Morphology of a New Deep-Sea Acorn Worm (Class Enteropneusta, Phylum Hemichordata): A Part-Time Demersal Drifter with Externalized Ovaries

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ABSTRACT   Ten individuals of an enteropneust in the family Torquaratoridae were videotaped between 2,900 and 3,500 m in the Eastern Pacific—one drifting a few centimeters above the bottom, two exposed on the substrate, and seven partly burrowed, reflecting a benthopelagic life style. Here, we describe a captured specimen (26 cm living length) as the holotype of *Allapasus aurantiacus* n. gen., n. sp. The small proboscis is dome-shaped, and the collar is only slightly wider than deep; both of these body regions are more muscular than in other torquaratorids, which presumably facilitates burrowing. The proboscis complex, in contrast to that of shallow-living enteropneusts, lacks a pericardial sac and is located relatively posteriorly in the proboscis stalk. The stomochord is separated from the main course of the gut by the intervention of a small, plate-like proboscis skeleton lacking posterior horns. The most anterior region of the trunk houses the pharynx, in which the pharyngeal skeletal bars are not connected by synapticles. The postpharyngeal trunk comprises three intestinal regions: prehepatic, hepatic (with conspicuous sacculations), and posthepatic. On either side of the worm, a flap of body wall (lateral wing) runs the entire length of the trunk. The two lateral wings can wrap the body so their edges meet in the dorsal midline, although they often gape open along the pharyngeal region. The holotype is a female (presumably the species is gonochoric) with numerous ovaries located in the lateral wings along the pharyngeal region. Each larger ovary contains a single primary oocyte (up to 1,500 μm in diameter) and bulges outwards in an epidermal pouch attached to the rest of the body by a slender stalk. Such externalized ovaries are unprecedented in any animal, and nothing is yet known of their role in the reproductive biology of *A. aurantiacus*. J. Morphol. 000:000–000, 2012.   © 2012 Wiley Periodicals, Inc.

KEY WORDS: Hemichordata; Enteropneusta; Torquaratoridae; *Allapasus aurantiacus*

INTRODUCTION

Hyman (1959), in reviewing acorn worms (class Enteropneusta, phylum Hemichordata), generalized that they typically live as benthic infauna at depths from the intertidal zone to the continental shelf—the only exceptions she noted were one shallow-living species that sometimes swims by undulating the body and a single deep-sea species dredged by the "Challenger" expedition. During the closing decades of the twentieth century, these older ideas needed to be modified because deep-sea photographs (summarized in Table 1 of Smith et al., 2005) revealed more and more enteropneusts living entirely exposed on the ocean floor. During that period, it was difficult to image an animal at great depth and then collect the same specimen, although this was accomplished in 1979 when the manned submersible "Alvin" collected a deep-sea acorn worm later described as *Saxipendium coronatum* by Woodwick and Sensenbaugh (1985). Fortunately, during the last decade, the use of remotely operated vehicles (ROVs) has facilitated in situ video recording of deep-living animals followed by collection of the same specimens for anatomical and molecular phylogenetic studies.

By now, two enteropneust species brought to the surface by ROVs have been named—*Torquarator bullocki* (Holland et al., 2005) and *Tergivelum baldwinae* (Holland et al., 2009)—and others have been collected but not yet formally described. In a recent molecular phylogenetic study of named and unnamed acorn worms, Osborn et al. (2012) demonstrated that most enteropneusts living in the deep sea belong to a single clade, the family Torquaratoridae. In this article, the torquaratorid

TABLE 1. *Collection data for* Allapasus aurantiacus

Specimen	Date	Dive no.	Depth (m)	Latitude N	Longitude W	Behavior[a]
A[b,c]	12 Jun '02	T438	2,994	36°34′48″	122°29′30″	Drifting
B	13 Jun '02	T439	3,492	36°19′41″	122°53′51″	Epibenthic
C	11 Mar '05	T829	3,455	36°14′21″	122°53′27″	Burrowed
D	16 Dec '05	T930	3,266	35°48′34″	122°34′5″	Epibenthic
E	21 Jan '06	T938	3,266	35°48′38″	122°34′5″	Burrowed
F	10 Jan '07	T1069	2,893	36°36′48″	122°26′9″	Burrowed
G	19 Dec '07	T1162	2,891	36°36′48″	122°26′8″	Burrowed
H[c]	19 Nov '09	D098	2,893	36°36′33″	122°26′3″	Burrowed
I	19 Nov '09	D098	2,891	36°36′32″	122°26′3″	Burrowed
J	19 Nov '09	D098	2,891	36°36′32″	122°26′3″	Burrowed

[a]All the "Burrowed" worms were partially emergent onto the sea floor.
[b]Morphological holotype (Voucher ID: SIO-BIC-H20).
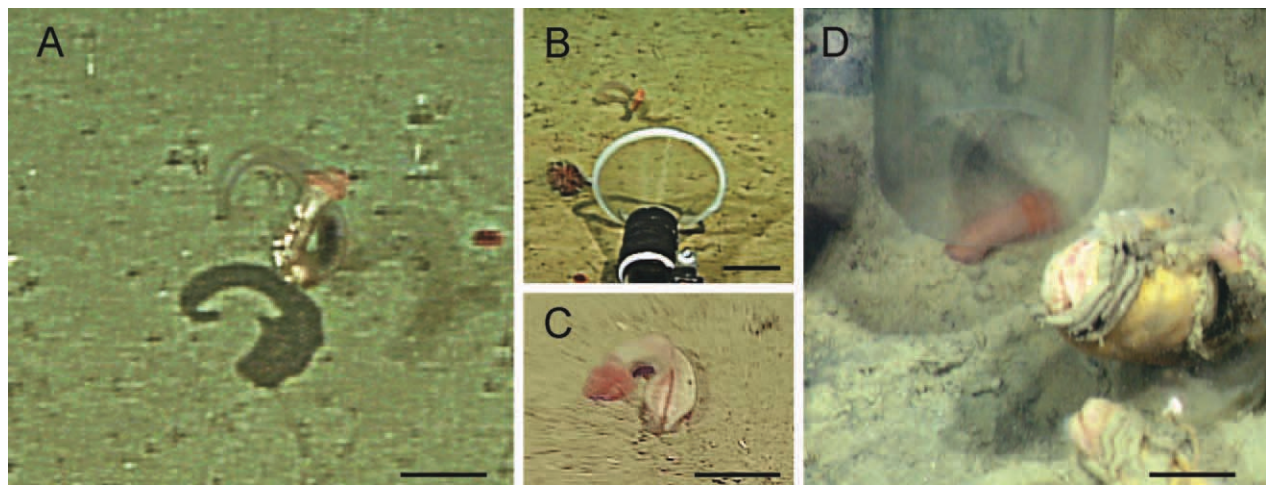[c]Molecular sequence (in Osborn et al., 2012).

Fig. 1.   *Allapasus aurantiacus* alive in situ; single frames from video recordings. (**A**) Holotype (A in Table 1) drifting in current near bottom. (**B**) Holotype entering collecting funnel. (**C**) Burrowed worm (C in Table 1) with its anterior fourth protruding. (**D**) Partially burrowed worm (H in Table 1) being collected with push-core (bivalves at bottom right). Scale bar in A,C,D = 5 cm; in B = 10 cm.

that Osborn et al. (2012) designated as Genus D, species 1 is described from deep-sea recordings as well as histological reconstructions and named *Allapasus aurantiacus* n. gen, n. sp. We directly demonstrate that these worms can drift demersally a short distance above the bottom, although we more often observed them extended on the surface of the ocean floor or partly burrowed in the sediment.

MATERIALS AND METHODS

Between 2002 and 2009, the ROVs "Tiburon" and "Doc Ricketts" of the Monterey Bay Aquarium Research Institute (MBARI) videotaped 10 specimens of an enteropneust in the Monterey Submarine Canyon and at the base of Davidson Seamount between 60 and 100 km offshore of Monterey, California, at depths between 2,900 and 3,500 m (Table 1, specimens A–J). Only two of the imaged animals were brought to the surface. One was recovered in good condition (Fig. 1A,B; specimen A in Table 1) and is the holotype described here. The other (Fig. 1D; specimen H in Table 1) was badly damaged during collection and was suitable only for molecular sequencing (Osborn et al., 2012).

The holotype was videotaped for 3 min as it drifted a few centimeters above the ocean floor (Fig. 1A) before being captured in a funnel-mouthed suction sampler facing into the approaching current (Fig. 1B). After the captured worm had been brought to the surface, several large oocytes were removed and frozen in liquid nitrogen for molecular analysis (Osborn et al., 2012), and the body, which had broken into two pieces at the level of the anterior trunk, was fixed in 10% formalin-seawater. The fixed specimen was photographed under a dissecting scope to show the overall morphology as well as details of the pharyngeal pores, pharyngeal skeletal bars, and parts of the gonadal region. For light microscopic reconstruction of the internal morphology, we embedded selected regions of the body in paraplast and prepared them as 15-μm serial sections stained in 0.1% aqueous azure A (Spicer, 1963). After paraplast embedding, the larger oocytes and hepatic region of the intestine tended to shatter when sectioned. Therefore, these tissues were embedded in Spurr's resin, sectioned at 4 μm with a glass knife, and stained in 0.1% aqueous azure A.

RESULTS
Systematics

Class Enteropneusta Gegenbaur, 1870; Family Torquaratoridae Holland et al., 2005 (as rediagnosed by Osborn et al. (2012), limiting it to enteropneusts whose proboscis skeleton is either absent or reduced

to a small medial plate and whose adult stomochord is either absent or not in communication with the main course of the gut).

Genus *Allapasus* n. gen. Type and only species: *Allapasus aurantiacus* n. sp.

Etymology: name of genus derives from Latin masculine noun *Allapasus* = gliding approach. Diagnosis: Small, plate-like proboscis skeleton intervening between stomochord and main course of gut, lateral wings running along entire length of the trunk, and with sacculate hepatic intestine shorter than posthepatic intestine.

Allapasus aurantiacus n. sp. (Figs. 1–5). Capture site and labeling of holotype: ROV Tiburon, MBARI dive T438, 36°34'48"W, 122°29'30"N, 2994 m, 12 June 2002, Karen J. Osborn collector; formalin-fixed female prepared as histological sections conserved along with unsectioned body parts in the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC-H20); no paratypes. Diagnostic features same as for genus. In life, proboscis and collar light orange, with trunk ranging from beige to light orange depending on individual. Etymology: species name derives from Latin masculine adjective *aurantiacus* = orange, in reference to the light orange color of the proboscis and collar.

Behavior, Living Appearance, and General Anatomy

In the 3-min videotape of the living holotype of *A. aurantiacus* (Fig. 1A), the worm was drifting with the current a few centimeters above the bottom. During the recording, there was no evidence of active swimming: the loosely curled posture of the worm underwent no detectable change, and no peristaltic or undulatory movements were observed. Moreover, there appeared to be no gut contents in the intestinal region running through the relatively translucent posterior part of the worm. The living body length, as estimated by a comparison with the dimensions of the collecting funnel (Fig. 1B), was about 26 cm. Other individuals of *A. aurantiacus* were videotaped on the sea floor—of these, two were entirely exposed, and seven were partly burrowed beneath the surface (Table 1; Fig. 1C,D).

From anterior to posterior, the main body regions are the proboscis, collar, and trunk. A lateral wing (a wide flange of body wall) runs along either side of the entire length of the trunk. In life, the right and left wings typically wrap around the body and appose their free edges dorsally, except in the pharyngeal region of the trunk, where they often leave a gap dorsally (Fig. 1A,C). In the drifting holotype (Fig. 1A), the gaping of the lateral wings in the pharyngeal region exposed the numerous externalized ovaries (described below) as cream-colored spheres.

The body of the holotype (Fig. 2A,B), broken into two parts at collection, lost its pigmentation soon after fixation and shrank to a total length of about 18 cm. Along with the fixed worm, the collecting jar contained a large clump of flocculent material (Fig. 2A, bottom left), evidently a precipitate of mucus that either had surrounded the living animal as a transparent sheath or had been secreted in response to the fixative. Moreover, the collection jar contained no feces, and the gut lumen was empty after fixation. Thus, all the evidence indicates that the gut of the drifting holotype was truly empty.

The major regions of the holotype are visible in Figure 2A,B, and the external details of the anterior end of the body are shown in Figure 2C–E. All measurements refer to the fixed specimen. The proboscis is a pointed dome (0.6 cm anterior to posterior, 0.6 cm dorsal to ventral, and 1 cm wide) and is deeply indented by a narrow groove running along the ventral midline. The collar measures 0.5 cm anterior to posterior, 0.6 cm dorsal to ventral, and 1 cm wide. The trunk measures about 17 cm anterior to posterior, 0.5 cm dorsal to ventral, and 1 cm wide (except along the region of the hepatic intestine, which is about 0.3 cm from dorsal to ventral and 1.4 cm wide). The right and left lateral wings, running along either side of the trunk, arise dorsolaterally in the pharyngeal region and ventrolaterally in the intestinal region; the apposition of these flaps obscures the dorsal side of the trunk in the fixed holotype. All along the ventral side, the trunk is indented by a groove in which runs the ventral nerve cord (Fig. 2A, arrowhead). The dorsal nerve cord (Fig. 2B, arrowhead) is visible running along the dorsal midline of the trunk.

Histological Structure

Proboscis and collar. A cross section near the anterior tip of the proboscis shows a core of smooth muscle fibers overlain by the epidermis (Fig. 2F).The muscles are organized as a moderately compact network with a tendency for radially oriented fibers to predominate. The epidermis of the proboscis, like that of the body generally, comprises supporting cells, gland cells (many containing acid mucopolysaccharides), and elements of the intraepithelial nervous system, most conspicuous as a basal fibrous layer (arrowed in inset in Fig. 2F). Within the muscle fiber mass of the proboscis, the protocoel opens up (Fig. 2G,H, arrows). Proboscis pores are absent, a lack that seems to be a feature of torquaratorids in general (Holland et al., 2005, 2009).

Figure 2I,J shows where the proboscis stalk begins to merge with the more dorsal collar tissues. The dorsal nerve cord (single arrow) is associated with a pair of proboscis vessels (arrowheads) and is underlain by the anterior tip of the
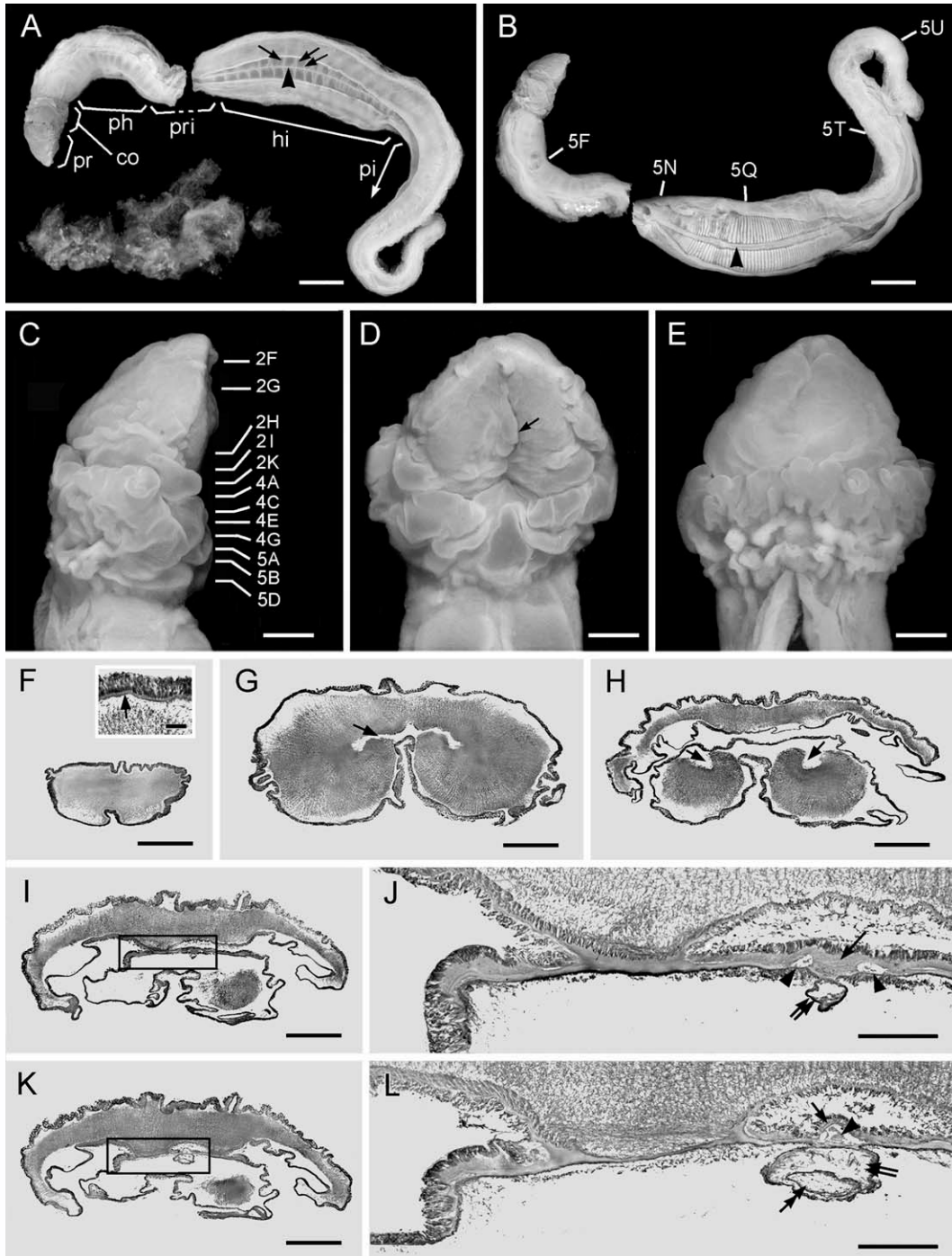
Fig. 2. *Allapasus aurantiacus* holotype. (**A**) Formalin-fixed worm broken into anterior fourth (left-side view) and posterior three-fourths (ventral view at left twists to left-side view at right); pr, proboscis; co, collar; ph, pharynx; pri, prehepatic intestine; hi, hepatic intestine; pi, posthepatic intestine; arrowhead indicates ventral nerve cord and arrows indicate folds in ventral body wall (possibly artifacts); extraneous mucus collected with living worm is at lower left. (**B**) Same, broken into anterior fourth (right-side view) and posterior three-fourths (dorsal view at left twists to right-side view at right); arrowhead indicates dorsal nerve cord; levels of cross sections in Figure 5 are indicated. (**C**) Enlarged right-side view of anterior end; levels of cross sections in Figures 2, 4, and 5 indicated. (**D**) Ventral view of anterior end; arrow indicates ventral groove in proboscis. (**E**) Dorsal view of anterior end. (**F**) Cross section of proboscis (inset: enlargement of epidermis with basal nerve fiber layer indicated by arrow). (**G**) Cross section of proboscis through protocoel (arrow). (**H**) Cross section showing anterior rim of collar (top) and proboscis (containing muscle masses with posterior extension of protocoel (arrows). (**I,J**) Rectangle in former enlarged in latter, showing dorsal nerve cord (single arrow), proboscis vessels (arrowheads), and anterior extremity of heart (twin arrow). (**K,L**) Rectangle in former enlarged in latter, showing dorsal nerve cord (single arrow), proboscis vessel (arrowhead), heart (twin arrow), and anterior extremity of stomochord (tandem arrow). Scale bar in A,B = 1 cm; in C–I, K = 2 mm; in J,L = 500 μm; for inset in F = 100 μm.
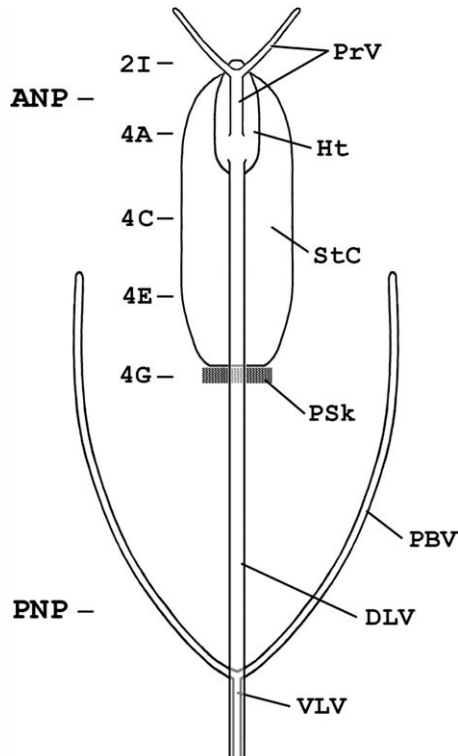
Fig. 3. Diagram of *Allapassus aurantiacus* proboscis complex (actually located in anterior half of collar) and related haemal vessels. At left are indicated levels for the anterior neuropore (ANP), posterior neuropore (PNP), and Figures 2I and 4A,C,E,G. At right, abbreviations (top to bottom) are: PrV, proboscis vessel; Ht, heart; StC, Stomochord; PSk, proboscis skeleton; PBV, peribuccal vessel; DLV, dorsal longitudinal vessel; and VLV, ventral longitudinal vessel.

heart (twin arrow). The anatomical relations of these features are shown in Figure 3, which diagrams the proboscis complex and related haemal vessels. In a slightly more posterior section (Fig. 2K) the anterior extremity of the stomochord is visible ventral to the heart. The enlargement (Fig. 2L) shows that the paired proboscis vessels have joined at this cross sectional level to form a single proboscis vessel. As diagrammed in Figure 3, the latter arises from dorsal side of the heart. More posteriorly, the dorsal side of the heart also gives rise to the dorsal longitudinal vessel, which runs in a posterior direction (Fig. 3). The proboscis complex of *A. aurantiacus*, in comparison to that of spengelids, harrimaniids, and ptychoderids, lacks a pericardial sac and is located relatively posteriorly in the proboscis stalk. This situation is intermediate between the absence of a proboscis complex in most torquaratorids (Holland et al., 2009) and its prominence in spengelids, harrimaniids, and ptychoderids (Hyman, 1959).

Figure 4A,B, a short distance posterior to the level of the anterior neuropore, shows the collar nerve cord, heart, and stomochord. The last is packed with vacuolated cells and has no obvious

lumen anywhere along its length. In contrast to its location in nontorquaratorid hemichordates, the glomerulus is more closely associated with the stomochord than with the heart. Just posterior to the heart (Fig. 4C,D), the proboscis complex comprises only the stomochord and a narrowed posterior extension of the glomerulus. At this level, the collar nerve cord is underlain by a pair of muscle-filled perihaemal coeloms, which are separated by a mesentery containing the dorsal longitudinal vessel.

Approximately midway between the anterior and posterior limits of the collar (Figs. 3 and 4E,F), a peribuccal vessel arises in the muscle mass of the body wall on either side of the worm and runs posteriorly. Figure 4G–J shows the reduced proboscis skeleton, as a small, nearly vertical plate that lacks the posterior horns characterizing the proboscis skeleton of shallow-living enteropneusts. The plate-like proboscis skeleton of *A. aurantiacus* completely separates the stomochord from the main course of the digestive tract.

Underlying the conspicuous collar tissues, the posterior region of the proboscis stalk (Fig. 4G–J) hangs down into the roof of the buccal cavity as sacculations, probably equivalent to the racemose (= cauliflower) organ of some other enteropneusts. More posteriorly in the collar (Fig. 5A), no elements of the proboscis stalk remain. The body wall of the collar, like that of the proboscis, comprises a moderately compact meshwork of muscle fibers among which no peribuccal coeloms were detected. At cross sectional levels shown in Figure 5B–F, left and right parabranchial ridges protrude dorsolaterally into the pharyngeal lumen, partially dividing it into a dorsal branchial region and a ventral digestive region; presumably, in feeding animals, the gut contents are limited to the ventral region of the pharynx. No coelomopores are present in the collar of *A. aurantiacus*, as may be typical of torquaratorids generally (Holland et al., 2005, 2009; our unpublished observations). The most anterior tissues of the trunk appear dorsal to the collar-trunk septum near the level of the posterior neuropore (Fig. 5B,C).

Pharyngeal region of trunk (and ovary). Figure 5D shows trunk tissues and collar tissues, respectively, dorsal and ventral to the collar-trunk septum. Left and right lateral wings arise as outfoldings of the dorsolateral body wall, and the peribuccal vessels have joined medially beneath the gut to form the ventral longitudinal vessel. At a level a little more posteriorly, cross sections show the ventral nerve cord (Fig. 5F,G), which begins at the anterior end of the trunk. The body wall of the pharyngeal region of the trunk, like that of the proboscis and collar, includes a moderately compact meshwork of muscle fibers. The branchial region of the pharynx (Fig. 5E) opens to the exterior via about a hundred pairs of slot-like pharyngeal pores
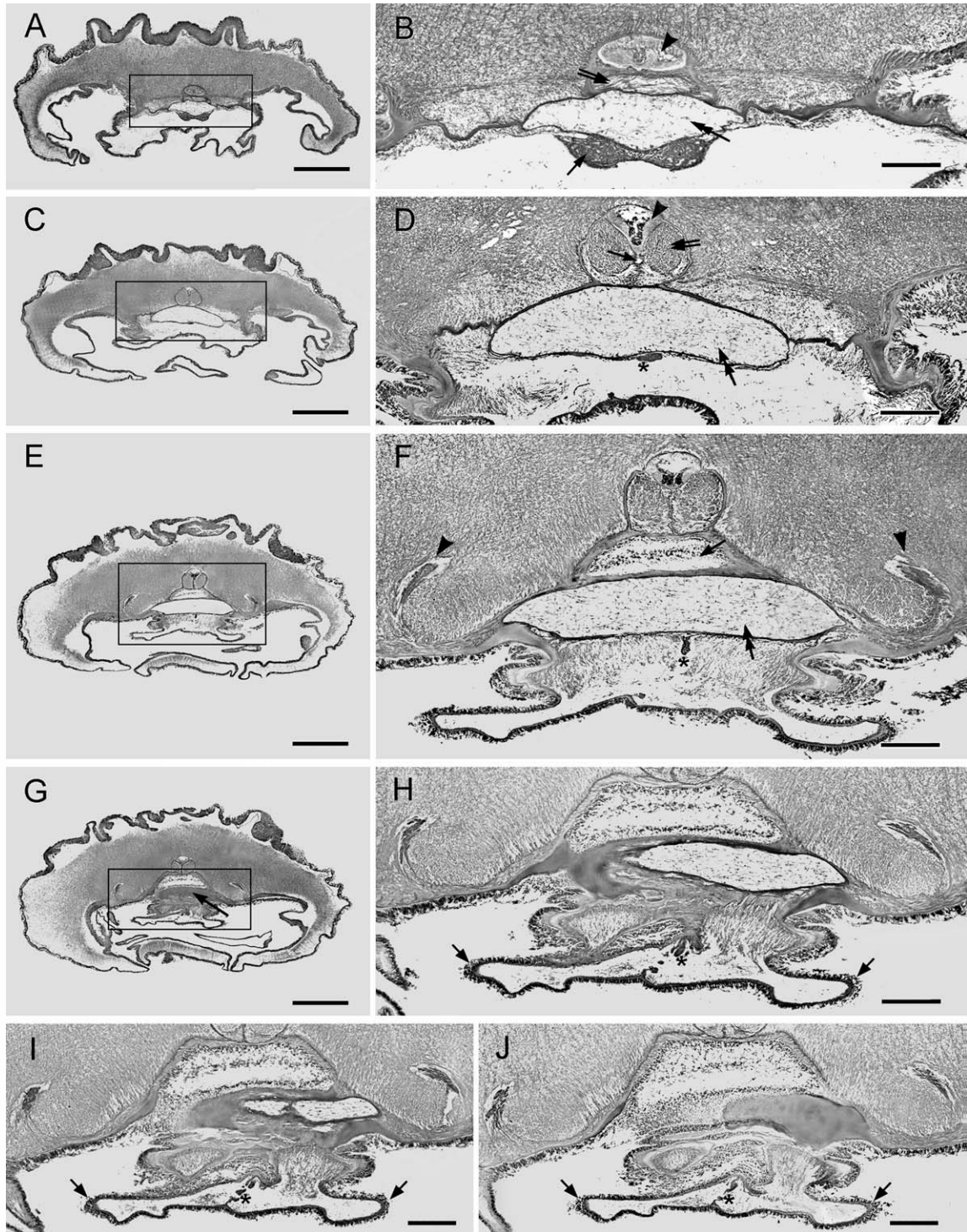
Fig. 4. *Allapasus aurantiacus* holotype. (**A**,**B**) Rectangle in former enlarged in latter, showing stomochord (tandem arrow) underlain by glomerulus (single arrow) and overlain by heart (twin arrow) and collar nerve cord (arrowhead). (**C**,**D**) Rectangle in former enlarged in latter, showing stomochord (tandem arrow) underlain by posterior extension of glomerulus (asterisk); collar nerve cord (arrowhead) is underlain by muscle-filled perihaemal coeloms (twin arrow) separated by mesentery containing dorsal longitudinal vessel (single arrow). (**E**,**F**) Rectangle in former enlarged in latter, showing peribuccal vessels (arrowheads); stomochord (tandem arrow) underlain by posterior extension of glomerulus (asterisk) and overlain by space (single arrow) continuous with buccal cavity. (**G**) Composite based on successive sections H-J showing posterior end of stomochord separated from buccal cavity by proboscis skeleton comprising a thin plate (single arrow). (**H–J**) Enlargements of rectangle in G showing three contiguous sections; posterior extremity of glomerulus indicated by asterisks; in H, sacculations of racemose (= cauliflower) organ indicated by single arrows. Scale bar in A,C,E,G = 2 mm; in B,D,F,H-J = 500 μm.

(Fig. 5H) and is strengthened by primary and secondary pharyngeal skeletal bars not connected by synapticles (Fig. 5I,J). Water exits the pharyngeal pores into a dorsal space bounded on either side by the lateral wings and open to the surrounding sea water by a mid-dorsal gap. The epidermis facing this dorsal space in this region of the trunk is associated with numerous ovaries (Fig. 5F, arrow).

Each immature ovary, which lies just beneath the epidermis, comprises a germinal epithelium surrounding a small mass of nongerminal cells (Fig. 5L, arrowhead). Somewhat more developed ovaries still lie just beneath the epidermis, but the germinal epithelium now encloses single, small primary oocyte (Fig. 5L, single arrow). Each oocyte contains a large germinal vesicle that includes a prominent nucleolus. At a later stage, ovaries containing medium-sized or large oocytes (Fig. 5K,L) bulge outward, beyond the surface of the animal. Such ovaries are surrounded by two very thin membranes, the inner comprising the germinal epithelium and the outer continuous with the epidermis. Each externalized ovary remains attached to the outside of the worm by a narrow stalk (Fig. 5K, arrow). We know of no comparable instance of externalized ovaries in any other animal group. The largest oocytes, of which there were several dozen (Fig. 5M), had a diameter of around 1,500 μm, equaling the size of those in *Tergivelum baldwinae* (Holland et al., 2009). In the present study of *A. aurantiacus*, the only specimen available for histological study was the female holotype: presumably, the sexes are separate in this species, and males will ultimately be discovered.

Intestinal region of trunk. The body wall of the intestinal region of the trunk contains only a sparse meshwork of muscle fibers. The right and left lateral wings, which arise ventrolaterally along either side of this trunk region can fold dorsally and meet in the dorsal midline of the living worm (shrinkage of the fixed tissues causes the lateral wings to pull slightly apart dorsally, as in Fig. 5N). The dorsal and ventral nerve cords (and their underlying dorsal and ventral longitudinal vessels) continue all along this region of the body (Fig. 5O,P). In many enteropneusts, the gut region just posterior to the pharynx has been called the esophagus; however, in *A. aurantiacus* there is no justification anatomically or histologically for making such a distinction, and we will simply call this region the prehepatic intestine.

In the pre- and posthepatic intestine (Fig. 5N,T,U), the lining epithelium is relatively flat ventrally, but is corrugated into plicae dorsally and laterally. From the dorsal midline, each plica runs ventro-anteriorly at an angle of about 45°. The hepatic intestine intervenes between the pre- and posthepatic regions and is morphologically distinct from them, at both the gross anatomical and cytological levels of organization. In the hepatic region (Fig. 5Q–S), the lining epithelium is relatively flat on the ventral side, but the dorsal side bulges with large-scale outpocketings (hepatic sacculations) oriented perpendicular to the long axis of the animal. The epithelium lining the sacculations of the hepatic intestine includes many cells filled with inclusions that range from light to dark brown in unstained sections.

DISCUSSION
Enteropneusts with a Bentho-Pelagic Life Style

Travel in the water column. Postlarval enteropneusts living in shallow water almost never float or swim above the bottom; the only known exceptions are individuals of the genus *Glandiceps* that can swim by undulating the body (Ikeda, 1908; Yoshimatu and Nishikawa, 1999). In contrast, deep-living enteropneusts, as adults, are much more prone to spend time in the water column. The first report of a deep-living enteropneust leaving the sea floor was by Gaillard (1991) who reported that the animal, probably in Genus A of Osborn et al. (2012), "swam away" in some unspecified manner when disturbed. Subsequently, a more passive drifting behavior has been directly observed in three more deep-sea acorn worms (Osborn et al., 2012). In addition, and is strong circumstantial evidence that a fourth species, *Tergivelum baldwinae* can similarly drift in the water column, because time-lapse photography showed one of these worms appearing abruptly, foraging for a day and a half, and suddenly disappearing, presumably into the water column (Smith et al., 2005). It would not be surprising if further work shows that most, if not all, torquaratorids are part-time demersal drifters.

Nothing is yet known about how deep-sea enteropneusts control their ascent into the water column, although rising from the bottom is presumably facilitated by emptying the gut and possibly also by the secretion of a thick coating or balloon of mucus (Osborn et al., 2012). It is also not known how a demersally drifting worm descends to a new benthic feeding site. Moreover, there is no information to indicate the horizontal or vertical distances typically traversed in a given drifting episode or the overall proportion of time spent above the bottom.

Life on the bottom: burrowing versus surface crawling. Although many shallow-living enteropneusts inhabit burrows in soft substrates, this generality has been overstated by some (like Barrington, 1965), who concluded that "Enteropneusts, then are essentially subterranean and burrowing animals ..." On the contrary, some shallow-water species can live epibenthically either temporarily or permanently. Benham (1899) found specimens of *Balanoglossus otagoensis* crawling on
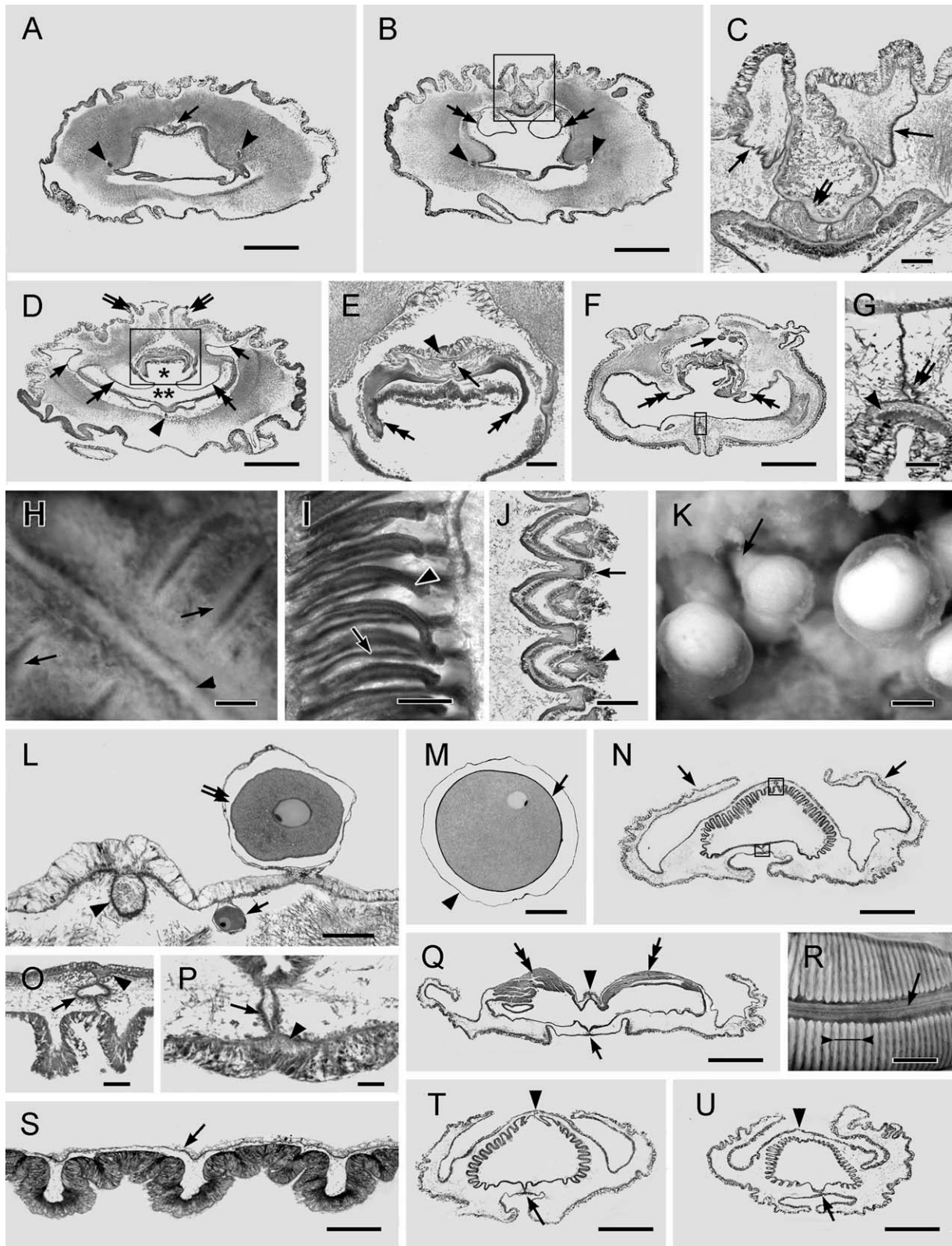
Fig. 5.

the surface of kelp holdfasts; Ikeda (1908) claimed that *Glandiceps hacksii* is "a creeper, but not a burrower as other enteropneusts are"; Stiasny (1910) reported, with some skepticism, that fishermen told him that *Balanoglossus clavigerus* crawls out of its burrow to sun itself when cold; Knight-Jones (1953) found that *Glossobalanus minutus* leaves its burrow and crawls on the surface when oxygen is limiting; Cameron et al. (2010) proposed that *Saccoglossus rhabdorhynchus* might crawl on the surface of rocks; and Hadfield (personal communication) informed us that a still-undescribed ptychoderid lives on the surface of hardened lava at a depth of 25 m at Ahihi-kinau, Maui, Hawai'i. In spite of these exceptions, however, it is fair to generalize that enteropneusts living at shallow depths are essentially burrowers.

In contrast to their shallow-living relatives, most torquaratorids, when associated with the bottom, are seen in their entirety, fully exposed on the ocean floor (Holland et al., 2005; Smith et al., 2005; Cannon et al., 2009; Holland et al., 2009; Anderson et al., 2011; Osborn et al., 2012). Moreover, the worms are often observed at the head of a fecal trail laid out on the ocean floor. Depending on the species, trail patterns may be spiral, looped in approximate raster patterns, or loosely meandering. *Allapasus aurantiacus* contrasts with other torquaratorids, first because no individual has yet been observed leaving a fecal trail on the surface of the sea floor and second because worms of this species are frequently encountered partly burrowed in the substratum (Fig. 1C,D); the burrowing is presumably facilitated by its proboscis and collar muscles, which are well developed in comparison to those of other torquaratorids. At pres-ent, it is not known if the burrow of *A. aurantiacus* has a conspicuous wall, as in *Saccoglossus inhacensis* (van der Horst, 1934), or is indistinct, as in *Balanoglossus simodensis* (Miyamoto and Saito, 2007).

Feeding in shallow- versus deep-living enteropneusts. It was once assumed that most enteropneusts swallow the substratum indiscriminately and pass it through the gut earthworm-style (van der Horst, 1939). Subsequently, however, Barrington (1940) and Carey and Mayer (1990) claimed that shallow-living enteropneusts deposit feed by protruding the proboscis from the burrow entrance and using ciliary-mucoid tracts to pick up the top few millimeters of sand plus organic matter for transport to the mouth. It is widely believed that this kind of feeding is not selective: thus the size spectrum of sand particles in the gut lumen mirrors that in the top few millimeters of substratum (Knight-Jones, 1953; Colin et al., 1986; Dobbs and Guckert, 1988; Miller, 1992). Such surface deposit feeding stuffs the gut lumen with sand and results in the production of sandy fecal casts. Although Karrh and Miller (1996) claimed that *Saccoglossus kowalevskii* is an obligate deposit feeder incapable of suspension feeding, many other enteropneusts in shallow depths have been found to augment their deposit feeding by sucking suspended particles directly in at the mouth and capturing them within the pharynx (Thomas, 1972; Cameron, 2002; Gonzalez and Cameron, 2009). Finally, according to Ikeda (1908), *Glandiceps hacksii* deviates strikingly from other shallow-living enteropneusts in having sand-free gut contents comprising only "microorganisms." The absence of ingested sand could indicate that

Fig. 5. *Allapasus aurantiacus* holotype. (**A**) Cross section of collar showing peribuccal vessels (arrowheads) and collar nerve cord (single arrow). (**B,C**) Rectangle in former enlarged in latter; level of posterior neuropore, showing peribuccal vessels (arrowheads), parabranchial ridges (tandem arrow) and dorsal nerve cord (twin arrow) underlain by paired perihaemal spaces; single arrows indicate collar-trunk septum. (**D**) Collar-trunk septum (single arrows) delimiting anterior extremity of trunk (towards top) from posterior extremity of collar (at bottom); lateral wings indicated by twin arrows; parabranchial folds (tandem arrow) separate pharynx into dorsal branchial part (single asterisk) and ventral digestive part (twin asterisks); ventral longitudinal vessel (arrowhead) underlies pharynx. (**E**) Enlargement of rectangle in D; dorsal nerve cord (arrowhead) overlies dorsal longitudinal vessel (single arrow); tandem arrows indicate pharyngeal skeletal bars. (**F**) Pharynx divided by parabranchial ridges (tandem arrows) into respiratory and digestive parts; dorsal to pharynx, lateral wings almost completely enclose space including some oocytes (single arrow). (**G**) Enlargement of rectangle in F, showing ventral nerve cord (arrowhead) and ventral longitudinal vessel (twin arrow). (**H**) Dorsal surface of pharynx showing dorsal nerve cord (arrowhead) flanked on either side by pharyngeal pores (single arrows). (**I**) Inner surface view of one side of respiratory pharynx (dorsal toward left) showing primary and secondary skeletal bars (arrow and arrowhead, respectively). (**J**) Cross section of primary and secondary skeletal bars (arrow and arrowhead, respectively). (**K**) Inner surface view of lateral wings in pharyngeal region; medium sized oocytes bulge outward, but remain connected to underlying tissue by a stalk (single arrow). (**L**) Section through inner side of lateral wing in pharyngeal region showing ovaries containing either nongerminal cells (arrowhead) or small oocytes (single arrow); twin arrow indicates medium-sized oocyte in extruded ovary connected to underlying tissue. (**M**) Section of oocyte of maximum size freed from outer epithelial layer, but surrounded by thin jelly layer (single arrow) and germinal epithelium (arrowhead). (**N**) Cross section of prehepatic intestine with lateral wings indicated by single arrows. (**O**) Enlargement of upper rectangle in N, showing dorsal nerve cord (arrowhead) and dorsal longitudinal vessel (single arrow). (**P**) Enlargement of lower rectangle in N, showing ventral nerve cord (arrowhead) and ventral longitudinal vessel (single arrow). (**Q**) Cross section of hepatic intestine with sacculations (tandem arrow), dorsal nerve cord (arrowhead) and ventral nerve cord (single arrow). (**R**) Dorsal surface view of hepatic intestine showing sacculations flanking the dorsal nerve cord (single arrow); line between arrowheads indicates orientation of section in S. (**S**) Parasagittal section of hepatic sacculations; gut lumen is toward bottom; sacculations overlain by thin epidermis (single arrow). (**T,U**) Cross sections of anterior and posterior regions, respectively, of posthepatic intestine with dorsal nerve cord (arrowhead) and ventral nerve cord (single arrow). Scale bar in A,B,D,F,N,Q,T,U = 2 mm; in I,M = 500 µm; in K = 400 µm; in R = 4 mm; in C,E,H,J,S = 300 µm; in L = 200 µm; in G,O,P = 100 µm.

this species is an exclusive suspension feeder and/ or a highly selectively deposit feeder that can pick up organic matter while excluding mineralized sediment particles.

Most torquaratorid enteropneusts are epibenthic deposit feeders that differ from most of their shallow-water relatives in having gut contents virtually devoid of sand grains (Holland et al., 2005; Smith et al., 2005; Holland et al., 2009; Holland, unpublished observations). Evidently, these deep-living worms can select particles rich in organic material from the surface of the deep sea without an appreciable admixture of mineralized sediments. It is likely that much of the selection takes place in ciliary-mucoid tracts on the ventral side of the collar lips, which are exceptionally wide in many torquaratorids. As already mentioned, *A. aurantiacus* has a tendency to burrow not seen in other torquaratorids; this raises the possibility that its gut contents may include a relatively high proportion of mineralized sediments. At present, however, nothing is known about the diet *A. aurantiacus*, because the floating holotype had an empty gut and the one partially burrowed individual was captured too badly damaged to yield any information on gut contents.

Reproduction compared for shallow- and deep-living enteropneusts. Hadfield (1975), in his review of reproductive biology in shallow-living enteropneusts, generalized that the sexes are separate, with females having hundreds of small ovaries and males having hundreds of small testes distributed in the anterior trunk region. Depending on the species, nongerminal "yolk" cells may or may not be present in the gonads. In females of species with indirect development, each ovary contains numerous primary oocytes that are relatively small when spawned (on the order of 100 μm in diameter). In contrast, females of species with direct development, commonest in the family Harrimaniidae, have only a few, large oocytes per ovary (the largest oocytes of *Harrimania kupfferi* measure 1,300 μm × 1,000 μm, the maximum sized oocytes known for any shallow-water enteropneust). Typically, when shallow-living enteropneusts spawn, the gametes pass through small pores in the epidermis and enter the sea water where fertilization takes place. No specialized copulatory interactions between males and females have been reported.

Aspects of the reproductive biology for deep-living enteropneusts have recently been considered by Osborn et al. (2012). Many have separate sexes, but some are hermaphrodites, which is one solution for facilitating fertilization of sparsely distributed animals in the deep-sea. Because torquaratorids are so sparsely distributed in the deep sea, it would also not be surprising to find that males and females associate at spawning time (for example, either by copulating or by surrounding themselves with a common mucous cocoon) to help insure fertilization. To date, there is no direct evidence for such interactions.

In torquaratorid species for which the gonadal morphology has been studied, the ovaries contain one or a few oocytes with large maximum diameters: 500 μm in *Torquarator bullocki* (Holland et al., 2005) and 1,500 μm in both *Tergivelum baldwinae* (Holland et al., 2009) and *A. aurantiacus* (present study). The large oocytes of torquaratorids could indicate that these worms are conventional direct developers. Alternatively, however, they might be unconventional indirect developers: that is, the large oocytes of torquaratorids may be the source of giant (up to 28 mm) *Planctosphaera* larvae. Hadfield and Young (1983) have previously suggested that such larvae might someday be traced back to the adult stage of enteropneusts living in the deep sea. As pointed out by Osborn et al. (2012), this possibility could be conclusively tested by preserving specimens of *Planctosphaera* appropriately for molecular analysis.

Certainly the most remarkable feature of the reproductive biology of *A. aurantiacus* is the externalization of the ovaries. Each ovary containing a medium- to large-sized oocyte hangs off of the outside of a ripe female in a stalked, hollow bag. The wall of the bag evidently comprises the remains of the germinal epithelium closely invested with a thin-stretched epidermis. Such an arrangement is without precedent, not only in enteropneusts, but in animals generally. At present, it is difficult to imagine a role for the externalized ovaries at mating time. Perhaps the sperm in this species are invasive, as they are in salps (Boldrin et al., 2009), and can burrow through nongerminal tissues to fertilize female gametes within the mother's body. Should this be so, the extreme peripheral location of the oocytes would shorten the invasion route. Finally, although, the externalized ovaries of the holotype contained only oocytes, capture of additional specimens of *A. aurantiacus* might reveal that the fertilized female gametes are retained in the externalized ovaries and undergo embryonic development there. Viviparity in enteropneusts has a precedent; one shallow living harrimaniid was found brooding a larva within the body of the mother (Gilchrist, 1925).

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