

A new deep-sea species of epibenthic acorn worm (Hemichordata, Enteropneusta)

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ABSTRACT

Individuals of an enteropneust, *Tergivelum baldwinae* n. gen., n. sp. were videotaped at a depth of about 4 km in the eastern Pacific and collected by a remotely operated vehicle. The living worms range in length from 9 to 28 cm and are dark brown anteriorly and beige posteriorly. The proboscis is shaped like a shallow dome, indented on either side by a laterodorsal fossa housing a prominent proboscis nerve. The collar comprises a thin transverse crest dorsally and two laterally projecting lips on either side of the mouth ventrally. The mouth is oriented parallel to the substratum and is flanked by large left and right buccal muscles (contrasting with the rudimentary musculature elsewhere in the body). The respiratory pharynx of the trunk extends far anteriorly so that much of it lies dorsal to the mouth opening. The gill bars are not joined by synapticles. The laterodorsal body wall at the anterior extremity of the trunk extends as two conspicuous flaps (back veils) that run posteriorly as unattached coverings over the anterior 30-50% of the trunk. On either side of the midline, the body wall of the trunk is extended as a narrow lateroventral fold. Within

KEY WORDS

Hemichordata,
Enteropneusta,
Acorn worm,
new genus,
new species.

the trunk runs the intestine, which lacks hepatic sacculations and opens at an anus at the posterior end of the body. Frame analysis of videotapes suggests that the worm can secrete a mass of mucus around the body to facilitate demersal drifting from one epibenthic foraging site to the next. We include a preliminary phylogenetic analysis based on rDNA sequences from *T. baldwinae* n. gen., n. sp. and additional deep-sea enteropneusts not yet formally described taxonomically (sequence data place them unexpectedly close to ptychoderids). Until more is known about the group as a whole, it is prudent to leave family level classification of *T. baldwinae* n. gen., n. sp. as *incertae sedis*.

RÉSUMÉ

Une nouvelle espèce d'entéropneuste épibenthique (Hemichordata) de mer profonde. Des spécimens du ver entéropneuste, *Tergivelum baldwinae* n. gen., n. sp. ont été enregistrés sur bande-vidéo à une profondeur d'environ 4 km dans l'est du Pacifique et collectés au moyen d'un véhicule téléopéré. Ces vers ont une longueur comprise entre 9 et 28 cm et une couleur brun foncé antérieurement et beige postérieurement. Le proboscis a la forme d'un dôme peu marqué, possédant de chaque côté un sillon latéro-dorsal contenant un nerf bien développé. Le collier se compose, en position dorsale, d'une étroite crête transverse et en position ventrale de deux lèvres de chaque côté de la bouche. La bouche est orientée parallèlement au substrat et est entourée à gauche et à droite par les muscles buccaux proéminents (par opposition aux autres muscles du corps qui sont très rudimentaires). Le pharynx respiratoire du tronc s'étend loin antérieurement de sorte qu'il se trouve en majeure partie dans la région dorsale de l'ouverture buccale. Les arcs branchiaux ne sont pas connectés par des synaptiques. À l'extrémité antérieure du tronc, la paroi du corps forme latéro-dorsalement deux pans (ou voiles) flottants qui couvrent 30-50% de la partie antérieure du tronc. De chaque côté de la ligne médiane, la paroi du tronc forme un pli latéro-ventral étroit. L'intestin, qui ne présente pas de diverticules hépatiques, s'étend le long du tronc et s'ouvre vers un anus situé dans la partie terminale du corps. L'analyse vidéo suggère que les vers secrètent une masse de mucus autour de leur corps, qui faciliterait leur dérive démersale d'une zone de fourragement à une autre. Nous présentons aussi une analyse phylogénétique préliminaire basée sur des séquences d'ARNr de *T. baldwinae* n. gen., n. sp. et d'autres espèces d'entéropneustes abyssaux (non encore décrites). Jusqu'à ce que de plus amples études soient faites sur le groupe entier, la classification au niveau de la famille sera laissée *incertae sedis*.

MOTS CLÉS

Hemichordata,
Enteropneusta,
Stomocordés,
genre nouveau,
espèce nouvelle.

INTRODUCTION

At the time Hyman (1959) reviewed the enteropneusts (acorn worms), they were thought to live exclusively as infauna. Moreover, almost all of the species then known had been collected at relatively shallow depths,

excepting several damaged specimens dredged from the deep sea by the *Challenger* (of these, only *Glandiceps abyssicola* Spengel, 1893 was ever formally described taxonomically) (Thomson & Murray 1885; Spengel 1893). These older views of enteropneust biology began to broaden when Bourne & Heezen (1965)

published a photograph of an enteropneust living epibenthically in the deep sea and producing a spiral fecal trail; since the specimen was not collected, it was not described taxonomically. This discovery by Bourne & Heezen (1965) was followed by many sightings of enteropneusts crawling on the deep sea floor, and, by the end of the 20th century, several dozen such worms had been photographed or videotaped at widely scattered locations in the world ocean (reviewed in Smith *et al.* 2005). However, with the exception of the spaghetti worm, *Saxipendium coronatum* (Woodwick & Sensenbaugh, 1985), none of these animals was recovered and described.

Over the last five years, the remotely operated vehicle *Tiburon* of the Monterey Bay Aquarium Research Institute has videotaped several kinds of enteropneusts living epibenthically in the deep sea and has captured some of them. These specimens have been fixed in formalin for morphological study and/or ethanol for molecular analysis. From preliminary studies, it is clear that the specimens collected by the *Tiburon* are morphologically diverse. In addition, many but not all of them have a disproportionately wide collar region, a feature not known for any shallow water enteropneust. One wide-collared form has already been described as *Torquarator bullocki* Holland, Clague, Gordon, Gebruk, Pawson & Vecchione, 2005. In the present paper, we describe the anatomy of a second wide-collared, epibenthic species of deep-sea enteropneust and name it *Tergivelum baldwinae* n. gen., n. sp. We include a phylogenetic analysis based on rDNA sequences in order to justify our taxonomic arrangement. In addition, from videotape analysis, we suggest that this species can secrete a mucous cocoon to facilitate lift into the water column for transit from one benthic foraging site to the next.

MATERIALS AND METHODS

During 2006 and 2007, the ROV *Tiburon* videotaped seven epibenthic enteropneusts (all apparently belonging to the same species) at depths around 4 km in the Eastern Pacific approximately 120 km off of the California coast (Fig. 1). After videotaping, specimens were collected by slurp-

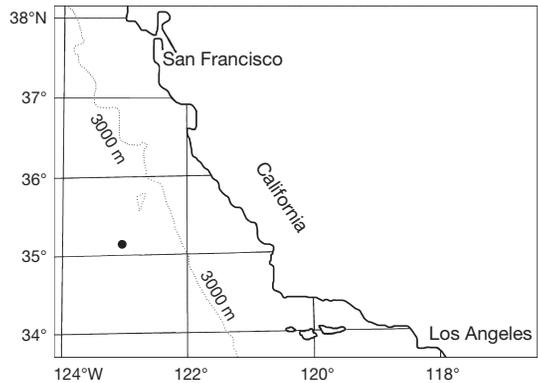


FIG. 1. — Collection site (depth: 3952–3953 m) for holotype and paratypes I–VI of *Tergivelum baldwinae* n. gen., n. sp.

gun, brought to the surface, fixed in ethanol or 10% seawater-formalin, and assigned MBARI collection codes (Table 1). During fixation, the fragile bodies tended to fragment. We selected one specimen (from MBARI dive number T1067) to be the holotype because it had broken into only three pieces; the other specimens were designated as paratypes I–VI (Table 1). The fixed holotype, after being photographed through the dissecting microscope, was dehydrated in an ethanol series, embedded via xylene in paraffin, oriented for cross sectioning, and prepared as serial sections 15 μ m thick. Paratypes I and II were similarly prepared as paraffin cross and sagittal sections, respectively. Most of the serial sections were stained in Mayer's hematoxylin and eosin, and a few were stained for acid mucopolysaccharides in 0.1% aqueous azure A (Spicer 1963). The best oriented sections were selected for illustrating the histology (Figs 5 and 6B, C from paratype I; Fig. 6A from paratype VI; and Fig. 6D–J from the holotype). For sex determination, a small sample of gonad-containing tissue was taken from the holotype and each of the paratypes, dehydrated in ethanol, embedded in Spurr's resin, sectioned 4 μ m thick with a glass knife, and stained in 0.1% aqueous azure A. Spurr-embedding prevented the oocytes from shattering when sectioned.

Genomic DNA was isolated from 50 mg ethanol-preserved tissue from paratypes II and IV–VI with the DNeasy kit, according to the manufacturer's protocol (Qiagen Inc., Valencia, CA). Gene regions

TABLE 1. — Collection data for holotype and paratypes of *Tergivelum baldwinae*, n. gen., n. sp. (Hemichordata, Enteropneusta); all these specimens were collected from a depth of 3952–3953 m at latitude 123°01'W, longitude 35°08'–35°10'N; collector Kenneth L. Smith Jr.

Specimen	MBARI no.	Collection date	Fixation	Collection numbers	Remarks
Holotype	T1067	13.XII.2006	Formalin	SIO-BIC H4	Paraffin cross sections
Paratype I	T1141(2)	21.IX.2007	Formalin	SIO-BIC H5	Paraffin cross sections
Paratype II	T1076(1)	31.I.2006	Ethanol	SIO-BIC H6	Paraffin sagittal sections, rDNA sequenced for molecular analysis
Paratype III	T1141(1)	21.IX.2007	Ethanol	SIO-BIC H7	Fragmented, not prepared for paraffin sectioning
Paratype IV	T1078	2.II.2006	Ethanol	MNH E24	Fragmented, not prepared for paraffin sectioning, rDNA sequenced for molecular analysis
Paratype V	T1076(2)	31.I.2006	Ethanol	MNH E23	Fragmented, not prepared for paraffin sectioning, rDNA sequenced for molecular analysis
Paratype VI	T1094	5.VI.2007	Ethanol	SIO-BIC H8	Fragmented, not prepared for paraffin sectioning, rDNA sequenced for molecular analysis

were amplified by PCR. Each 25 µl PCR reaction contained 100 ng of template DNA, 2.5 mM MgCl₂, 10 µM of each primer, 2.5 units Taq polymerase (Promega Inc., Madison, WI), and 0.4 mM dNTPs. Universal primers to amplify an approximately 2000-bp fragment of the 18S rDNA gene were 18e (5'-CTGGTTGATCCTGCCAGT-3') and 18P (5'-TAATGATCCTTCCGCAGGTTACCT-3') (Halanych *et al.* 1998). Primers to amplify an approximately 550-bp fragment of the 16S rDNA gene were 16Sar (5'-CGCCTGTTTAAACAAAA-CAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi 1996). PCR products were bidirectionally sequenced. Previously published GenBank rRNA sequences for *Balanoglossus carnosus* (16S, AF051097; 18S, D14359) and for *Saccoglossus kowalevskii* (16S, L19302; 18S, L28054) were aligned with 16S and 18S sequences determined in the present study — namely: the echinoid *Echinocrepis rostrata* Mironov, 1973 (16S, EU520492; 18S, EU520504), *Saxipendium coronatum* (16S, EU520493; 18S, EU520505), *Tergivelum baldwinae* n. gen., n. sp. T1076(1) (16S, EU520494; 18S, EU520506), T1078(1) (16S, EU520495; 18S, EU520507), T1076(2) (16S, EU520496; 18S, EU520508), T1094 (16S, EU520497; 18S, EU520509), “narrow-lipped” enteropneusts T1012(A8) (16S, EU520498; 18S,

EU520510), T886(A4) (16S, EU520499, 18S, EU520511), “extrawide-lipped” enteropneusts T879(A8) (16S, EU520500; 18S, EU520512), T879(A10) (16S, EU520501; 18S, EU520513), T1013(A8) (16S, EU520502; 18S, EU520514), T1011 (16S, EU520; 18S, EU520515). For alignment, we used ClustalX (Thompson *et al.* 1994) followed by manual corrections. Secondary structure of rRNA (i.e. stems and loops) was inferred using the program GeneBee (Brodsky *et al.* 1995). Bayesian inference of phylogeny was performed using MrBayes v3.0B4 (Huelsenbeck & Ronquist 2001) with data partitions using RNA secondary structure. Six chains were run simultaneously for 1 000 000 generations and trees sampled every 1000 generations. The first 1000 trees were discarded as “burn in” and Bayesian posterior probabilities were estimated on the 95% majority rule consensus.

ABBREVIATIONS

an	anus;
cen	circumenteric nerve;
co	collar;
coc	collar cord;
cts	collar-trunk septum;
dnc	dorsal nerve cord (of trunk);
flpn	fossa of left proboscis nerve;
frpn	fossa of right proboscis nerve;
gb	gill bar;
gp	gill pore;

gs	gill slit;
in	intestine;
lbm	left buccal muscle;
lbv	left back veil;
lcc	lumen of collar cord;
ldp	left dorsal protuberance;
li	lip;
llf	left lateroventral fold;
lpn	left proboscis nerve;
MBARI	Monterey Bay Research Institute;
MNHN	Muséum national d'Histoire naturelle, Paris;
mo	mouth;
pc	proboscis coelom;
PCR	polymerase chain reaction;
ph	pharynx;
pr	proboscis;
rbm	right buccal muscle;
rbv	right back veil;
rdp	right dorsal protuberance;
rlf	right lateroventral fold;
rpn	right proboscis nerve;
ROV	Remotely Operated Vehicle;
SIO-BIC	Scripps Institution of Oceanography Benthic Invertebrate Collection, La Jolla;
tc	transverse crest;
TL	total length;
tr	trunk;
vnc	ventral nerve cord (of trunk).

SYSTEMATICS

Class ENTEROPNEUSTA Gegenbaur, 1870
Family *incertae sedis*

Genus *Tergivelum* n. gen.

TYPE AND ONLY SPECIES. — *Tergivelum baldwiniae* n. sp.

ETYMOLOGY. — Name of genus derives from neuter Latin words, *tergum* (= back) + *velum* (= veil), referring to back veils.

DIAGNOSIS. — Enteropneust hemichordates with paired back veils, each a long projection of the body wall, arising at anterior extremity of trunk and extending unattached along anterior 30-50% of trunk. Additional apomorphies are conspicuous right and left buccal muscles flanking mouth.

Tergivelum baldwiniae n. sp.
(Figs 2-6)

HOLOTYPE. — ROV *Tiburón*, dive T1067, 123°01'W, 35°08'N, 3952 m, 13.XII.2006, Kenneth L. Smith Jr.,

♂, TL (living) 9 cm, formalin-fixed and prepared as serial cross sections (SIO-BIC H4).

PARATYPES. — Collection data for paratypes I-VI (SIO-BIC H5 through SIO-BIC H8 and MNHN E23 and MNHN E24) in Table 1.

ETYMOLOGY. — Species name commemorates Roberta Baldwin (1940-2006), an oceanographer with a special fondness for deep-sea biology.

DESCRIPTION

Anterior body (proboscis, collar, and back veils) dark brown (Fig. 2). Posterior body beige (colour of gut contents showing through relatively transparent tissues). In life, smallest specimen (holotype) 9 cm TL and 2 cm wide at collar, and largest (paratype I) 28 cm TL and 4.5 cm wide at collar. Fixed worms shrank to about two-thirds of living size. Figure 3A diagrams external anatomy, and Figure 3B shows distinctive anterior nerves and muscles. These diagrams emphasize that dorsal side of collar is reduced rostrocaudally to narrow transverse crest, while branchial region of trunk extends far anteriorly, being situated dorsal to mouth. Figure 4B indicates levels of cross sections in Figures 5 and 6.

Proboscis in living specimens a shallow, rounded dome (Fig. 2A), but may temporarily become slightly pointed anteriorly (Fig. 8). Histological fixation wrinkles proboscis (Figs 4A-E; 5A, C). Either side of proboscis indented by a laterodorsal fossa (Figs 2A, white arrowhead; 3A; 4E, arrow) housing lateral proboscis nerve (Figs 3B; 5C). Proboscis nerves on either side extend dorsally and join in midline just anterior to collar cord (Fig. 3B); they also extend ventrally to merge on ventral side of proboscis (just posterior to section in Fig. 5D). Proboscis coelom includes clusters of black pigment granules and an anteroposterior muscle cell tract (Fig. 5A). Elsewhere proboscis coelom (like and most other coelomic spaces of body) contains delicate meshwork of widely scattered connective tissue and muscle cells (Fig. 5B). Histological examination of proboscis revealed no stomochord, proboscis skeleton, heart, pericardium, glomerulus or proboscis pores (although our material was limited, we think most of these features are absent and not simply inconspicuous). The posteroventral region of proboscis (Fig. 5G) forms anterior margin of mouth. Epidermis covering

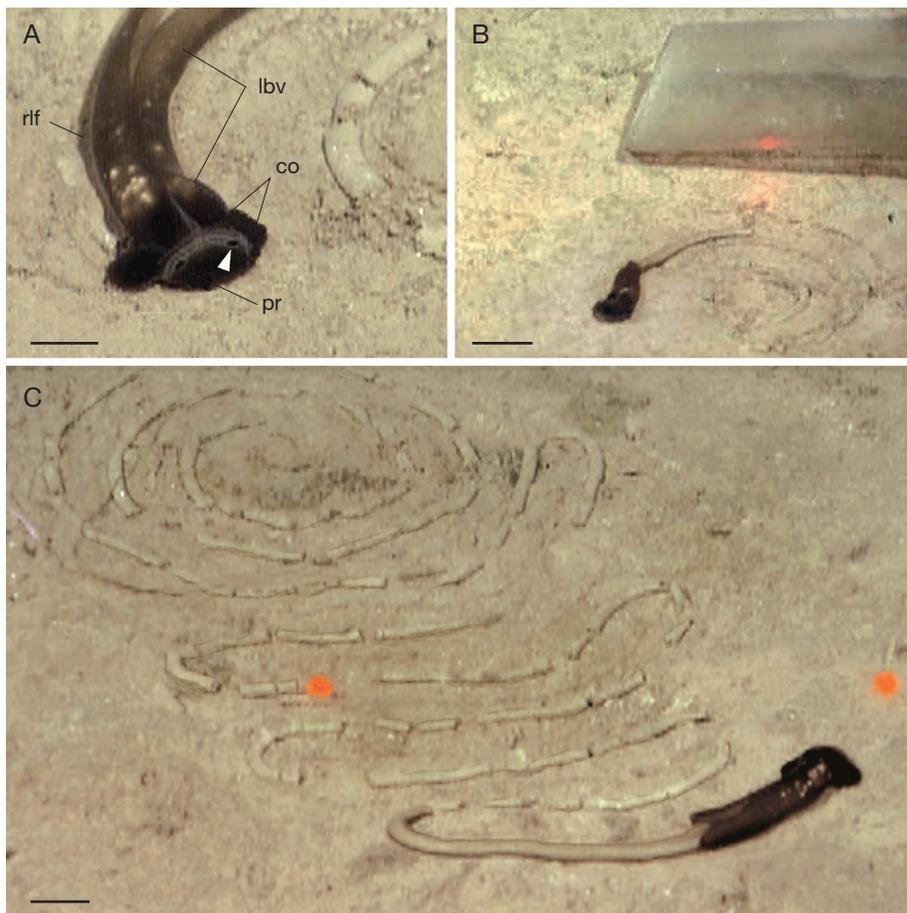


FIG. 2. — Single video frames of living *Tergivelum baldwiniae* n. gen., n. sp.: **A**, anterior of paratype IV, white arrowhead indicates fossa of left proboscis nerve; **B**, holotype laying down fecal trail; suction sampler in background; **C**, paratype II laying down fecal trail, 29 cm between red laser dots. Abbreviations: **co**, collar; **lbv**, left back veil; **pr**, proboscis; **rlf**, right lateroventral fold. Scale bars: A, 2 cm; B, C, 3 cm.

proboscis (and much of rest of body) comprises slender support cells and gland cells overlying thin basal concentration of diffuse intraepidermal nervous system (Fig. 5B, arrow).

Collar (Fig. 3A, co) represented dorsally by narrow transverse crest composed of highly glandular epidermal cells that swell artifactually when fixed. Mid-dorsally, beneath transverse crest runs a short collar nerve cord (Figs 3B; 5D, E) having a spacious lumen and overlying paired perihæmal coeloms. Posteriorly, the collar cord continues as the lumenless dorsal nerve cord of trunk (Fig. 5G, H). Ventral

region of collar comprises lips bordering mouth laterally and posteriorly. Lips of living animal flush with substratum (Figs 2; 3A), but, after fixation, curling dorsally (Fig. 4C, arrowheads). Lips are associated with circumenteric nerves and buccal muscles (Fig. 5D), left rectangle, shows these structures only on right side because cross section cut slightly obliquely; corresponding structures appear on left side in more posterior cross sections (Fig. 5G). Figure 5F shows collar-trunk septum associated at its gastral side with buccal muscle and at epidermal side with circumenteric nerve. Anteriorly, circumenteric nerves

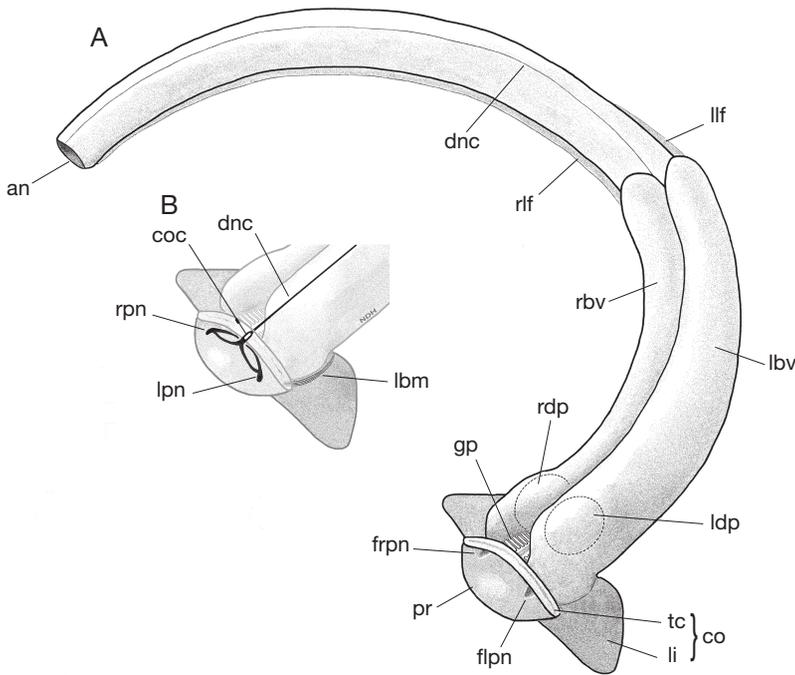


FIG. 3. — *Tergivelum baldwinae* n. gen., n. sp. in approximate dorsal view: **A**, surface details; **B**, locations of major nerves and muscles associated with proboscis and collar. Abbreviations for structural features: see text.

and peribuccal muscles dwindle and disappear near anterior limit of collar lips. Posteriorly, right and left buccal muscles diminish (Fig. 5I) and merge in posterior lip, while right and left circumenteric nerves approach midline and join, becoming ventral nerve cord of trunk. The prominence of the buccal muscles raises the question of their function(s) — one possibility is that these muscles are employed by the animal to steer a precise spiral or meandering course while foraging.

Trunk projecting much farther forward dorsally than ventrally, so that its dorsal region (Fig. 5G-I) is encountered first as one sections animal from anterior end. This anterodorsal trunk region includes branchial (respiratory) pharynx as well as dorsal nerve cord overlying a dorsal blood vessel flanked by coelomic spaces relatively free of muscle cell meshwork present elsewhere in trunk coelom. On either side, branchial pharynx perforated by about 30 slot-shaped gill pores (Fig. 4F) associated with primary and secondary gill bars (not connected

by synapticles) supported by skeletal elements; in addition, each secondary bar includes an extension of the trunk coelom (Fig. 6A). Gill pores in register with gill slits (Fig. 6A). Because glandular epidermis of this region becomes swollen and disrupted by histological fixation, we could not determine whether each gill pore is associated with its own atrium (= branchial sac) as diagrammed for other enteropneusts (Benito & Prados 1997). On ventral side, trunk begins just behind posterior lip, where left and right circumenteric nerves join to form ventral nerve cord underlain by ventral blood vessel (Fig. 6B, C).

Digestive tract within trunk comprises a pharynx anteriorly and a considerably longer intestine posteriorly, without any intervening esophagus. In addition to branchial region already described, pharynx also has a digestive region ventrally (Figs 5I, arrowheads; 6B, D) with a relatively smooth lining. In contrast, intestinal lining is corrugated by about two dozen plicae on either side of midline (Fig. 6E-J).

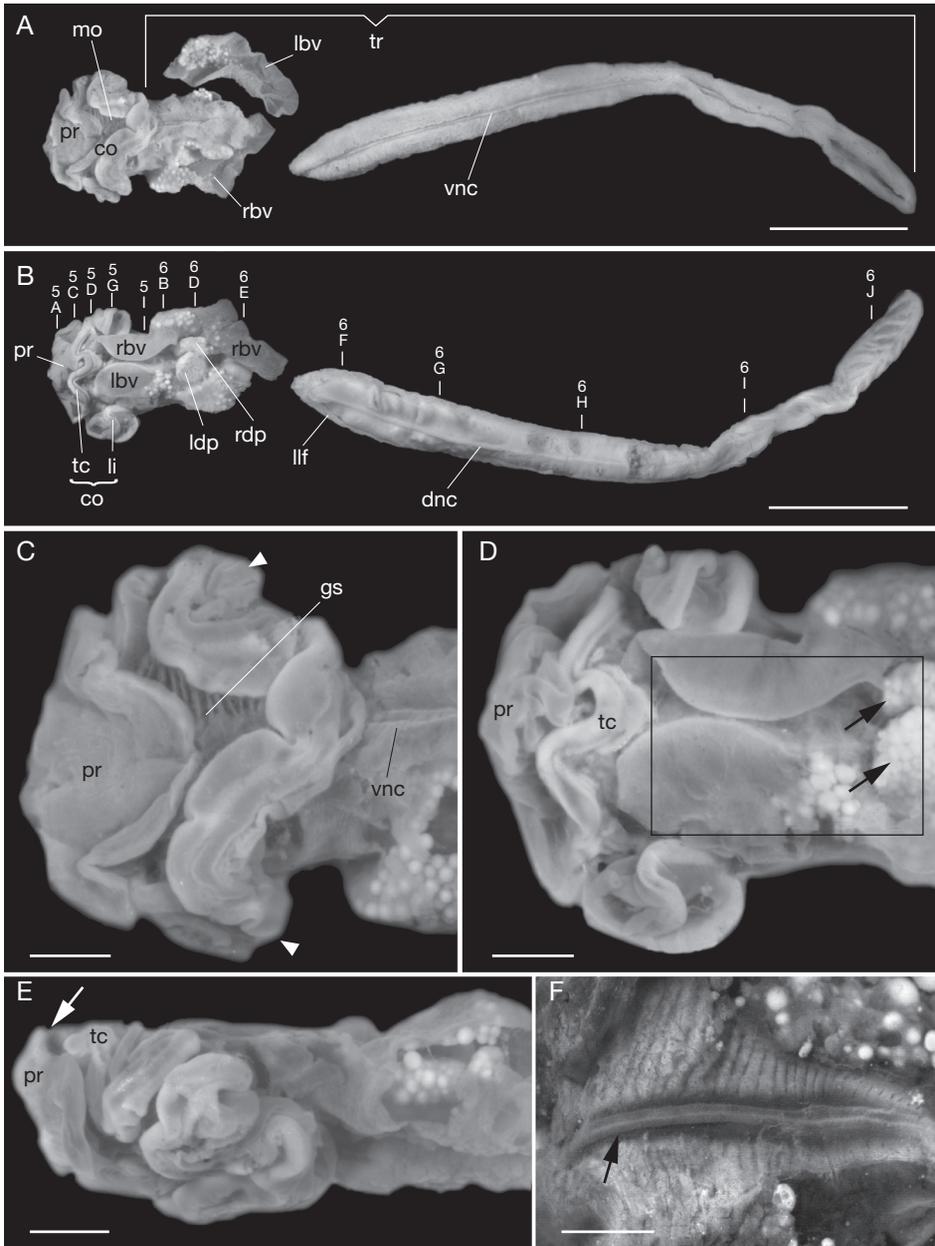


FIG. 4. — Fixed specimens of *Tergivelum baldwinae* n. gen., n. sp.: **A**, holotype (ventral view) which broke into three parts when fixed — namely the left back veil (**lbv**), most of intestinal region of trunk (at right), and anterior body (at left, comprising proboscis, collar, and anterior extremity of trunk); **B**, holotype (dorsal view, excluding left back veil) with numbered letters indicating levels of cross section in Figures 5 and 6; **C**, enlargement of anterior portion of holotype in ventral view; lateral lips of collar (arrowheads) curl dorsally as a preservation artifact; **D**, enlargement of anterior portion of the holotype in dorsal view; arrows indicate dorsal protuberances; **E**, enlargement of anterior portion of holotype in left side view with arrow indicating fossa of left proboscis nerve; **F**, dorsal view of anterior region of trunk of paratype II (corresponding to area in rectangle in panel 4D); back veils and dorsal protuberances dissected away revealing dorsal nerve cord (arrowed) flanked by slot-shaped gill pores. Abbreviations for structural features: see text. Scale bars: A, B, 1 cm; C-F, 2 mm.

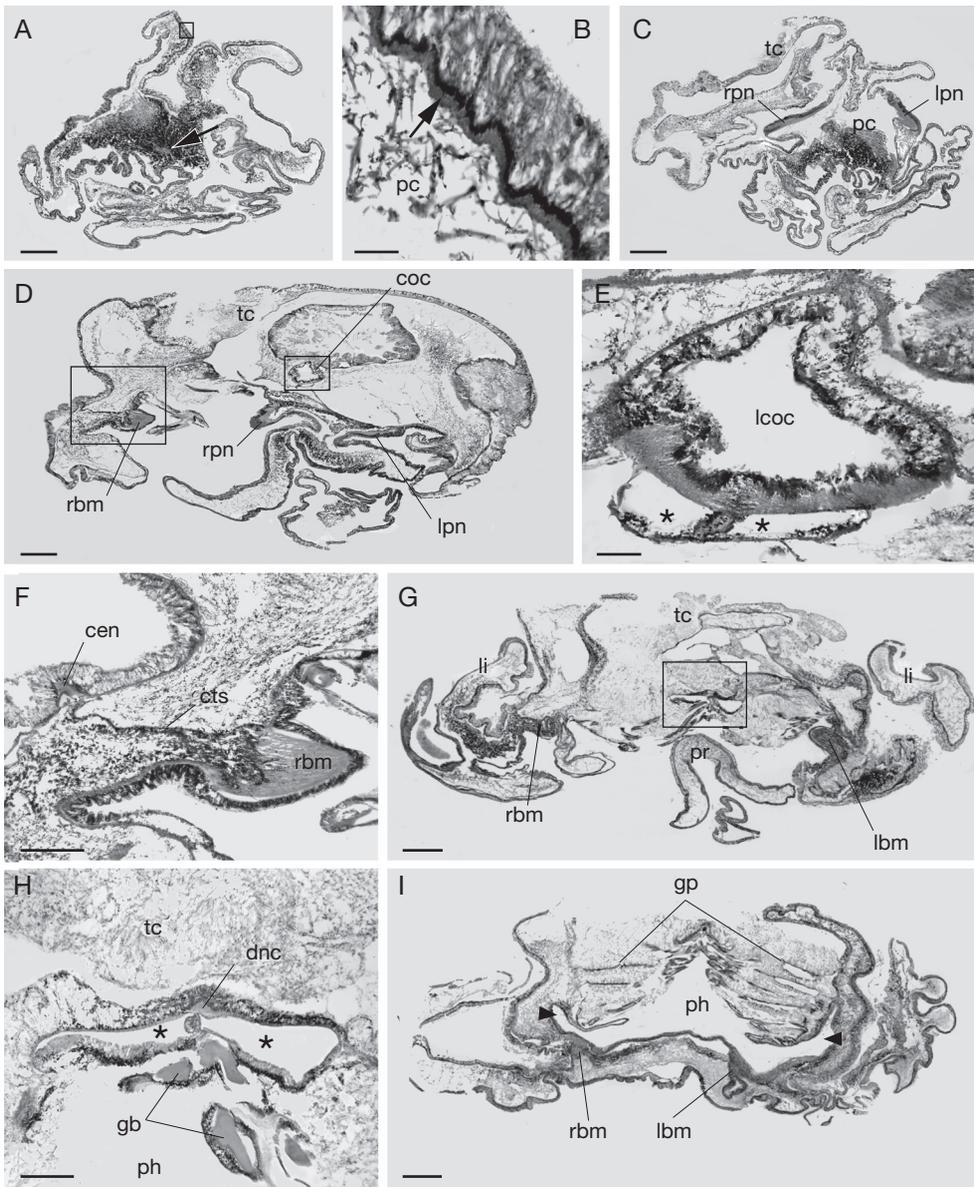


FIG. 5. — Cross sections of *Tergivelum baldwiniae* n. gen., n. sp. at levels indicated in Figure 4B: **A**, near anterior tip of proboscis, arrow indicates tract of longitudinal muscle fibers; **B**, enlargement of rectangle in 5A showing epidermis (toward right) underlain by basal concentration (arrowed) of diffuse intraepidermal nervous system; proboscis coelom contains meshwork of connective tissue and muscle cells; **C**, more posterior section of proboscis; **D**, section through collar showing anterior end of right buccal muscle; specimen oriented with left side somewhat in advance of right, so left buccal muscle not yet in view; **E**, detail of the center rectangle in 5D; showing lumen of collar cord separating dorsal non-neural and ventral neural regions; perihemal coeloms (marked by asterisks) lie on either side of dorsal blood vessel; **F**, detail of left-hand rectangle in 5D. Collar-trunk septum separates trunk coelom (above) from collar coelom (below); **G**, section through level of the mouth; lateral lips comprise ventral region of collar; posteroventral extremity of proboscis forms anterior margin of mouth; **H**, detail in the rectangle in 5G; beneath dorsal nerve cord is dorsal blood vessel with parts of trunk coelom (asterisks) on either side; **I**, section at level of posterior rim of mouth; buccal muscles approaching each other and will merge a few sections more posteriorly; pharynx comprising ventral digestive region (arrowheads) and dorsal branchial region (obliquely sectioned) penetrated by gill pores. Abbreviations for structural features: see text. Scale bars: A, C, D, G, I, 1 mm; B, 50 μ m; E, 100 μ m; F, 300 μ m; H, 200 μ m.

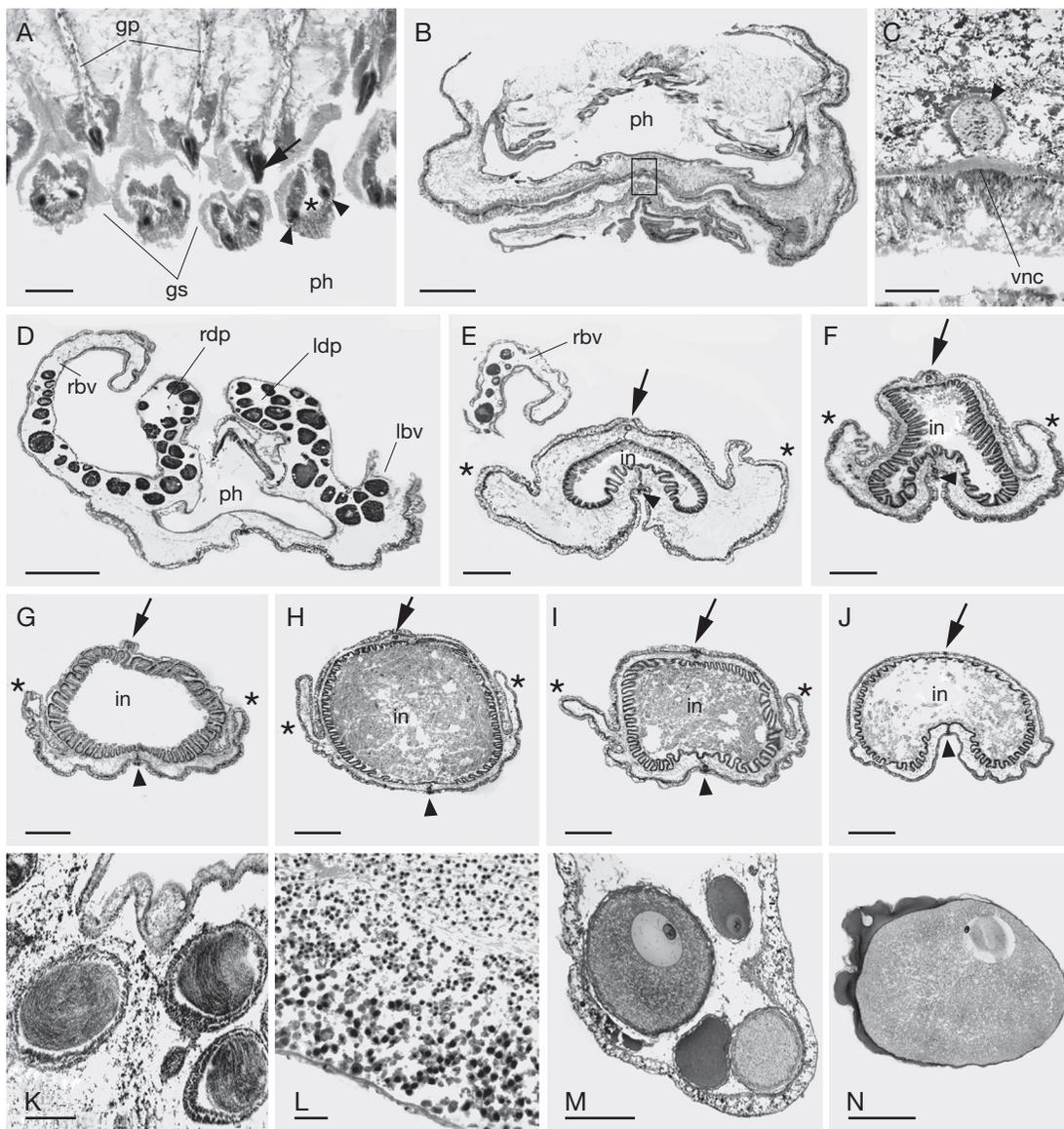


FIG. 6. — Cross sections (B–J) of *Terpigvelum baldwinae* n. gen., n. sp. at levels indicated in 4B: **A**, parasagittal section in region of gill pores showing primary gill bar with skeleton (arrowed) and secondary gill bar with skeleton (arrowheads) and coelomic space (asterisk); **B**, cross section of trunk near anterior extremity of ventral nerve cord (in rectangle); **C**, detail from rectangle in 6B with ventral nerve cord underlain by ventral blood vessel (arrowhead); **D**, cross section of anterior trunk showing dorsal protuberances and back veils (the left one broken off); dark spheres are testes; **E–J**, intestinal region of trunk; asterisks mark lateroventral folds; dorsal and ventral nerve cords indicated, respectively, by arrows and arrowheads; **K**, section through three testes; **L**, detail of testis with spermatozoa-filled lumen toward top right; **M**, medium-sized oocytes; **N**, large oocyte. Abbreviations for structural features: see text. Scale bars: A, 100 μ m; B, D, 1 mm; C, 50 μ m; E–J, N, 500 μ m; K, M, 200 μ m; L, 20 μ m.

From dorsal midline of the intestine, each plica runs ventro-anteriorly to ventral midline at a 45° angle. There are no large-scale outpocketings (he-

patatic sacculations) of intestinal wall. Intestinal lumen filled with abundant soft granular material plus fragmented skeletal remains of diatoms,

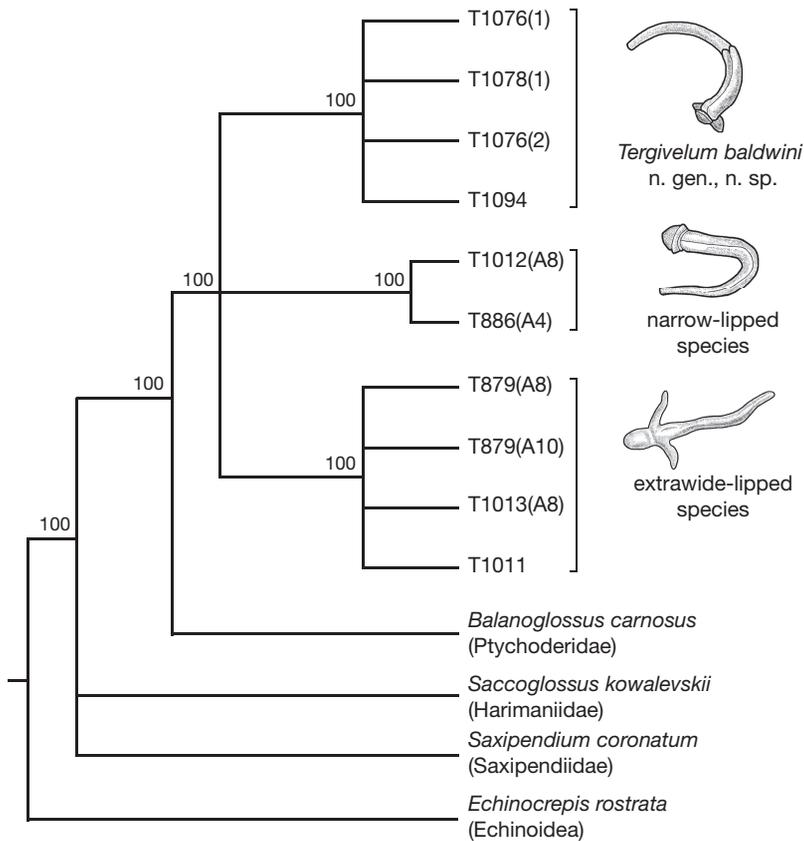


FIG. 7. — Bayesian cladogram based on enteropneust 18S and 16S rRNA sequences, rooted using an echinoid echinoderm (*Echinocrepis rostrata* Mironov, 1973). Numbers at nodes represent posterior probabilities (p). All nodes with $p < 0.95$ were collapsed into polytomies.

foraminiferans, coccoliths, holothurians, sponges, and crustaceans.

Three kinds of projections arise from trunk as outfoldings of epidermis over a core of trunk coelom containing loose meshwork of connective tissue and muscle cells. The most conspicuous of these projections are right and left back veils arising at anterior extremity of trunk and extending posteriorly as unattached dorsal coverings over anterior 30–50% of trunk (Fig. 2). The second kind of trunk projection comprises left and right dorsal protuberances (Figs 4B, D; 6D), which are convexities of dorsal wall partly obscuring the gill pores. In living animal, back veils overlie dorsal protuberances (Fig. 3A). The third, and least obvious trunk projections are left and right

lateroventral folds running along most of the trunk (Figs 2A; 3A; 6E–I), except at posterior extremity (Fig. 6J). Presumably, coordinated beating of cilia on epidermal cells of lateroventral folds (along with those on ventral side of trunk generally) power gliding along substratum. Videotapes of living animals never showed peristalsis.

Numerous gonads are located in dorsal protuberances and in anterior region of back veils (Figs 2A, C; 6D). Sexes are separate, although not easily distinguished with naked eye. Histological examination shows holotype and paratype V are males; whereas paratypes I–IV and VI are females. Testes of males and ovaries of females both appear as white spheres (each several hundred micrometers in diameter) contrasting strongly with surrounding dark brown

TABLE 2. — Collection data for voucher specimens of the unidentified narrow-lipped enteropneusts and the extrawide-lipped enteropneusts of Figure 8. All these specimens were collected by David A. Clague. At least part of each specimen was fixed in ethanol for molecular analysis (the ethanol-fixed material is available from William J. Jones).

Specimen	MBARI no.	Collection	Depth	Latitude/longitude	SIO-BIC no.
Narrow lips	T1012(A8)	3.VIII.2006	2273 m	130°40'W, 45°30'N	H9
Narrow lips	T886(A4)	25.VIII.2005	3081 m	126°47'W, 42°41'N	H10
Extrawide lips	T878(A8)	13.VIII.2005	2344 m	130°08'W, 45°08'N	H11
Extrawide lips	T879(A10)	13.VIII.2005	2345 m	130°08'W, 45°08'N	H12
Extrawide lips	T1013(A8)	4.VIII.2006	2030 m	130°30'W, 45°27'N	H13
Extrawide lips	T1011	2.VIII.2006	1675 m	130°27'W, 45°25'N	H14

of non-germinal tissues. Neither testes nor ovaries contain non-germinal cells rich in nutritive stores – namely “yolk cells” characterizing gonads of some other enteropneusts (Hadfield 1975). In ripe testes, germinal epithelium (comprising spermatogonia, spermatocytes and spermatids) surrounds testicular lumen containing spermatozoa with spherical heads about 3 µm in diameter (Fig. 6K, L). Each ovary contains one or a few primary oocytes of diverse sizes (Fig. 6M). The largest primary oocytes (totaling about a dozen per female) are approximately 1.5 mm in diameter and surrounded by a jelly layer rich in acid mucopolysaccharides (Fig. 6N). These are the largest primary oocytes yet found in any enteropneust – previous record held by *Harrimania kupfferi* (von Willemoes-Suhm, 1871), in which Spengel (1893) found primary oocytes up to 1.2 mm in diameter. The paucity of large oocytes in each female of *T. baldwinae* n. gen., n. sp. strongly suggests that males and females spawn in close proximity and that oocytes emitted from ovaries do not immediately float away in the sea water. Instead, it is likely that there is some provision – possibly a mucous cocoon (see below) – for keeping male and female gametes together at least until fertilization is ensured. No female in present study was brooding embryos; however, the possibility of brooding remains open due to the small number of individuals of *T. baldwinae* n. gen., n. sp. sampled so far.

PHYLOGENETIC ANALYSIS

A cladogram based on 16S and 18S rDNA (Fig. 7) places *Tergivelum baldwinae* n. gen., n. sp. in

an unresolved trichotomy with two additional groups of epibenthic, deep-sea enteropneusts. One group of these yet-undescribed enteropneusts has relatively narrow collar lips and the other has exceptionally wide collar lips (collection data and locations of voucher specimens given in Table 2). Both groups differ from *T. baldwinae* n. gen., n. sp., in lacking back veils and conspicuous buccal muscles (our preliminary studies). Given the considerable morphological diversity of these three kinds of epibenthic enteropneusts, it is surprising that the molecular data unite them so closely – evidently as a sister group to the Ptychoderidae (Fig. 7). Further sampling of Ptychoderidae is critical to assess if these yet-undescribed deep-sea enteropneusts are in fact nested inside Ptychoderidae. Until the morphologies of the narrow-lipped and extrawide-lipped species have been described in detail, we think it prudent not to create a new family to accommodate *T. baldwinae* n. gen., n. sp., but instead to leave the family level classification of this species as *incertae sedis*. The suspected ptychoderid affinities of many deep-sea, epibenthic enteropneusts (excluding *Saxipendium coronatum*) raise the possibility that the previously described *Torquarator bullocki* (Holland *et al.* 2005), for which no sequence data are yet available, might also prove to be closely related to ptychoderids; should this be so, the family level taxonomy of *T. bullocki* would probably need to be revised. In sum, for all of these recently discovered enteropneusts, a satisfactory higher-level classification will require a combination of reliable morphological and molecular data.

DISCUSSION

All the specimens of *Tergivelum baldwiniae* n. gen., n. sp. yet collected have been found at depths around 4 km on the Monterey deep-sea fan, a benthic habitat previously described by Smith & Druffel (1998). Even before the first collection of *T. baldwiniae* n. gen., n. sp., aspects of its behaviour had been studied *in situ* by time-lapse photography (Smith *et al.* 2005) showing that the worms crawl forward at about 8 cm per hour. The fecal trail laid down by the worm may be clockwise (Smith *et al.* 2005), counter-clockwise (Fig. 2A, B), or meandering (Fig. 2C). Although the finer details of feeding are not known, we presume that mucus produced by the ventral surface of each lateral lip picks up particles from the substratum and transports them via a ciliary-mucoid tract to the mouth. As already mentioned, the gut contents are chiefly soft granular material containing a small amount of plant and animal skeletal remains. Because it is likely that much of this mineralized material had no nutritive value when ingested, the enteropneust apparently ingests the surface layer of the substratum with little or no selectivity.

In addition to likely roles in feeding and reproduction (already mentioned), the epidermal mucus of *T. baldwiniae* n. gen., n. sp. might serve other functions, like deterring predation, as previously proposed for shallow water enteropneusts (Kirk 1938; Nørrevang 1965). Mucus might also help the worms drift demersally – the results of Smith *et al.* (2005) strongly indicate that *T. baldwiniae* n. gen., n. sp. spends part of its time drifting above the bottom, because time-lapse photographs, taken at one frame per hour, demonstrated that one of these worms appeared suddenly in the field of view (presumably from the water column), foraged for 39 hours, and then abruptly disappeared (presumably back into the water column). In the present study, we made an observation suggesting that mucus might facilitate demersal drifting. Frame analysis of an 8-min videotape of paratype IV showed that the enteropneust reacted to turbulence caused by the ROV by accelerating its forward speed and by elevating the anterior third of the body a few millimeters above the substratum (Fig. 8). During

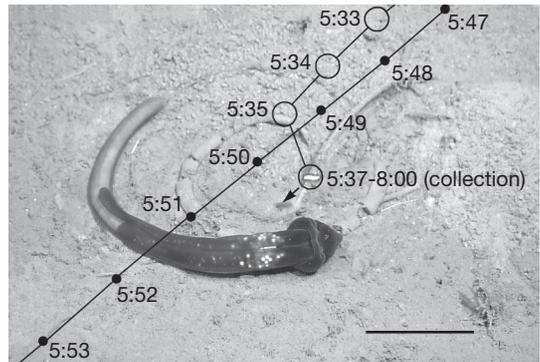


FIG. 8. — Single frame (at 5 min: 37 sec) of 8-min videotape of *Tergivelum baldwiniae* n. gen., n. sp. paratype IV. Filled circles show path of a particle transported unobstructed about 5 cm above the bottom (from 5:47 to 5:53). Open circles show path of particle (small polychaete) drifting about 2 cm above the bottom and evidently getting stuck (at 5:36) in substantial mucus surrounding acorn worm; arrow indicates shadow cast on sea floor by the immobilized particle. Scale bar: 5 cm.

this time, the prevailing current was transporting suspended particles past the worm at about 4 cm per second. One of these suspended particles (a small polychaete worm) was transported into the field of view and then suddenly stopped about 2 cm upstream from the anterior end of the enteropneust and 2 cm above the substratum. The particle was evidently caught in a voluminous mass of clear mucus surrounding the enteropneust. This observation suggests that specimens of *T. baldwiniae* n. gen., n. sp., when preparing to ascend into the water column, secrete a cocoon of mucus around the anterior part of the body to help them drift above the bottom to the next foraging site.

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