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The Development and Structure of Feeding Arms in Antarctic Species of Pterobranchs (Pterobranchia, Hemichordata)

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Senior Honors Research

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Abstract. Pterobranchs are of particular interest to evolutionary biologists because as members of the phylum Hemichordata, they share characteristics with vertebrate animals and other chordates. The focus of this study is an examination of the development, structure, and function of the feeding arms in several species of pterobranchs collected from depths greater than 500 m from waters surrounding Antarctica. Pterobranch zooids in the genus *Cephalodiscus* feed using a crown of arms held over the dorsal surface of the body to filter particles from the water. Larvae released from adult tubes are ciliated, but lack feeding arms and are thought to derive energy from internal yolk stores. However, we have observed larvae of at least one species respond to the presence of particulate food, suggesting that these developmental forms may feed without the aid of adult structures. The feeding arms develop on the dorsal side of the animal, often beginning with a pair near the central axis followed by pairs of arms to the left and right. Each arm develops from a trilobed bud. The adult feeding apparatus consists of up to twelve arms held in a sphere on the dorsal side of the animal. Each arm has multiple tentacles, which are paired along the length of the arm until the apical-most tip. Scanning electron microscopy reveals that a single tentacle has two tracts of cilia along its outer face which may beat to draw water across the tentacular net or capture food particles. Food particles, including bacteria and single-celled algae, may then be conveyed down the tentacle to a deep, thickly ciliated groove on the outer face of the arm central to the paired tentacles, and eventually to the mouth. Scanning electron and light microscopy have elucidated the structures associated with feeding, as well as unusual refractive spheres at the tip of each arm in some species.

Introduction

Pterobranchs are small colonial animals that live in tubes attached to hard substrata on the sea floor. Most live in deep waters; the species examined here are from waters off the coast of Antarctica. The tubes are constructed of an as yet unidentified substance in which sand or mud is often embedded (Fig 1 A,B). Adult pterobranchs use the anterior-most region of the body, the oral shield, to construct the tubes (Fig 1C) (9). The oral shield is also used to crawl throughout the colony of tubes, much as a snail or slug might crawl using the foot (6). The zooids are semi-permanently attached to the base of the tubes by basal disc connected to a tethering stalk. Pterobranchs reproduce both asexually and sexually. Zooids are added to the colony by budding from the basal disc, resulting in multiple zooids stemming from one disc (Fig 1C) (4, 7). Zooids are separate sexes (gonochoristic) or hermaphroditic. Eggs are laid within the tubes and larvae may live within the tubes for a time before becoming free-living. After an undetermined time spent in the water column, the larvae settle, develop into adults, and form colony tubes (9). They may also invade tubes of other pterobranchs (3, 7).

The phylum Hemichordata includes two classes, the Enteropneusta and Pterobranchia, and is recognized by many researchers as having primitive characteristics also shared with chordates (sea squirts, amphioxus, and vertebrates) and echinoderms (sea stars and their relatives) (12). Hemichordates and echinoderms have three major body regions; in pterobranchs, these are the oral shield, the collar, and the trunk (Fig 1C. Development, especially of the collar region that supports the feeding arms, is similar in echinoderms and pterobranchs. Hemichordates share with chordates the presence of gill slits (5).

Because their body organization and tissue structure are similar to those of other invertebrate animals, they are thought to represent a basal taxon embodying a number of primitive traits (5, 10). Further, although the development of the collar and feeding arms is similar to development of the second body region in echinoderms, the feeding arms have in contrast been considered homologous to the feeding apparatus of bryozoans, brachiopods, and other filter-feeding invertebrates, thus uniting the hemichordates not with echinoderms and chordates, but with protostome phyla. (6, 8). The contradiction of these two evolutionary scenarios is possibly resolved by identifying characters that existed in the ancestor of the two major lineages, and are present in pterobranchs (Fig. 2′) Alternatively, further investigation may provide additional information on characters to resolve the evolutionary relationship among these taxa.

Previous work has shed little light on pterobranch phylogeny, with much of the published information being counterintuitive and even contradictory. Further, fewer than half a dozen studies have examined the development of the arms, structures that have been used to link pterobranchs to other phyla. We examined the feeding structures of adult pterobranchs to establish the ciliation pattern and possible mode of feeding. Larvae were observed to elucidate the development of feeding structures and possible feeding behavior.

Methods

Adult and larval specimens of four species of *Cephalodiscus* were collected using bottom dredges from waters surrounding Antarctica in December 2005 (for description of species see Table 1). Living larvae were transported to the laboratory at IWU and maintained in culture in seawater at 5 °C. To observe tentacle development and putative feeding behavior, living pterobranch larvae at various stages of development were observed using light microscopy.

Specimens were fixed for microscopy using standard methods while on board the R/V Laurence M. Gould (see Balser 1998). For SEM, larvae were fixed in osmium tetroxide, dehydrated in ethanol and liquid CO_2, critical-point dried, then sputter-coated with an alloy of gold and palladium. For light microscopy, living larvae were gently removed from dishes to avoid distressing them, then placed on a clean glass depression slide or a slide with clay feet. Some were relaxed with 7.5% MgCl in seawater, which did not affect ciliary action but stopped muscular contractions in the body wall.

Larval and adult pterobranchs were examined using a JEOL JSM 5800 scanning electron microscope to elucidate the structures of the feeding crown, arms, and tentacles. To reveal the internal structures of the arms, tentacles, and refractive beads, epon-embedded specimens were sectioned at 1 µm using a Sorval RMC MT2C, stained with 1% toluidine blue and photographed with a Nikon E600 compound microscope equipped with a DSM5 digital camera.

Results

Development

A pterobranch leaves its parental colony as a round, ciliated lecithotrophic larva and lives for a time in the water column (Fig 2A). The young larva swims for a variable period as it lengthens along its anterior-posterior axis and develops a clear bilateral symmetry; as this process occurs, the yolk sac can be seen withdrawing from the translucent body wall (Fig 2A). The larva eventually settles and begins to develop feeding structures. The time required to settle varies and may extend beyond eighteen months. The first structure seen is the oral shield, which develops ventrally and can be seen as a flattened portion of the squash-shaped larva, often with a dark band of pigment at its posterior edge (Fig 2B). The feeding arms later develop dorsally in pairs beginning at the center line of the body and take the form of three-lobed tentacle buds (Fig 2C). While the oral shield impinges slightly on the ventral tentacle buds, it does not cover them. The young arm does not form a refractive bead, nor have they been found elsewhere in the developing larvae.

Larvae contain substantial yolk stores, but we have observed behavioral modifications in response to particulate food. A swimming-stage larva sitting on the bottom of a dish begins to swim in a rapid spiral, rising and falling through the food cloud, while a swimming larva under the same conditions increases the rapidity of its loops. A trail of food particles trapped in mucus leads from the larvae to the food cloud once the larva has moved away.

Larvae of *Cephalodiscus nigrescens* have settled and begun to secrete a translucent white system of tubes. Single pterobranchs have built small, rounded structures, while those which settled near other pterobranchs have a larger complex composed of the tubes secreted by several individuals. Other larvae have not begun to build or inhabit tubes, even when provided with appropriate materials or tubes collected from the same sites. Many adhere to the glass substrate or filter matting by the developing stalk at the posterior end, but do not secrete tubes or gather tube-building materials.

<u>Morphology of adult arms and tentacles</u>

The arms of an adult pterobranch project from the collar region and form a hollow sphere on the dorsal side of the animal. A single arm is shaped like a shallow V with the apex of the V pointing inward and the opening outward. Arms are seen in various stages of development in the SEMs, from mature arms bearing refractive beads to younger arms with only a few pairs of tentacles. The arms, which are paired, have numerous bilateral extensions, or tentacles, with a single tentacle or refractive bead at the apex. The tentacles interdigitate somewhat. Two tracts of cilia lead down the outer face of the tentacle to a densely ciliated groove in the outer face of the arm (Fig 3B). In addition to this ciliation pattern, we have observed heavily ciliated regions between the tentacles.

The arms are supported internally by coeloms (fluid filled body cavities), as expected. A coelom runs longitudinally through each arm and is continuous throughout the feeding crown and collar. Sections of the tentacles reveal a central coelom continuous with that of the arm as well as a crescent-shaped blood vessel (Fig 3D).

Refractive beads are found on the ends of mature arms, but are not seen during development. The surface of the bead is irregular, with large bumps studding the surface. Sections of the bead examined by light microscopy reveal a similarly amorphous internal structure. Unlike the arms and tentacles, the bead does not have clear tissue layers and is not coelomated. Heavily stained structures are distributed throughout the bead (Fig 4). These structures are not found elsewhere in the body of the adult pterobranch.

Discussion

<u>Development of feeding structures and behavior</u>

Swimming larvae do not have visible feeding structures. The first structure to develop is the oral shield, which begins as a ventral thickening of the body wall, followed by the tentacles, which develop dorsally (4). While the tentacle buds begin development at different times, this disparity is not reflected in their later morphology; ventral tentacle buds form before the dorsal buds have grown markedly larger. Refractive beads are not present on the tentacle buds or young arms. This suggests that the beads are the final step in arm formation and that the arms develop apically, adding new tentacles toward the tip until the full length is reached, then forming the refractive bead.

Larval settlement was expected within forty-eight hours based on previous reports of *Rhabdopleura normani* (9). However, larvae in culture remained in the swimming stage, presumably planktonic, for up to eighteen months with no deleterious effects or development of either oral shield or tentacle buds. If this is consistent with planktonic development in their natural habitat, it suggests not only an extremely effective dispersal within the plankton, allowing for gene flow and invasion of new habitats on a far grander scale than predicted, but requires some way of taking in nutrients from the water. Because the larvae respond to the presence of particulate food, we suspect that they feed either by absorbing nutrients across the body wall or by other means, including immature feeding structures. Alternatively, the larvae may react to the particles as a fouling nuisance and rely only on stored yolk for nutrition under normal circumstances; they may settle more quickly in the wild for a variety of reasons.

The construction of a colony by several individuals suggests that there is not strong intraspecific competition. Individual pterobranchs living in close proximity to each other, whether in tubes or loosely anchored to the substrate, do not appear to suffer deleterious effects or morphological differences. Pterobranchs living in single dishes are not qualitatively different from those raised with others; the cue to develop a system of tubes and become adults does not appear to be based on the presence or absence of other individuals or their metabolites.

Morphology of arms and method of feeding

We suspect that the pattern of ciliation on the adult pterobranch arm indicates that water flows into the feeding sphere across the tentacles, then out the upper opening of the sphere where the feeding arms meet. This is similar to the system of currents described by Lester (1985). The exact method of particle capture is uncertain. Halanych, working with *Rhabdopleura normani,* found that ciliary reversal, rather than impingement on the arms and tentacles or muscular flicking of individual tentacles, was responsible for most particle capture (6); the greater size and nutritional needs of *Cephalodiscus* may require a different method of feeding, much as they may have driven the formation of multiple feeding arms rather than the two of *Rhabdopleura* (5). The interlocking tentacles may provide a larger surface area for impingement of food particles (2). The ciliated intertentacular regions seen in sections may serve to create a current or to sort particles for rejection or transport to the mouth, as reported by Halanych (6). Gilmour's (1979) work with *Rhabdopleura* revealed that the genus could sort particles by size, relying on a sudden change in the direction of the current, certainly another possibility for particle capture in *Cephalodiscus*. However, we have not ascertained the ciliation of the inner surface of the feeding arm, which we suspect causes the current through the feeding crown.

The refractive beads may be glandular in nature. While there is no clear tissue organization, the heavily stained structures may be secretory tissues (Fig 4). The beads do not, however, have a tissue organization similar to that of the oral shield, which is known to be secretory (3, 9). They may also contain bacteria or other symbionts for nutrition or defense. Similar structures are not found elsewhere in the body, implying that they are linked to the function of the refractive bead. The beads are reported to be constructed of ectodermal cells containing "a large clear globule of an ovoid shape" which may be irregularly ejected from the cell (10), but we have not seen intracellular evidence of this in the epithelial layer. Based on distribution of the beads themselves, which were found on the arms of adults and throughout the ectoderm of larvae and juveniles, John posited that they were defensive in nature (4), but we have not observed the same structures in living larvae. The beads also do not seem to be a form of compound eye or photoreceptor (10).

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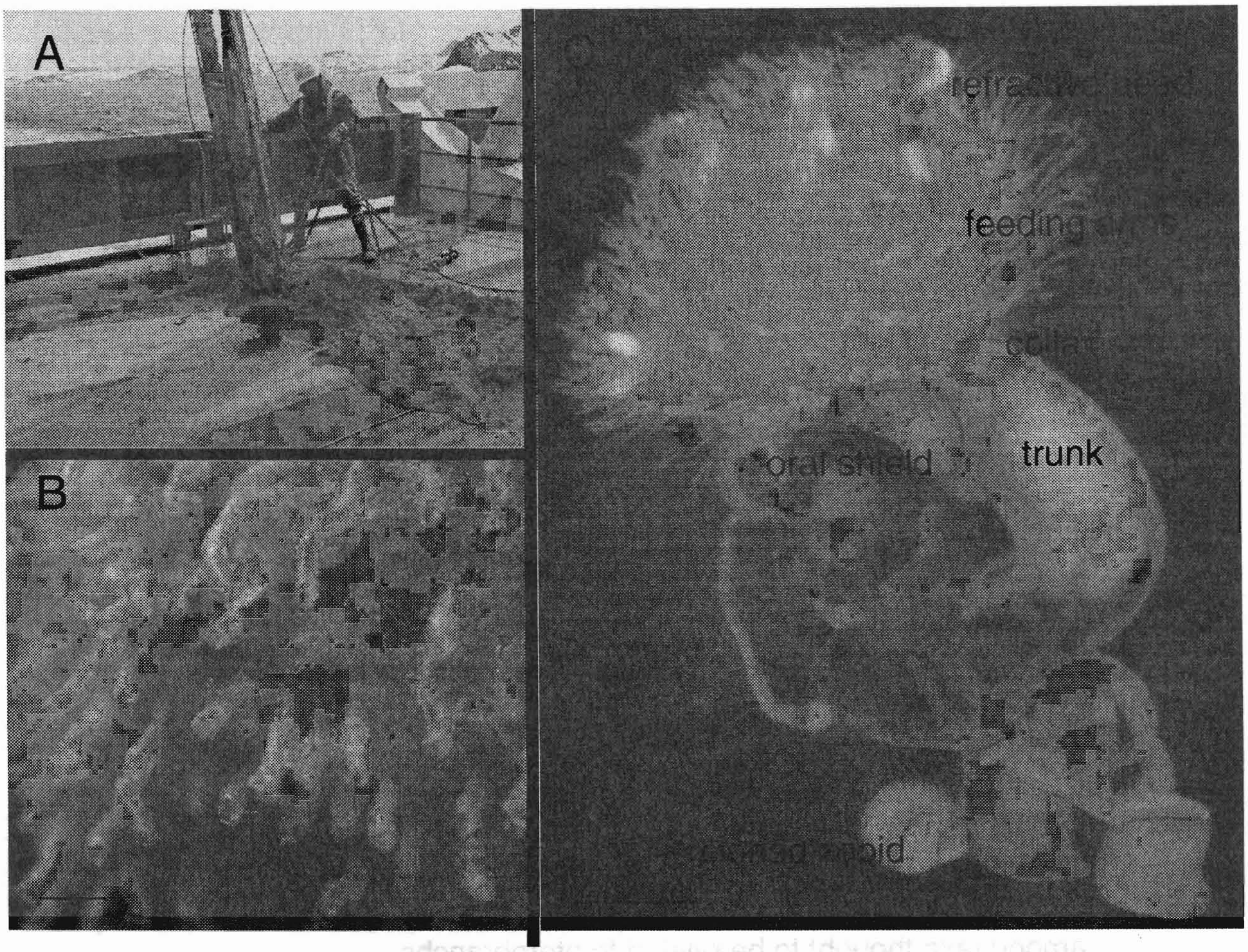

Fig. 1. Photographs of *Cephalodiscus* spp. A) Pterobranch colonies were collected using a bottom dredge from depths of ~500m off the coast of Antarctica. The large, branched structures in the mud are colonies containing adult and larval pterobranchs. B) Tubes in which zooids of the colony live. The feeding crown is extended from the opening of the tube, which may be embedded with sand or mud. C) Adult removed from its tube. Asexually produced clones are attached to the parent zooid by a long stalk. The zooids develop oral shields before other feeding structures. Note the arms, tentacles, and refractive beads of the feeding crown, which projects dorsally from the collar. Scale, 1 mm

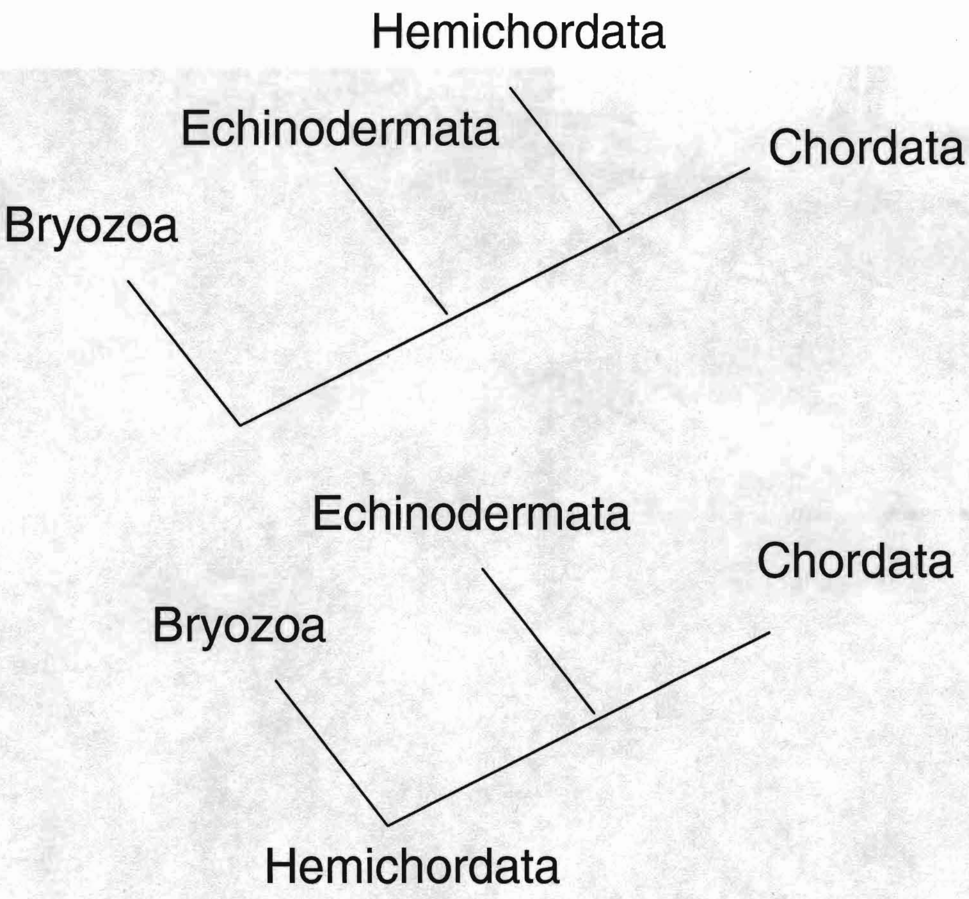

Fig. 2 Two possible evolutionary scenarios showing the relationship among taxa thought to be related to pterobranchs

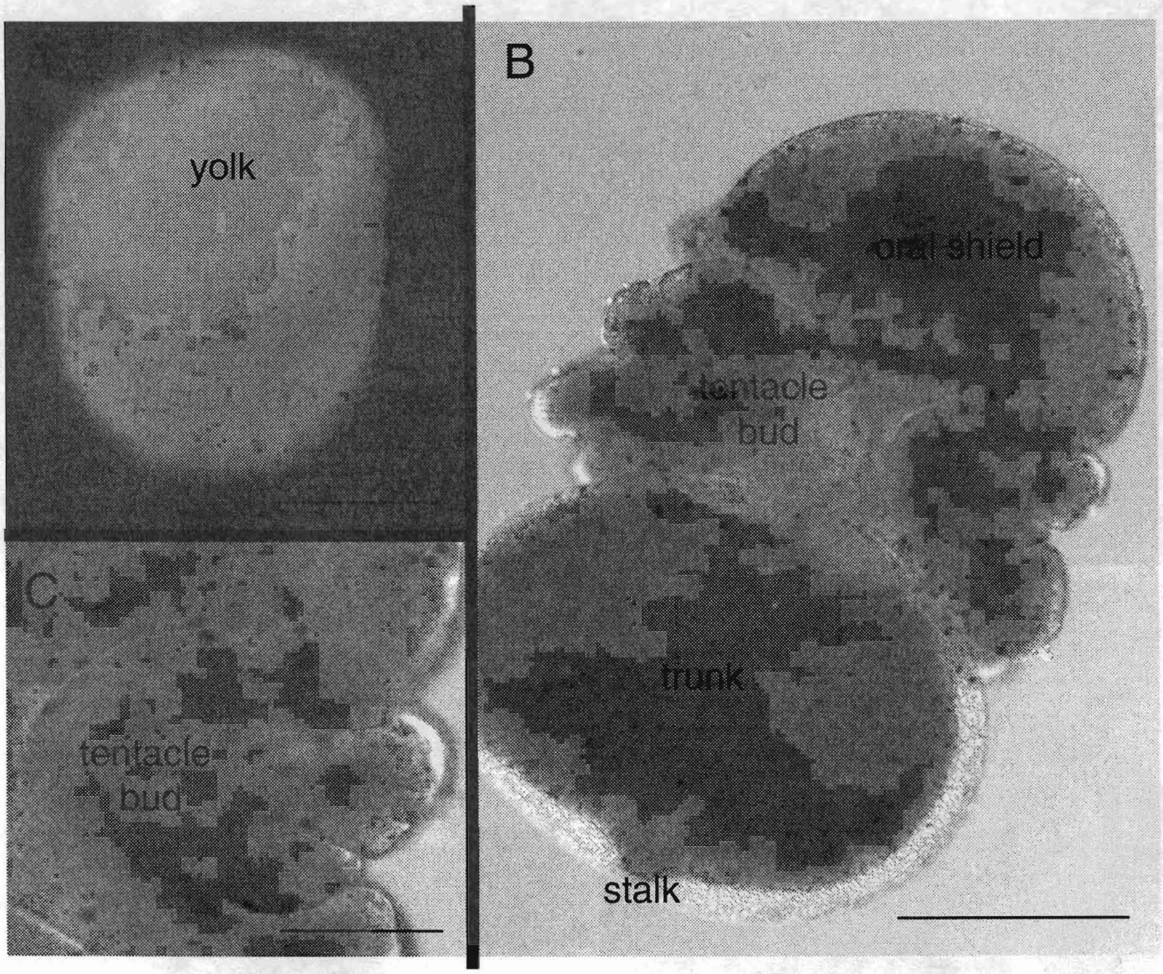

Fig. 2. A) A swimming larva with a shrinking yolk sac visible through the body wall. Pigment spots of unknown composition are characteristic of larvae. B) Ventral view of a larva developing feeding arms on the collar. The oral shield is already formed and the stalk is beginning to develop at the posterior-most region of the trunk. A pair of tentacle buds is clearly visible at the collar region. C) The feeding arm develops from a three-lobed tentacle bud. Scale, 1 mm

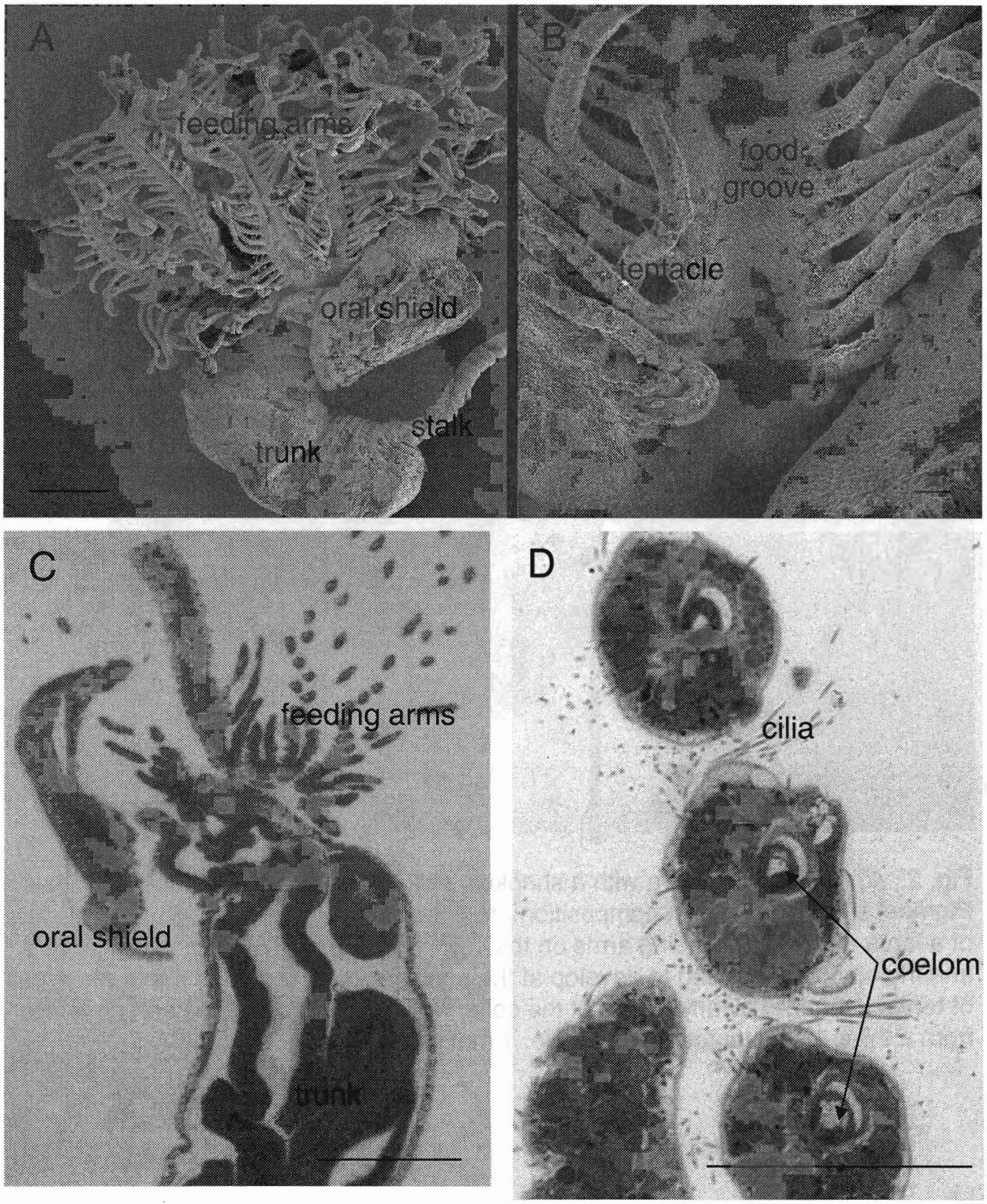

Fig 3: Fine structure of the feeding crown. The adult pterobranch feeds by filtering particles through its feeding arms. The typical feeding posture is as shown (Fig 1C, 3A). Each arm has numerous bilateral tentacles with a single tentacle or a refractive bead at the apex. Each tentacle has two tracts of cilia leading to a densely ciliated groove in the outer face of the arm (B). C) The arms and tentacles are coelomated. The coelom extends throughout the feeding crown. A crescent-shaped blood vessel can also be seen in cross sections of individual tentacles (D). Note the intertentacular ciliated regions. Scale, A 1 mm; C 0.5 mm; B, D 10 µm.

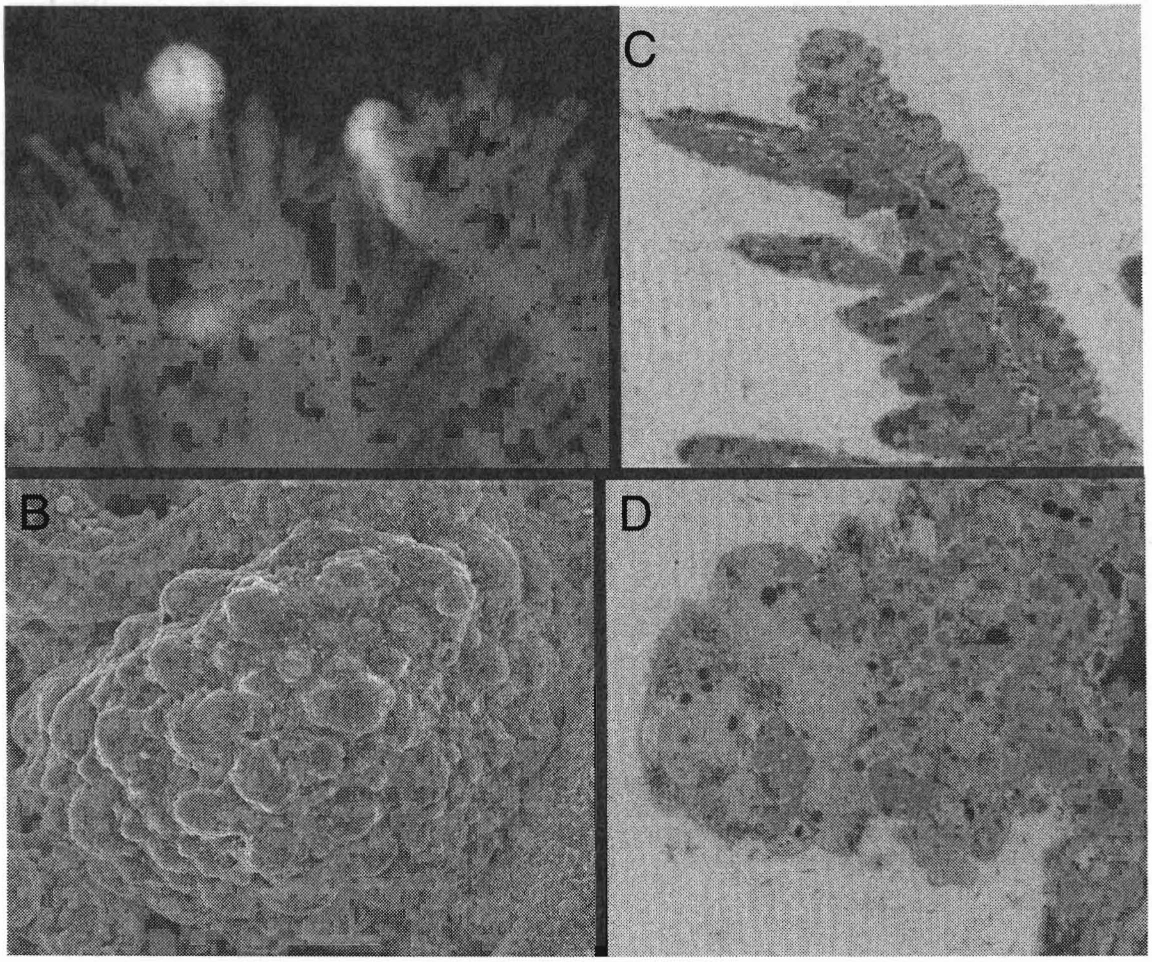

Fig. 4. Refractive beads. Refractive beads are shown by light microscopy in a living adult (A) and by SEM, which reveals the granular surface (B). Sections of a bead (C, D) show the bead's irregular shape and lack of organized tissue layers, as well as heavily stained structures confined to the refractive beads. {Needs labels and scales}