig. 4B).

**DISCUSSION**

The structure of the ciliary axoneme and basal body of the mantle cavity cilia of scaphopods described here reflects the general morphology of locomotory cilia. However, the presence of an anterior ciliary rootlet is surprising, because representatives of the Conchifera – the ‘higher’ molluscs, including scallopods – had been thought to possess only a vertical ciliary rootlet (see Lundin & Schander, 2001c).

Paired rootlets have previously been reported in locomotory cilia of different organs of several bivalve taxa, such as the lips and palps of adult Placopecten and Chlamys (Beninger et al., 1990), the gills of Thyasina (Passos et al., 2007) and probably also Ostrea (Bigas et al., 2001), as well as the velum of larval Pecten (Cragg, 1989). These rootlets, however, are not perpendicular in relation to the basal body, but are both vertically orientated and arise from the lower face of the basal body. These twin vertical rootlets have likely been derived from a single vertical rootlet that split into two parts, perhaps for enhanced mechanical stability. An anterior ciliary rootlet thus generally appears to be lacking in bivalves, including the protobranchs (Lundin & Schander, 2001a).

In Cephalopoda, locomotory cilia occur in postembryonic stages only, and locomotory cilia with bifurcated rootlets have been described in Loligo vulgaris and Sepia officinalis (Sundermann, 1983). Here, the origin from a single, split, vertical rootlet is even more obvious than in bivalves. Further and more detailed studies are still lacking and are desirable for further evaluation of the relationships between Scaphopoda and Cephalopoda.

In molluscs, an anterior rootlet is thus known only from Neomeniomorpha (Lundin & Schander, 2001b), Chaetodermomorpha (Lundin & Schander, 1999), Polyplacophora (Lundin & Schander, 2001c) and Scaphopoda (herein). The presence of a long anterior rootlet, as in Neomeniomorpha, has earlier been interpreted as the plesiomorphic state (Lundin & Schander, 2001c; Table 1). This assumption is supported by the widespread occurrence of long anterior rootlets in other lophotrochozoans (Tyler, 1979; Ax, 1995; Emschermann, 1996; Lundin & Schander, 2003; Hausen, 2005). Similar to Chaetodermomorpha and Polyplacophora, the anterior rootlet in the investigated representatives of Scaphopoda is rather short and thus presumably modified as compared to the state in the Neomeniomorpha.
The presence of an anterior rootlet in Scaphopoda shows that this character is plesiomorphic within the Mollusca and not a common trait of a clade ('Aculifera', 'Amphineura') uniting the aplacophoran groups Neomeniomorpha and Chaetodermomorpha with the Polyplacophora. As a plesiomorphic character, the presence of an anterior rootlet cannot be used to evaluate phylogenetic concepts within the ‘higher molluscs’, such as the Diasoma-concept.

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Figure 2. Epidermal ciliary rootlets of Scaphopoda, TEM. A. Antalis entalis; note the long vertical (second) rootlet (arrows) and part of the short anterior (first) rootlet (arrowhead). B. Antalis entalis; basal body in longitudinal section and part of the vertical rootlet of an epidermal cilium. C. Entalina tetragona; anterior (first) rootlets are to the left and the basal feet (arrows) to the right side of the basal bodies. The vertical (second) rootlets are obliquely directed. D. Entalina tetragona; epidermal ciliary basal bodies and rootlets in longitudinal section. E. Antalis occidentalis; cross-sections of several epidermal ciliary basal bodies with two anterior rootlets (arrowheads) and a basal foot visible (arrow). Scale bars = 0.5 μm.

Figure 3. Schematic drawing of the ciliary rootlet apparatus of multiciliated epidermal cells in species of the Scaphopoda. Abbreviations: ar, anterior rootlet; bb, basal body; bf, basal foot; cs, ciliary shaft; pr, vertical rootlet.
Table 1. Ultrastructural characters of the ciliary apparatus on multiciliated epidermal cells of studied species (adults) from major taxa of the Mollusca and Sipuncula.

<table>
<thead>
<tr>
<th>Character</th>
<th>Scaphopoda</th>
<th>Chaetodermomorpha</th>
<th>Neomeniomorpha</th>
<th>Polyplacophora</th>
<th>Bivalvia</th>
<th>Gastropoda</th>
<th>Sipuncula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cilium set in deep pit on cell surface</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes/No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Transition zone between cilium and basal body</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
</tr>
<tr>
<td>Basal plate (dense plate)</td>
<td>Blurry</td>
<td>Thin</td>
<td>Blurry</td>
<td>Blurry</td>
<td>Thick</td>
<td>Thick/Blurry</td>
<td>Thick/Blurry</td>
</tr>
<tr>
<td>Ciliary necklace with connecting strands</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Aggregation of granules below basal plate</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Centriolar triplet derivative in basal body</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Accessory centriole</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Basal foot with continuous tubular fibres</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Anterior ciliary rootlet; length</td>
<td>Short</td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
<td>Absent</td>
<td>Absent</td>
<td>Long</td>
</tr>
<tr>
<td>Anterior ciliary rootlet; shape</td>
<td>Conical</td>
<td>Flat</td>
<td>Conical</td>
<td>Flat</td>
<td>Absent</td>
<td>Absent</td>
<td>Conical</td>
</tr>
<tr>
<td>Vertical (posterior) ciliary rootlet; length</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
</tr>
<tr>
<td>Vertical (posterior) ciliary rootlet; shape</td>
<td>Conical</td>
<td>Conical</td>
<td>Conical</td>
<td>Conical</td>
<td>Conical</td>
<td>Conical</td>
<td>Conical</td>
</tr>
<tr>
<td>Brushborder of microvilli</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Microvilli on epidermal surface; shape</td>
<td>Slender/Variable</td>
<td>Slender/Variable</td>
<td>Slender/Variable</td>
<td>Slender/Variable</td>
<td>Slender</td>
<td>Slender</td>
<td>Slender</td>
</tr>
<tr>
<td>Microvilli on epidermal surface; branched</td>
<td>Few</td>
<td>No</td>
<td>Few</td>
<td>Few</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Glycocalyx</td>
<td>Layered</td>
<td>Layered</td>
<td>Homogeneous</td>
<td>Homogeneous</td>
<td>Layered</td>
<td>Lay./Hom.</td>
<td>Layered</td>
</tr>
</tbody>
</table>

Note that the twin vertical rootlets in species of lamellibranch bivalves is regarded as a split of a single rootlet and not included in the table.

Figure 4. A. Brush border of epidermal microvilli on Antalis occidentalis. TEM. Scale bar = 1.0 μm. B. Epidermal microvilli of Cadulus propinquus. Note the weakly layered glycocalyx. TEM. Scale bar = 2.0 μm.
REFERENCES


