Particle Retention and Flow in the Pharynx of the Enteropneust Worm *Harrimania planktophilus*: The Filter-Feeding Pharynx May Have Evolved Before the Chordates

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Abstract. An investigation of the feeding behavior of the acorn worm *Harrimania planktophilus* suggests a novel form of enteropneust feeding with significant phylogenetic implications. *H. planktophilus* is a holoinfaunal worm that feeds on deposited sediments, and filter feeds on suspended particles in interstitial pore water. To visualize the particle retention behavior involved in filter feeding, adult animals were held in chilled seawater under low light and fed food coloring and fluorescent particles. The behavior was recorded by videography. Most particles ingested were drawn into the mouth by an incurrent flow created by cilia on the pharyngeal bars and without the aid of mucus. Particles that passed freely through the gill pores averaged 3.04 μm, whereas particles retained in the gut and defecated in the feces averaged 13.9 μm. Food coloring entered the mouth and was pumped through the pharynx at a rate of 0.5–2.0 mm/s. There is no evidence of an endostyle or mucus-net capture mechanism in *H. planktophilus,* but instead particles are filtered and manipulated by a dense covering of cilia on the pharyngeal gill bars. This study suggests that the filter-feeding pharynx is not an innovation of the chordates, but evolved prior to the evolutionary divergence of the hemichordate-echinoderm clade from the chordates.

Introduction

The enteropneust worms and bryozoan-like pterobranchs constitute the small deuterostome phylum Hemichordata, which is generally regarded as an early offshoot from the chordate line of evolution (Ruppert and Barnes, 1994). Such a group might be expected to hold clues concerning early chordate pharyngeal evolution, but pharyngotremy and the feeding behavior of hemichordates have been little studied since the work of Barrington (1940; Barrington and Thorpe, 1963). What little work has been done on enteropneust feeding behavior has primarily focused on deposit feeding. The Enteropneusta have long been considered classical mucociliary feeders, collecting their food particles on the proboscis with mucous secretions, and transporting them on the surface of multiciliated cells to the mouth (Barrington, 1940; Knight-Jones, 1953; Burdon-Jones, 1962; Thomas, 1972). In addition to deposit feeding, some evidence of filter feeding in the Enteropneusta has also emerged from the research of Barrington (1940; *Glossobalanus minutus*), Knight-Jones (1953; *Saccoglossus horsti*), and Burdon-Jones (1962; *Balanoglossus gigas*). Barrington (1940) studied the path of carmine particles as they moved in and around the collar lip of *Glossobalanus minutus*. The majority of particles were transported with the aid of mucus, but some were pulled into the mouth with a ciliary current, suggesting that *G. minutus* is able to acquire food by filter feeding. Flow velocities and particle-size measurements were not reported in that feeding experiment, so the effectiveness of the feeding mechanism, including the size selectivity of suspended particles, could not be estimated. Respiratory currents were also observed to enter the mouth of burrowing *Saccoglossus horsti,* apparently to assist sediment-laden mucus to enter the mouth (Knight-Jones, 1953).

Balanoglossus gigas, the largest of the enteropneusts, grows to lengths exceeding 2 m (Horst, 1939), and creates deep burrow systems with many connections to the surface

(Burdon-Jones, 1962). When graphite suspensions were released at the margin of the collar lip of *B. gigas,* the particles were swiftly drawn into the dorsal branchial pharynx, with no indication that mucus was playing a role in transport. Particles were trapped in the branchial pharynx, and transported ventrally to two grooves on the parabranchial ridges, which separate the dorsal branchial pharynx from the ventral digestive pharynx. The parabranchial ridges rolled the graphite into mucus produced by the pharyngeal epithelium, forming a loose cord. This cord was then transported ventrally to the digestive pharynx, and then posteriorly to the esophagus, hepatic region, and eventually the intestine. This branchial sieving mechanism was very efficient and prevented particles of 1 to 2 μm from passing out through the branchial pores (Burdon-Jones, 1962). Notwithstanding these observations in support of filter feeding, this mechanism was regarded as secondary to deposit feeding (Knight-Jones, 1953; Burdon-Jones, 1962) or of minor importance (Thomas, 1972).

Among deuterostomes, gill slits occur in fossil echinoderms (Jeffries, 1986), hemichordates, and chordates. In enteropneusts they are generally believed to dispose of excess water that is transported into the pharynx with ingested sediment (Barrington, 1940; Knight-Jones, 1953; Burdon-Jones, 1962; Barrington and Thorpe, 1963), and are also assumed to be gas-exchange surfaces (Ruppert and Barnes, 1994). The chordate pharynx, on the other hand, collects and concentrates suspended food (ascidians and amphioxus), and provides a means of locomotion for salps and doliolids (Bone, 1998). The pharynx of lamprey ammocete larvae functions much like that of amphioxus, and the vertebrate pharynx functions as a surface for gas exchange (although filter feeding has been reinvented in some taxa). In higher vertebrates, the gill bars have been modified to form the jaws and the hyoid arches (Radinsky, 1987).

Given the unquestionable shared form of the hemichordate pharynx with that of fossil echinoderms and protochordates, I decided to investigate the potential for filter feeding in the enteropneust worm *Harrimania planktophilus*. My goals were (i) to confirm my preliminary observations suggesting filter feeding by this enteropneust (Cameron, 2002), (ii) to quantify the size range of particles that the pharynx can filter from seawater, and (iii) to examine the pharynx microscopically to better understand the movement of water and particles through it. The results of this study challenge the long-held view that the filter-feeding pharynx is a synapomorphy of the chordates, and suggest that it may have been present in the common ancestor to the hemichordates, echinoderms, and chordates.

Materials and Methods

Harrimania planktophilus was collected from the intertidal sand flats at Cape Beale (48° 47′ 30″ N, 125° 12′ 56″ W) Barkley Sound, Vancouver Island, Canada. Twenty-one worms were collected over the summer of 1999 and transported to the Bamfield Marine Station, where they were kept in specimen bowls containing their natural sediment under a flow of fresh seawater. One or two worms at a time were removed from their bowls for videomicrography, light microscopy, or scanning electron microscopy. The feeding behavior of *H. planktophilus* was recorded as follows. Animals were first placed in finger bowls without sediment, and then allowed to cool on ice until the water temperature was between 5 and 7 °C. Cooling was the most effective way to relax the muscular and mucus secretory responses of the animals, which are otherwise stressed from being removed from the sediment. An ice bath was also the most effective means of keeping the water cool during videorecording. *H. planktophilus* is strongly photonegative, and animals were therefore filmed with the lowest light levels consistent with maintaining a visible image. Orange, red, and blue filters were placed between the fiber-optic light source and the animal, but this did not appear to reduce its photosensitivity. To make images of the water current through the mouth and pharynx of *H. planktophilus,* a number of particles suspended in seawater were tried, including carbon dust, starch, Sephadex beads, carmine red, and fluorescent particles (Dayglo Color Corp.). Dyes in seawater solution were also tried, including diluted milk, methylene blue, fluorescein, and blue food coloring. *H. planktophilus* responded most positively to the orange fluorescent particles (Dayglo Color Corp.) and blue food coloring in seawater. The orange fluorescent particles were positively charged and had a size range of 1.6–30 μm, with 20% at 6 μm. The size of the particles cleared and captured by *H. planktophilus* was determined by direct measurement of the particles that passed freely through the gill slits, and that were bound into fecal material. Feeding trials were taped and measurements taken with a JVC 3-CCD color video camera mounted on a Wild dissecting microscope. Still pictures in Figures 2 and 3 were captured with imaging software (Optimus Corporation).

From these data, the Reynolds number was estimated as:

$$Re = \frac{IU}{\nu} ,$$

where I is the inside diameter of the pharynx (in meters), U is the flow velocity (m s^{-1}) of the dyestream front as it entered the mouth, and ν is the kinematic viscosity of seawater ($= 1.047 \times 10^{-6}$ m^2 s^{-1} @ 20 °C) (Vogel, 1981).

In preparation for light microscopy, animals were allowed to defecate their gut contents and were cleaned externally of sediment. They were then relaxed in 7% MgCl$_2$, fixed in Bouin's solution, and dehydrated through a graded series of ethanol dilutions. Once in 100% ethanol, these specimens were dissected into small pieces, transferred to
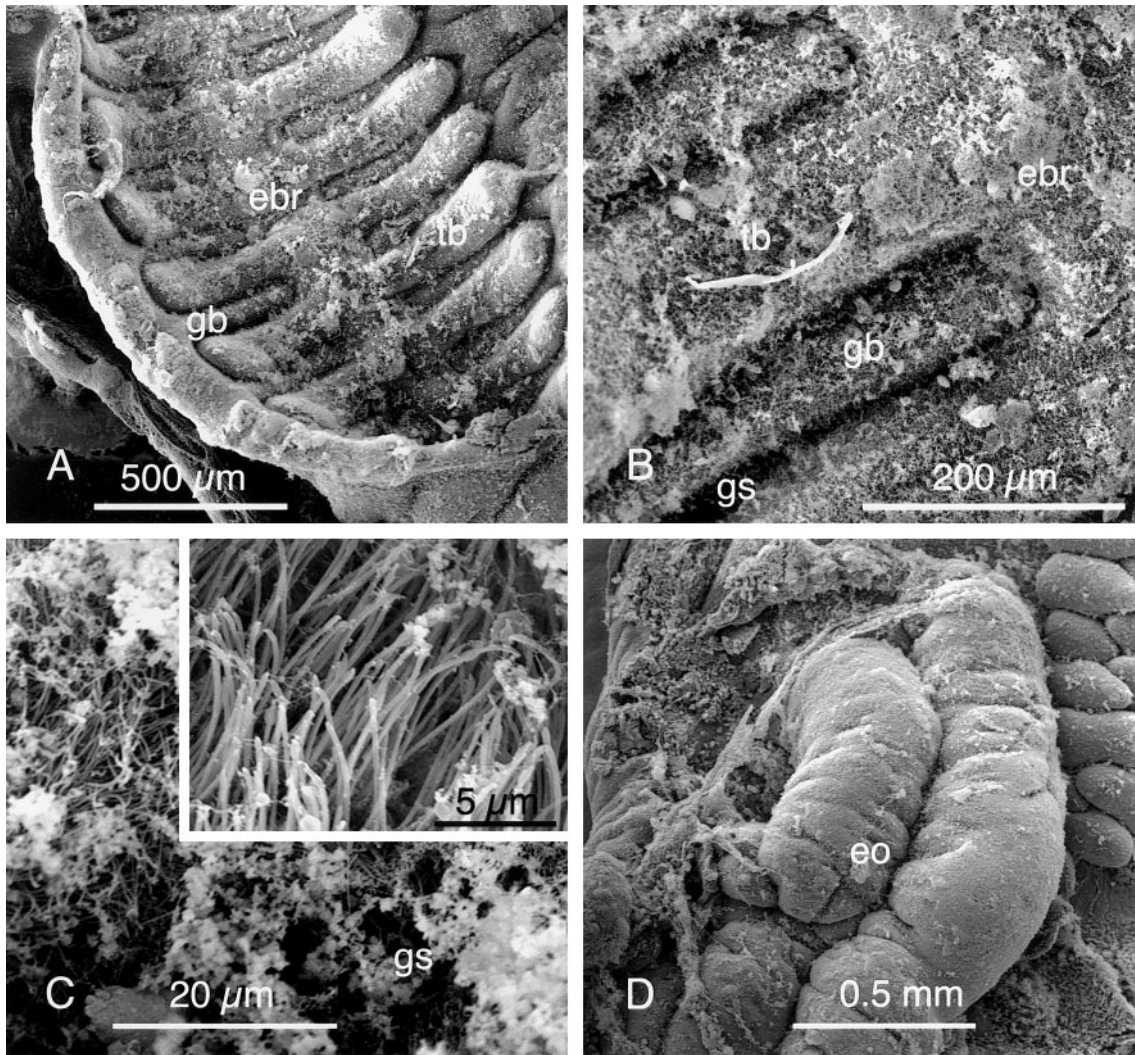
Figure 1. Scanning electron microscope images of sectioned material of *Harrimania planktophilus*. (A) The dorsal branchial pharynx, showing the primary bars, tongue bars, and gill slits. (B) The gill bars and slits in the dorsal branchial pharynx. (C) Cilia and mucus on a gill bar. (C inset) High-magnification image of cilia on a tongue bar. (D) The esophageal organ concentrates food in passage from the pharynx to the hepatic region of the gut. ebr, epibranchial region; eo, esophageal organ; gb, primary or gill bar; tb, secondary or tongue bar; gs, gill slit.

xylene, and then infiltrated with paraffin wax. Sections were cut on an American Optical Corporation 820 Spencer microtome and stained with either Delafield's hematoxylin or eriochrome-cyanin, and viewed and photographed with an Olympus OM-4T 35-mm camera mounted on an Olympus BH2 compound microscope.

For scanning electron microscopy, pieces of tissue were dissected from a relaxed animal so that the dorsal pharynx was exposed. The specimen was then fixed in 2.5% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer at pH 7.4 for 1.5 h and postfixed in 1% osmium tetroxide in 0.2 *M* Millonig's buffer for 1 h. The specimen was then dehydrated through a graded series of ethanol solutions, followed by critical point drying. After sputter coating with colloidal gold, the specimen was examined with a Hitachi S-3500N scanning electron microscope.

Results

Histology and microstructure

Sections were cut through the pharynx and particular attention was given to the dorsal branchial pharynx (Figs. 1A, 2, and see fig. 1B in Cameron, 2002), where the feeding current was presumed to be maintained in *Balanoglossus gigas* (Burdon-Jones, 1962). Long cilia were observed on the lateral side of the primary and secondary gill bars (Fig. 1C inset), and at the pharyngeal edge of the brachial sac.
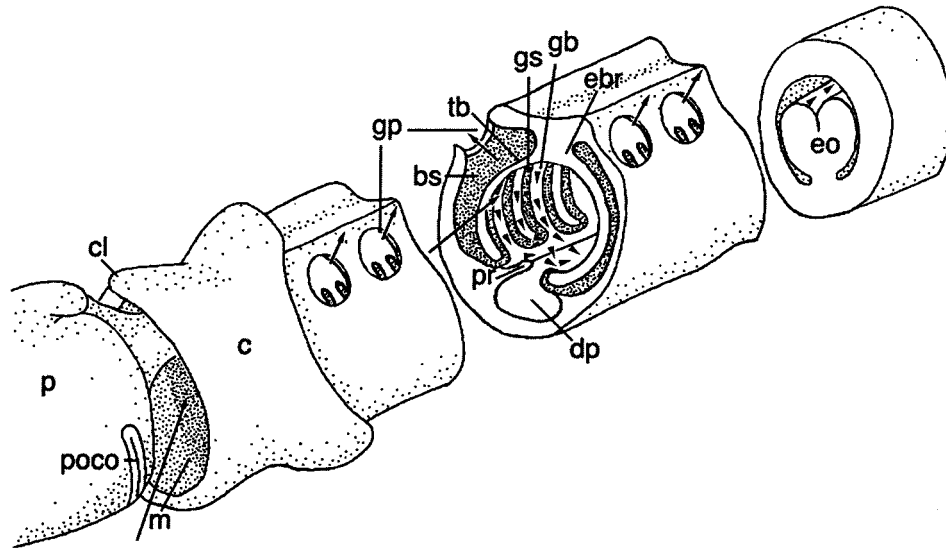
Figure 2.   Organization of the filter-feeding morphology of *Harrimania planktophilus*. Anterior is at the left of the figure. Particle-laden seawater (long arrows) enters the mouth on a current that is generated by cilia. Particles (arrowheads) are trapped in dense cilia that line the gill slits; transported ventrally down the gill bars, and carried over the parabranchial ridge to the digestive (ventral) pharynx. Once in the digestive pharynx, particles are transported posterior to the esophagus organ and intestine (not shown). Filtered seawater (short arrows) exits the pharynx through the gill slits, flows into the branchial sac, and reaches the outside through a gill pore. bs, branchial sac; c, collar; cl, collar lip; dp, digestive pharynx; ebr, epibranchial region; eo, esophagus organ; gb, gill bar; gp, gill pore; gs, gill slit; m, mouth; p, proboscis; poco, pre-oral ciliary organ; pr, parabranchial ridge; tb, tongue bar.

Pharyngeal cilia did not show evidence of modified tips (Fig. 1C inset). Scanning electron micrographs revealed no synapticles bridging the tongue bars to the primary gill bars, and consequently the tongue bars project far into the pharynx lumen (Fig. 1A). The pharyngeal region of *H. planktophilus* is 5 times the length of the extended proboscis, and has 36.2 ± 9.7 ($n = 6$) pairs of large gill slits that connect to muscular gill pores. The gill pores are located in paired dorsolateral grooves on the outside of the body (see Cameron, 2002, fig. 1). In some animals the gill skeleton is pigmented dark black, rather than the more common collagenous-opaque white color, allowing it to be easily visualized through the body wall. The esophagus region has a large bilobed thickening of the ventrolateral gut muscle, here called the esophageal organ (Fig. 1D). In live animals the esophageal organ is brick red, as observed through the body wall. This organ may be involved in squeezing excess water from the food before the latter is transported to the digestive gut. Just posterior to the esophageal organ, the gut is darkly pigmented and sinuous. The gut straightens before reaching the anal sphincter.

Observations of flow

Fluid velocity through the mouth was estimated from video recordings of blue food coloring (Fig. 3) that was introduced around the mouth with a handheld pipette (Fig. 3B). The average rate of flow into the mouth and through the pharynx is 0.5–2.0 mm/s. The Reynolds number is approximately between 0.2 and 1.0 in the pharynx (~0.5 mm diameter), and therefore water flow is laminar (the breakover point for purely laminar flow is $Re = 1.0$ (Vogel, 1981)). *H. planktophilus* was observed to regulate its feeding flow in three ways: (1) by contracting the gill pore sphincter muscles; (2) by plugging the mouth, either by withdrawing the proboscis or constricting the collar lip around the neck; and (3) by ciliary arrest in the branchial pharynx. The feeding flow through the mouth of *H. planktophilus* was stopped by ciliary arrest when the animal began to burrow or when it had a pharynx full of particles. Under these conditions, the mouth and gill pores would be wide open, yet a feeding current could not be observed. A muscular sphincter, derived from the longitudinal trunk musculature, curves around each pore (Horst, 1939). When the worm bent its body to turn right, the gill pores on the inside (right) side closed and water exited only from the left pores, and *vice versa*.

Particle retention

Suspended fluorescent particles enter the mouth (Fig. 4) and pass through the collar region and into the dorsal (branchial) pharynx (Figs. 1A, 2, and see fig. 3D in Cameron, 2002) on a current created by the pharyngeal cilia. The
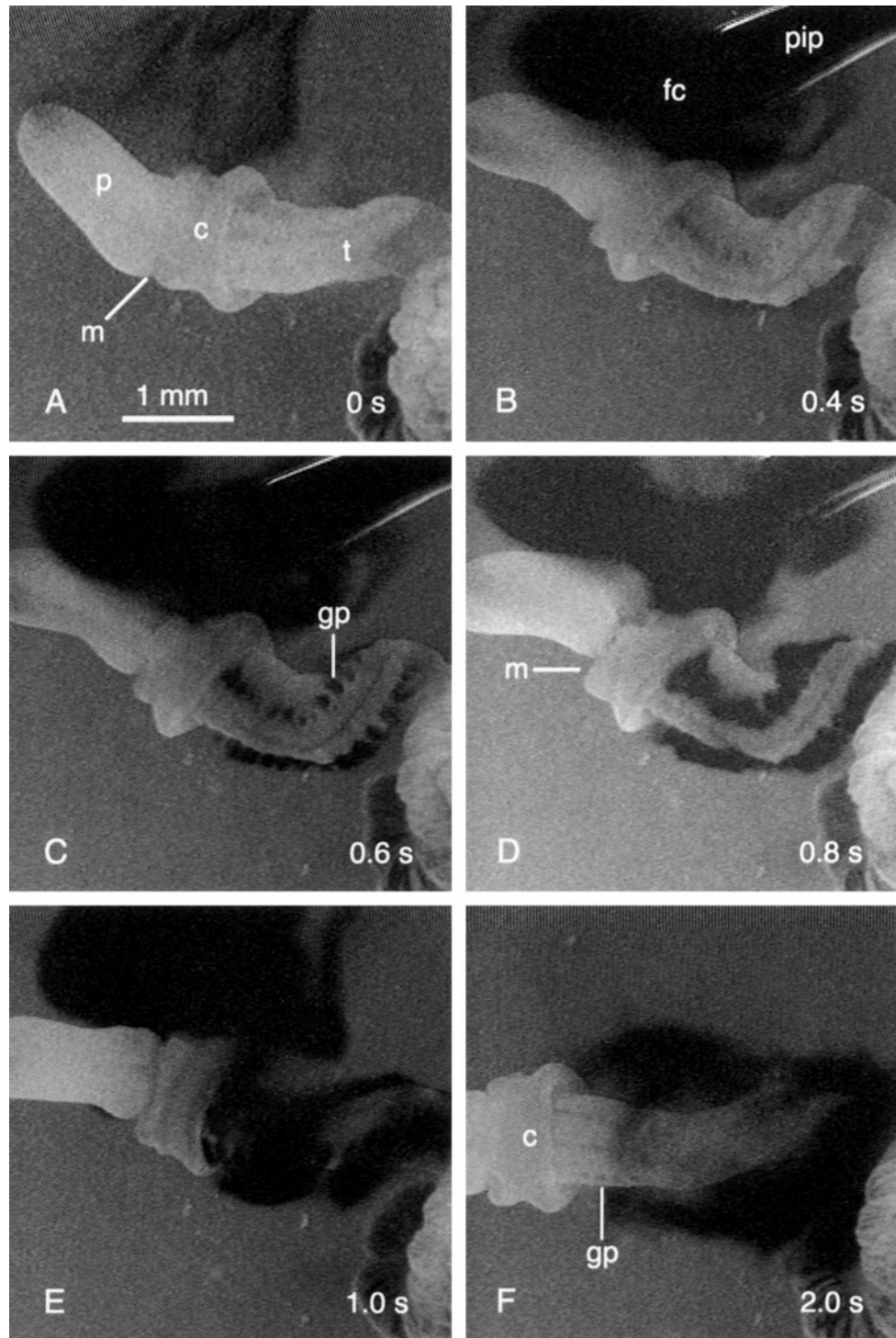
Figure 3. Photographs captured from a videotape, showing the flow of food coloring into the mouth and out of the gill pores of *Harrimania planktophilus*. Scale bar is indicated in A, time in tenths of seconds is indicated in frames A–F. c, collar; fc, food coloring; gp, gill pore; m, mouth; p, proboscis; pip, pipette tip; t, trunk.

particles are then filtered from the water by cilia on the primary gill bars and tongue bars (Figs. 1A, 1B, 1C inset, and 2). Excess water is pumped from the pharynx through the gill slits, into the branchial sac, and through the gill pore to the outside (Fig. 2). Particles that are trapped in the branchial pharynx are transported ventrally down the gill bars and over the parabranchial ridges (Fig. 2) to the digestive pharynx. Once in the digestive pharynx, the particles
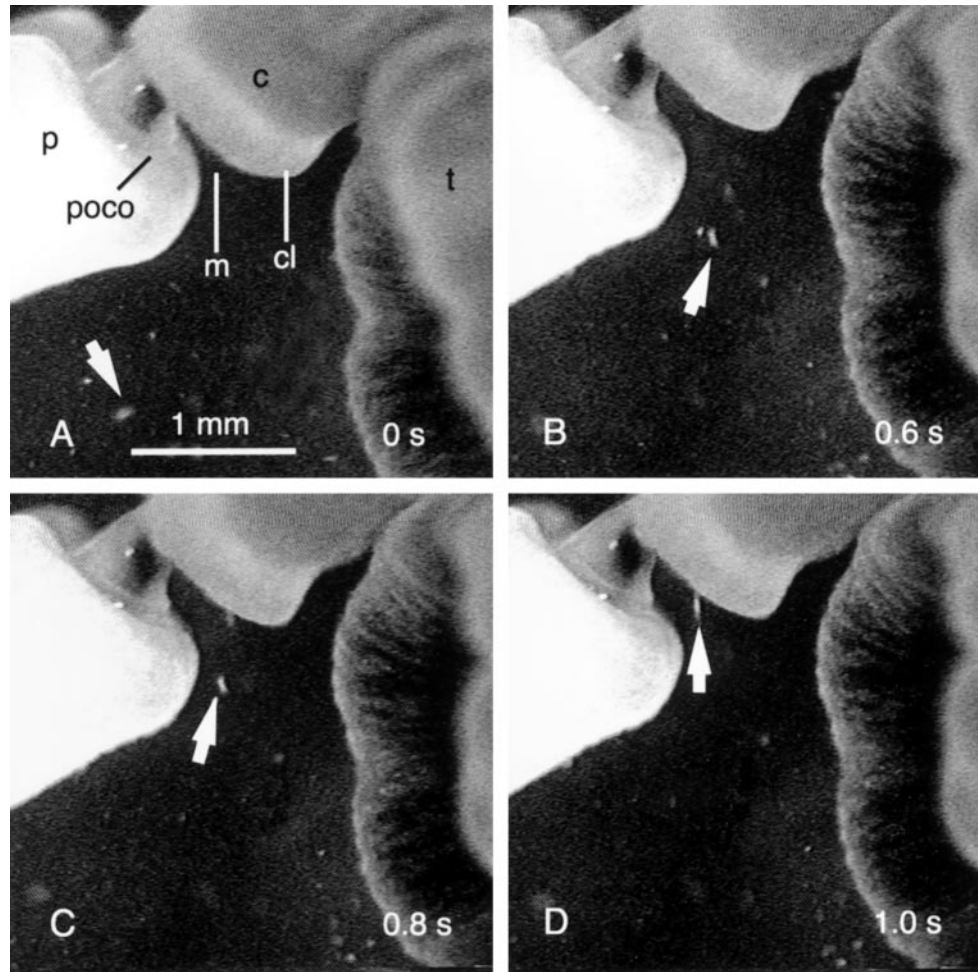
Figure 4. Photographs captured from a videotape of suspended particles moving towards the mouth of *Harrimania planktophilus*. Scale bar is indicated in A, time in tenths of seconds is indicated in frames A-D. c, collar; cl, collar lip; m, mouth; p, proboscis; poco, pre-oral ciliary organ; t, trunk. Arrows indicate the movement of a single particle in suspension as it is drawn towards the mouth on a feeding current.

are transported posteriorly to the esophagus organ and intestine. Mucus was observed in the pharynx (Fig. 1B, C), where it possibly aided in the trapping and transport of particles. Enteropneusts that were fed particles in suspension for more than a few minutes would slow their feeding rates and eventually stop feeding altogether because the pharynx would become plugged with particles. In other words, the branchial pharynx can capture particles at a rate that exceeds the processing efficiency of the gut. The "bottle neck" in the particle filtration system is at the esophagus organ (Fig. 1D) and in the intestine. Fluorescent particles passed through the gut in about 5 h in a 3-cm-long worm.

The size of particles that passed freely through the gill slits (Fig. 2) and gill pores of 6 worms measured 3.04 μm $\pm$ SD 1.52 μm ($n = 61$), the maximum particle size was 5.8 μm, and the minimum was 0.2 μm. Particles collected from the feces had a mean size of 13.9 $\pm$ SD 8.6 μm ($n = 48$) with a minimum size of 0.2 μm and a maximum of 28.4

μm. Particles in the feces were compacted in the gut and packaged in a mucous matrix, resulting in a tubular fecal casting. Particles had to be teased from the fecal casting for measuring and therefore the large size range may have been overestimated if particles stuck together, or underestimated if larger particles fragmented while the feces were being manipulated. Therefore particle sizes measured in the feces may not be a precise indicator of those that entered the mouth. Despite the problems with particle size estimations, *H. planktophilus* appears capable of ingesting particles ranging from 0.2 μm to 28.4 μm, and the largest size to pass through the gill slits, branchial sac, and gill pore was 5.8 μm. These data suggest that *H. planktophilus* is a facultative filter feeder that exploits a wide size range of particles available in sedimentary pore water.

In addition to being a suspension feeder, *H. planktophilus* is a deposit feeder. Food in the form of sediment, detritus, and interstitial plankton is trapped in mucus on the proboscis

and transported posteriorly over its surface toward the mouth. The preoral ciliary organ (POCO) (Figs. 2, 4A), a horseshoe-shaped organ located on the posterioventral face of the proboscis, does not have an obvious function in suspension feeding, but effectively sorts particles that are trapped while deposit feeding. The POCO functions to transport food to the ventral midline and into the mouth on mucous strings. Unwanted particles, on the other hand, are transported by the POCO to the dorsal proboscis, where they are rejected over the collar lip on mucous strings. Strangely, not all deposit-fed particles, or all rejected particles, are manipulated by the POCO. It is not known how the POCO distinguishes between food and nonfood particles.

Discussion

Harrimania planktophilus appears to have a feeding behavior similar to that described in protochordates, suggesting that a filter-feeding pharynx evolved before the divergence of the hemichordate-echinoderm clade from the chordates. Long cilia located on the lateral sides of the gill bars probably mediate the pumping behavior and capture most of the particles from suspension, but a fuller experimental study would be necessary to demonstrate this conclusively.

Burdon-Jones (1962) described a ciliary current within the collar and the anterior region of the pharynx of *Balanoglosus gigas*. Particles entering the pharynx of dissected animals are initially trapped by the dorsal, branchial pharynx and then transported ventrally to the digestive pharynx *en route* to the gut. The observations of particle movement in the present study were made through the body wall and closely resemble those reported by Burdon-Jones (1962). In neither case was there any indication of a mucous net associated with the pharynx as there is in the invertebrate chordates. Instead, heavily ciliated gill bars appear to secrete the mucus. Except for the long cilia mentioned above, cilia in the pharynx were unspecialized, and none exceeded about 8 μm in length. Such cilia are evidently sufficient for pumping and filtration of food-laden water.

The observations above agree with those of Burdon-Jones (1962), who found particles of 1–2 μm retained by the gill slits of *B. gigas*. *H. planktophilus* retained particles as small as 0.2 μm and as large as 28.4 μm in the pharynx, suggesting that the filtering adaptation makes use of microbial as well as interstitial plankton production. *H. planktophilus,* like appendicularians and amphioxus (Ruppert *et al.,* 2000), can feed on a wide size range of particles, exceeding that of the ascidians *Ascidiella aspersa, Molgula manhattensis, Clavelina lepadiformis,* and *Ciona intestinalis* (Randløv and Riisgård, 1979).

Recent experiments reveal that key developmental genes are expressed in the epithelia of differentiating gills of the ascidians and of the enteropneust *Ptychodera flava* (Ogasawara *et al.,* 1999), suggesting that the gill slits of enteropneusts are homologous to those of chordates. A test of this idea might be to look for the expression of members of this gene family in the pharyngeal pores of macrodasyid gastrotrichs (Ruppert and Barnes, 1994) to see if the gene is responsible for pores or openings in the pharynx in general, rather than having an expression that is specific to deuterostome gill pores. The evidence that the gill slits of enteropneusts and chordates are homologous is based on their shared location, developmental origin, and, at least in *H. planktophilus,* their shared function. How then do the gill skeletal arches and the endostyle fit into this picture?

The enteropneust and cephalochordate pharynx share serially paired gill slits in the pharynx that are framed on either side by collagenous gill arches, and parted down the middle by a secondary gill (or tongue) bar. In ptychoderid enteropneusts (but not in *Harrimania*), synapticles, or collagenous bridges, join the primary bars with the secondary bars. The cephalochordate pharynx differs from that of the Enteropneusta in that it possesses an endostyle on the ventral midline. Horst (1939) considered the ventral digestive gut of enteropneusts to be homologous to the chordate endostyle, but recent evidence indicates an endostyle-like organ in the *dorsal* pharynx of the enteropneust *Schizocardium brasiliense* (Ruppert *et al.,* 1999). Further support for the dorsoventral inversion hypothesis may be found in the direction of particle movement in the pharynx of *H. planktophilus.* The food is gathered on the gill bars and transported from dorsal to ventral (in chordates, particles move from ventral to dorsal), and then transported posteriorly to the gut in a mucous food cord. The direction of food transport in *H. planktophilus* suggests that the enteropneusts are inverted dorsoventrally with respect to the chordates, and this notion is supported by molecular evidence (Arendt and Nübler-Jung, 1994). If true, such an inversion means that the gill bars could not be considered homologous with those of amphioxus, because they would also be inverted dorsoventrally and therefore contradict the conformational consistency required to assign homology. The coelomic diverticula in the gill bars and tongue bars is different in the two groups, suggesting that the gills of the common ancestor may have lacked primary and secondary gill bars. This idea is consistent with the absence of gill skeletal structures in adult pterobranchs, the adult enteropneust *Protoglossus,* urochordates, and developmental stages of all other chordates. Clearly, more comparative research is needed before we can be confident about gill-arch homologies among the deuterostomes.

The enteropneust pharynx should not be considered less specialized than that of the chordate pharynx on the basis that it lacks an endostyle organ that produces a mucous net. The pharynx of *H. planktophilus* is effective at capturing a wide range of particle sizes, and although it does not have a mucous net, mucus is produced abundantly by the surface of the enteropneust body and surely plays a role in capture

and transport of ingested material. The enteropneust pharynx may represent an intermediate stage in the evolution of the chordate pharynx; that is, first came the filter-feeding gill slits, and later came the capacity to make a mucous net. It is not clear whether the mucous net was present in the ancestral deuterostome animal and lost in the lineage to the enteropneust worms, or absent in the common ancestor and gained in the lineage to the chordates. The lack of the chordate-like endostyle and mucous net in *H. planktophilus* may be due to the undue stress that the net would receive from sediment acquired while deposit feeding. In any case, *H. planktophilus* is an effective filter feeder, even in apparent absence of a mucous net and endostyle.

H. planktophilus is perhaps unique among the filter-feeding protochordates because it lives entirely beneath the sediment. In aquaria, the burrows of this worm do not approach the sediment surface. Initially, these filter-feeding experiments were tried with worms *in situ,* but the difficulty in viewing particles entering the mouth made it impossible to establish whether particles found in the gut of exhumed worms were ingested *via* deposit feeding or suspension feeding. Most investigations that quantify filter feeding have been concerned with epifaunal organisms (including sessile, tube-dwelling, and free-living organisms) or infaunal forms that have part of their body or tube in direct contact with the epibenthos (examples include the bivalve *Mya arenaria* and the polychaete *Chaetopterus*) (Wildish and Kristmanson, 1997). *H. planktophilus* apparently exploits nutrients from interstitial pore water, a world occupied by protozoans, diatoms, foraminifera, flatworms, gastrotrichs, nematodes, copepods, molluscs, tardigrades, and bacteria that thrives at the boundary of the reduced oxygen layer (Levinton, 1995). *H. planktophilus* is a very active enteropneust and responds negatively to low light levels. When it is removed from its burrow, its gut is full of sediment. Sediment obtained from deposits probably accounts for the bulk of the gut's content, but food filtered from pore water may be most important nutritionally (Barrington, 1940). Many deposit feeders display adaptations that improve their efficiency in sediment processing and food absorption (Lopez and Levinton, 1987), and the filtration of nutrient-rich pore water, such as observed in *H. planktophilus,* may be more common than formerly realized.

Harrimania planktophilus is a facultative filter feeder, and a detailed comparison between a wide range of hemichordates will undoubtedly reveal further evolutionary innovations of the branchial pharynx. Several species seem to be especially good candidates for filter feeders, including the swimming enteropneust *Glandiceps hacksii* (Spengel, 1909); the deep-sea rock-pendulum worm *Saxipendium coronatum,* which lives atop rocky outcrops and extends its proboscis into the water column (Woodwick and Sensenbaugh, 1985); *Harrimania maculosa,* which lives on the

underside of rocks in Alaska (Ritter, 1900); and species of *Schizocardium* because of their well-developed branchial pharynx (Ruppert *et al.,* 1999). Much remains to be learned about the form, function, and origin of the filtering pharynx of deuterostomes.

Clearly the evolution of slits in the pharynx is one of the most important events in the evolution of the deuterostomes. Protochordates and *H. planktophilus* have a filter-feeding pharynx. Given the occurrence of homologous pharyngeal slits (but possibly not gill arches) in enteropneusts and chordates, and given the sister group relationship between hemichordates and echinoderms (Cameron *et al.,* 2000), it is not surprising that echinoderm paleontologists have found fossil carpoid echinoderms with pharyngeal slits (Jeffries, 1986). The most parsimonious conclusion is that the carpoid pharynx was also a filter-feeding pharynx. This study suggests that the filter-feeding pharynx may not be a synapomorphy of the chordates, but a defining feature of the deuterostomes.

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