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Diversification of acorn worms (Hemichordata, Enteropneusta) revealed in the deep sea

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Enteropneusts (phylum Hemichordata), although studied extensively because of their close relationship to chordates, have long been considered shallow-water, burrowing animals. The present paper more than doubles the number of enteropneust species recorded in the deep sea based on high-resolution imaging and sampling with remotely operated vehicles. We provide direct evidence that some enteropneusts are highly mobile—using changes in posture and currents to drift between feeding sites—and are prominent members of deep, epibenthic communities. In addition, we provide ecological information for each species. We also show that despite their great morphological diversity, most deep-living enteropneusts form a single clade (the rediagnosed family Torquaratoridae) on the basis of rDNA sequences and morphology of the proboscis skeleton and stomochord. The phylogenetic position of the torquaratorids indicates that the group, after evolving from near-shore ancestors, radiated extensively in the deep sea.

Keywords: deep sea; epibenthic; demersal; Enteropneusta; Hemichordata; Torquaratoridae

1. INTRODUCTION

Hemichordates are a critical component in any study of early chordate evolution [1–3], yet relationships within the phylum remain unresolved [4,5], diversity is poorly known [6–9] and our perception of enteropneusts as coastal infauna needs revision. Other than a single species described from *Challenger* material [10], enteropneusts were unknown in the deep sea until 1965 when a specimen was photographed at the head of its distinctive faecal trace (but not collected) [11]. Over the next two decades, more than a dozen reports of similar animals were recorded based on variable quality still images (summarized by Smith *et al.* [12]). Subsequently, two deep-sea species were collected and described [6,13], and based on the former study, a new enteropneust family, Torquaratoridae, was tentatively established. In all, these photographs and collections included three distinct morphotypes in addition to the ones described, suggesting that substantial undocumented enteropneust diversity existed in the deep sea [6]. The feeding behaviour of an animal with the most common morphotype was described from time-lapse camera images [12] and the species was later described [7]; however, it could not be placed in a family because of the phylogenetic uncertainty within Enteropneusta. Here, we (i) show that enteropneusts are common and diverse in the deep sea, (ii) provide a phylogenetic framework for Hemichordata based on ribosomal DNA, (iii) show that some deep-sea enteropneusts use the water column to move between feeding sites (figure 1*a*–*c*), and (iv) provide individual species densities and habitat characteristics.

2. METHODS

(a) *Ecological studies and collections*

Observations were made with the Monterey Bay Aquarium Research Institute's (MBARI) remotely operated vehicles (ROVs) *Tiburon* and *Doc Ricketts* in the NE Pacific and Gulf of California, and with the UK National Oceanography Centre, Southampton's *Isis* ROV in the Atlantic, all equipped with high-definition cameras. Pacific observations were opportunistic in nature; enteropneusts were never the focus of the science being conducted and thus the locations tend to be areas of geological interest (i.e. seamounts, ridges or canyons). Likewise, biogeographic distributions shown for each species do not represent a systematic search, but instead are best representative of where the ROV has worked (both MBARI ROVs are operational to 4000 m depth; figure 2).

Quantitative estimates of animal density in the Pacific were obtained from 23 video transects in areas where enteropneusts were present using MBARI's ROVs equipped with paired lasers for scaling a field of view with 1 m width. Transects varied in length from 200 to 1600 m. In the Atlantic Ocean, similar surveys were performed at two stations north and two stations south of the Charlie Gibbs Fracture Zone

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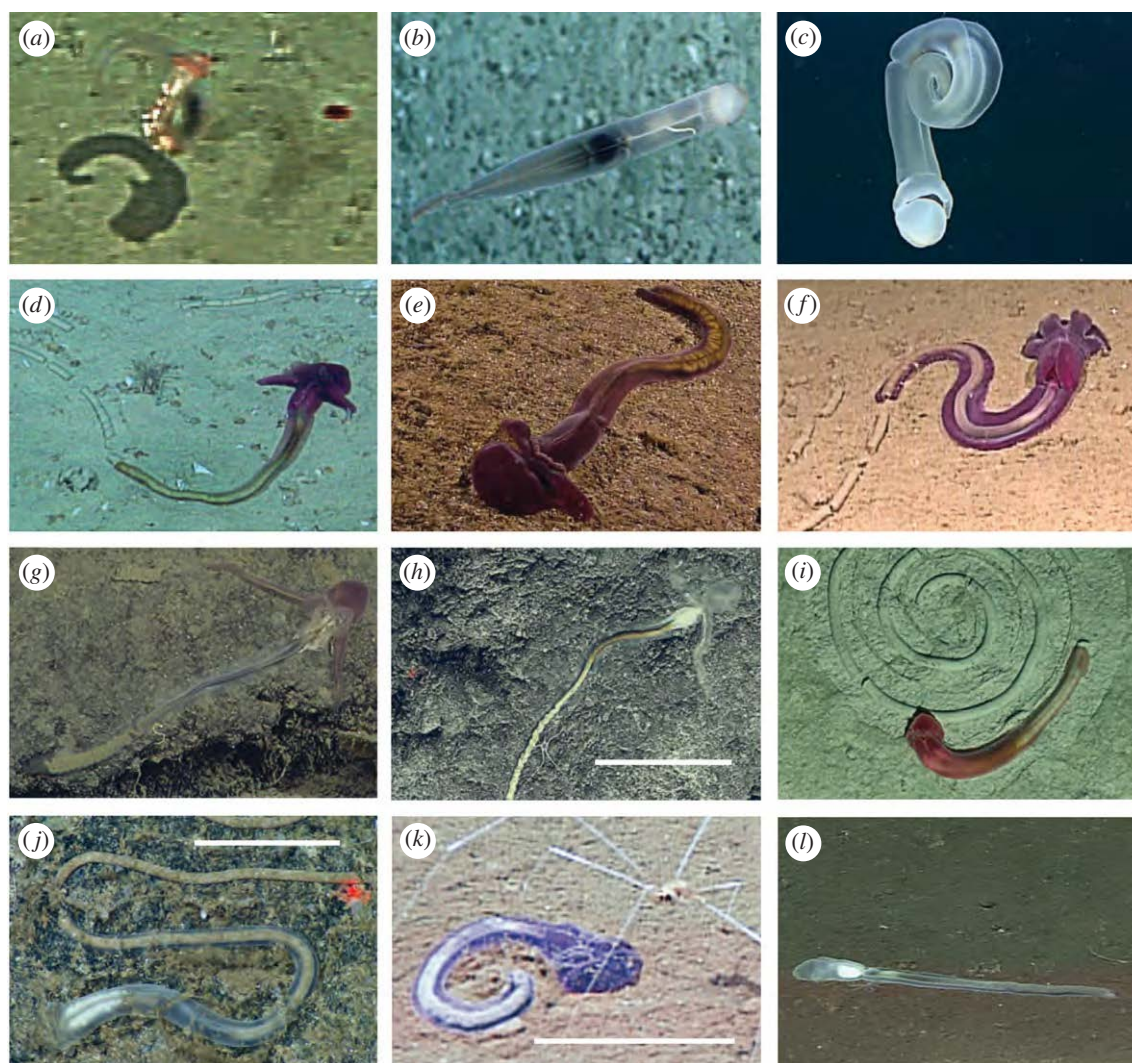
Figure 1. Video frame grabs of representative deep-sea torquaratorid enteropneusts *in situ*. (*a*) Genus D sp. 1, two shadows created by the lights of the remotely operated vehicle show that the animal is only a few centimetres above the sea floor. (*b*) Genus D sp. 2, animal shown just off the sea floor surface. (*c*) Genus D sp. 3 uncollected specimen, observed at least 20 m above the sea floor. (*d*) Genus A sp. 1. (*e*, *f*) Two uncollected species from the Celebes Sea. Images: NOAA Office of Ocean Exploration and Research. (*g*) Genus B sp. 1. (*h*) Genus B sp. 2. (*i*) *Tergivelum* sp. 1. (*j*) Genus C. (*k*,*l*) Two uncollected species from the Gulf of California, Torquaratoridae sp. 2 and 3. (*a*–*l*) Scale bars: 10 cm.

(figure 3) using the *Isis* ROV [14]. Density data are not yet available from these transects (D. Jones & C. Alt 2010, unpublished data). Observations and sampling on the Mid-Atlantic Ridge focused on three categories based on sea floor topography: flat plains ($0-2°$), slopes ($8-12°$) and cliffs (more than $30°$). Additional specimen-collecting dives were completed at each of the four main stations on the Mid-Atlantic Ridge.

Specimens were collected with suction samplers mounted on the ROVs. Upon recovery from the ROV, specimens were photographed, a tissue sample fixed in chilled 95 per cent ethanol and the remainder fixed in 4 to 20 per cent formalin.

MBARI's video archives were searched using the Video Annotation Reference System database [15] for all observations of enteropneusts. Each observation was reviewed, habitat and behaviours were recorded, and representative video frame grabs were taken. Ancillary data linked by video time code provided specific location and depth information for each observation.

(b) Phylogenetics

All available specimens were sequenced to confirm morphospecies designations and for inference of evolutionary relationships of all reliable hemichordate sequences. DNA extraction, amplification and sequencing methods varied in the following ways from those described by Osborn & Rouse [16]. In combination with AmpliTaq Gold Fast Master Mix (Life Technologies Corp., Carlsbad, CA), the following 18S primers were used for amplification and/or sequencing: 18e, 18h [17], 18p and 18qa [18]. All sequencing was carried out with the BigDye Terminator v. 3.1 sequencing kit and analysed on an ABI 3100 capillary sequencer (Life Technologies Corp.). JMODELTEST [19] was used for model choice. The 18S rDNA alignment consisted of 2012 bp and the 16S was 597 bp. Specimen information, GenBank and voucher accession numbers are given in electronic supplementary material, table S1.

3. RESULTS
(a) Ecology

We recorded 498 observations of deep, epibenthic enteropneusts off the west coast of North America, Hawai'i and in the Gulf of California from 2000 to the present by MBARI ROVs (table 1). In addition to *Torquarator bullocki* and
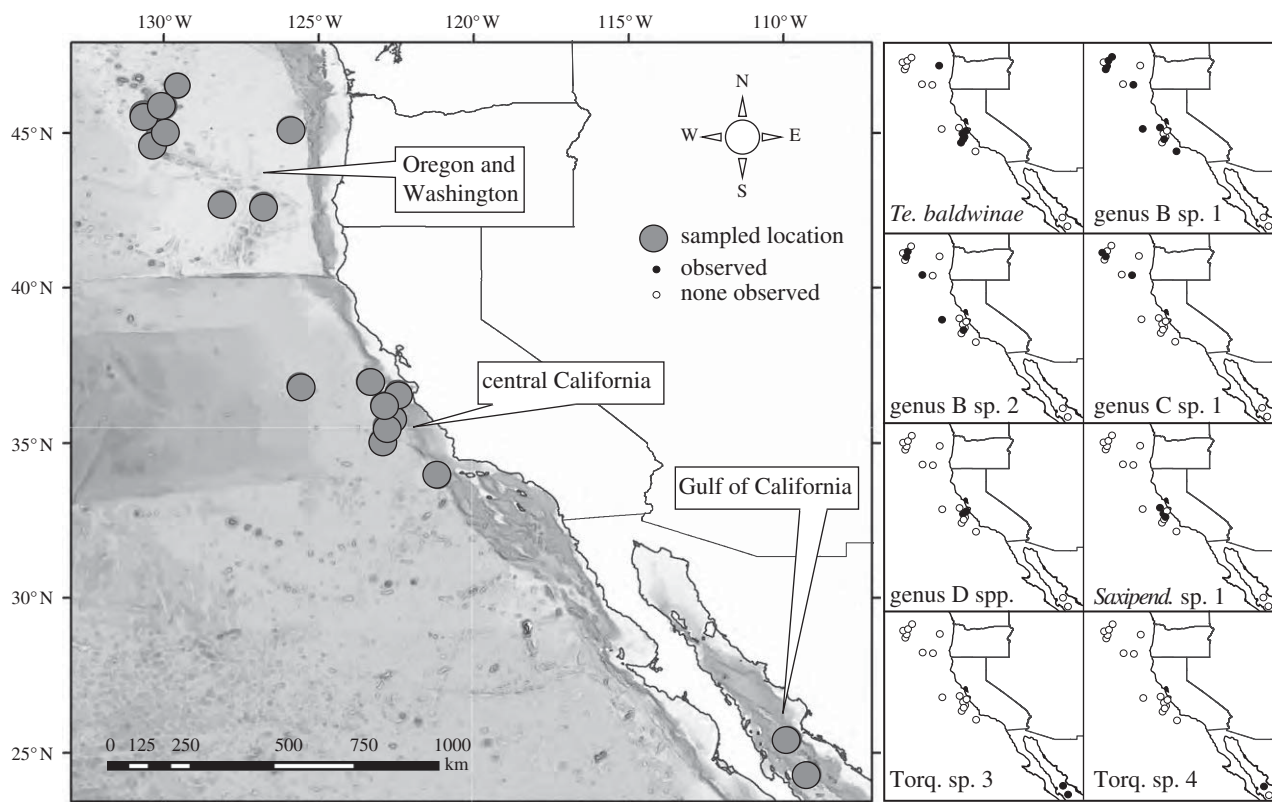
Figure 2. Northeast Pacific, showing locations of enteropneust observations by Monterey Bay Aquarium Research Institute's remotely operated vehicles and the location of the eight most abundant individual species.

Tergivelum baldwinae, at least nine species were identified from these videos (including those in figure 1a,c,g,h,j−l; electronic supplementary material, figure S2; and [6]). Morphologically distinct specimens recorded at depth but not collected will be designated here simply as Torquaratoridae sp. 1, sp. 2 and sp. 3. Pacific specimens were observed on sea floor that ranged from 1642 to 3954 m depth, with individual species ranges spanning as much as 1500 m depth (electronic supplementary material, figure S2; table 1). Atlantic exploration was restricted to sea floor approximately 2500 m deep. Individuals of most of the Atlantic and Pacific species were relatively sparsely distributed, although in certain observations one species (*Saxipendium* sp. 1) reached densities as high as five individuals per square metre. Average density was highly variable (0.1−9.5 individuals per 100 square metres) between locations and animals were patchily distributed along transects. Genus B sp. 1 (figure 1g) and *Te. baldwinae* were the most often observed in the Pacific, each with nearly twice the number of observations as any other species.

The eastern Pacific and Gulf of California observation sites for the deep-living enteropneusts are shown in figure 2 and ecological data are provided in table 1. Two species were observed only in the Gulf of California (Torquaratoridae sp. 2 and 3, shown in figure 1k and l, respectively), one only off Hawai'i (Torquaratoridae sp. 1 [6]), three only off central California (*Saxipendium* sp. 1, shown in electronic supplementary material, figure S1; genus D sp. 1 and 3, shown in figure 1a and c, respectively), and two only off Oregon and southern Washington (*To. bullocki*, and genus C sp. 1, shown in figure 1j). *Tergivelum baldwinae* and genus B sp. 1 and 2 (figure 1g and h, respectively) were observed off central California, Oregon and southern Washington.

We observed and collected three species on the Mid-Atlantic Ridge as part of the MAR-ECO project (figure 1b,d,i and table 1). A single specimen of genus D sp. 2 (figure 1b) was discovered drifting above the sea floor during a survey of flat sediment-covered plain at the SW station (figure 3). Genus A sp. 1 (figure 1d) predominated on flat and slope areas of the SE and SW stations (figure 3), and was observed rarely at the NE station. *Tergivelum* sp. 1 (figure 1i) was found both north and south of the Charlie Gibbs Fracture Zone, but only in low numbers at the SE and SW stations (figure 3). Enteropneusts or their traces were occasionally seen on small sediment-covered ledges in steep rocky areas of the Mid-Atlantic Ridge. They were not seen on vertical rock faces.

Exploration of the Celebes Sea off Indonesia using ROV *Little Hercules* allowed observation of two additional species (figure 1e,f). Gross morphological differences distinguish these specimens from others observed in the Pacific (collar shape, pigmentation of the posterior region, width of posterior lateral folds and plicate nature of posterior gut contents). Genetic differences distinguished morphologically similar Atlantic and Pacific species (1.4% uncorrected genetic distance for approx. 570 bp of 16S).

The extensive *in situ* observations allowed description of typical behaviour for these animals. Members of genus D were observed drifting in currents from just centimetres above the sea floor (figure 1a,b) to at least 20 m above the sea floor (figure 1c; electronic supplementary material, video S1). Various other torquaratorids were observed lifting their anterior bodies off the sea floor, suggesting that they also use the water column for long-distance movement. No drifting enteropneusts had material in
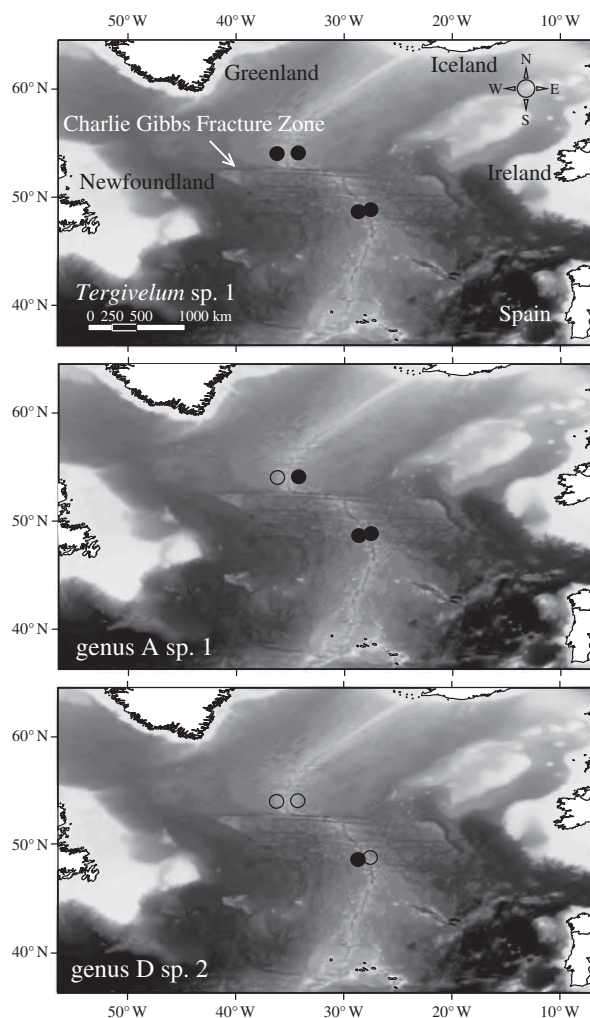
Figure 3. North Atlantic, showing the four stations explored by the *Isis* remotely operated vehicle (referred to in the text by their relative locations: NW, NE, SW and SE) and locations of observations/collections of each species along the Mid-Atlantic Ridge (filled circles, present; open circles, absent).

their guts (figure 1*a*−*c*) in contrast to the individuals observed on the sea floor, which had sediment-packed guts (figure 1*d*−*k*). Most torquaratorids are poorly muscularized, gelatinous and fragile in the extreme. Only the more robust genus D sp. 1 was observed with its posterior end inserted in the sediment.

Individual species were observed associated with specific habitat types (table 1). Most species were observed on the muddy sea floor, whereas four species were associated primarily with hard substrate. The number of observations was evenly split between the two substrate types within Pacific observations, but was predominantly on soft sediment in the Atlantic. Several species have eggs reaching 1.5 mm in diameter.

(b) Phylogenetics and systematics

All deep-sea enteropneusts considered here (excluding *Saxipendium coronatum* and a second *Saxipendium* species discovered during this project) were more closely related to each other than to other enteropneusts based on rDNA (figure 4), forming the clade Torquaratoridae. The close correspondence of rDNA sequences among the members of Torquaratoridae allowed identification

of morphological features uniquely characterizing the group. The diagnosis of Torquaratoridae is here amended to accommodate the increased diversity and to reflect the molecular phylogeny: enteropneusts whose proboscis skeleton is absent or reduced to a small medial plate and whose adult stomochord is either absent or separated from the buccal cavity of the collar.

Monophyly of Hemichordata, Torquaratoridae and Harrimaniidae was recovered with strong support, and that of Ptychoderidae with moderate support (figure 4). Pterobranchs, harrimaniids and a moderately supported clade consisting of spengelids, ptychoderids and torquaratorids formed a basal polytomy (figure 4). Deep-living enteropneusts (excluding the two *Saxipendium* species) fell within Torquaratoridae, the clade that our analyses strongly suggested was sister to Ptychoderidae.

Within Torquaratoridae, five clades were well supported by molecular data and gross morphology (figure 4; uncorrected genetic distance 3.5–13.5% between and 0–1.4% within generic-level clades for approx. 570 bp of 16S). We found sister taxa in the Atlantic and Pacific within two of the generic-level clades (probably three but no Pacific wide-lipped species were available for molecular analysis; figure 4).

4. DISCUSSION

(a) Ecology and natural history

Our observations of active drifting in deep-sea enteropneusts are the first to show that at least three species use demersal currents to move between locations not by chance, but in a controlled manner through changes in body posture. Further, our observations of empty guts in animals in the water column and recently settled, full guts in animals on the sea floor, and the time-lapse camera observations of *Te. baldwinae* emptying its gut completely before disappearing from a feeding site [12], suggest that gut contents (consisting of sea floor surface sediments) function in part as ballast. Using gut contents as ballast is also known from swimming sea cucumbers (Echinodermata, Holothuroidea) [20].

Circumstantial evidence suggests several of the other torquaratorids also drift in demersal currents. We observed animals lifting their anterior bodies off the sea floor, their feeding and faecal trails lack tracks leading to or away from them [11,12,21], and they secrete mucus [12] that may increase drag when in the water column (electronic supplementary material, video S1). Enteropneusts or their traces were occasionally seen on small sediment-covered ledges in steep rocky areas (but never on vertical rock faces) of the Mid-Atlantic Ridge, indicating that such topography is not a barrier to their movement. Based on these points, we hypothesize that movement between patches of suitable sediment-covered habitat is achieved by drifting in the water column. At least one shallow-water enteropneust swims seasonally [22–24] and there is one previous report of swimming by a provoked deep-sea specimen [25]. The ability to drift in the water column greatly increases individual ranges in comparison to shallow-water enteropneusts and may be an adaptation to limited or sporadic [26,27] food supply at depth.

While burrowing in the sediment or in crevices around rocks is the typical mode of life for shallow-water enteropneusts, many deep-sea species seem incapable of

Table 1. Collection, ecological and biogeographic information by species. ESM, electronic supplementary material; n.a., not available.

taxa	observed	collected	figure	biogeographic range	depth (m)	habitat	density (number per 100 square metres)
genus A (wide-lipped) sp. 1	272	4	1d	Mid-Atlantic Ridge	2227–2939	sediment	n.a.[b]
genus B (extra wide-lipped) sp. 1	155	5	1g	off S. Washington–central California	1712–3287	lava with sediment veneer	0.2–4.7
genus B (extra wide-lipped) sp. 2	9	3	1h	off S. Washington–central California	1674–2847	lava with sediment veneer	0.3
Tergivelum baldwinae	155	5	2–8 in [7]	off S. Washington–central California	2712–3954	sediment	0.1–9.5
Tergivelum sp. 1	92	4	1i	Mid-Atlantic Ridge	2227–2939	sediment	n.a.[b]
genus C (narrow-lipped) sp. 1	6	3	1j	off Oregon–Washington	2252–3124	lava with sediment veneer	0.3
genus D (plain-collared) sp. 1	8	2	1a	off central California	2891–3492	sediment/water column above sediment	n.a.
genus D (plain-collared) sp. 2	1	1	1b	Mid-Atlantic Ridge	2622	water column above sediment	n.a.
genus D (plain-collared) sp. 3[a]	2	0	1c	off central California	3460–3488	water column, more than 20 m above sea floor	n.a.
Torquaratoridae sp. 1[a]	1	0	3d in [6]	off Hawai'i	3035	lava with sediment veneer	n.a.
Torquaratoridae sp. 2[a]	6	0	1k	Gulf of California	2892–3177	sediment	n.a.
Torquaratoridae sp. 3[a]	31	0	1l	Gulf of California	2851–3167	sediment	n.a.
Torquarator bullocki[a]	10	1	1a–b in [6]	off Oregon–Washington	1643–1901	sediment near hard substrate	n.a.
Saxipendium sp. 1	92	1	S1 (ESM)	off central California	1774–3156	sediment ponds near hard substrate	n.a.
unidentifiable[a]	23	0	—	—	—	—	up to 2.0

[a]Not included in figure 4 because no tissue was available for genetics.
[b]D. Jones & C. Alt 2010, unpublished data, National Oceanographic Centre, Southampton UK.

Figure content (phylogenetic tree) — taxon labels:

0.2

IFREMER Enteropneust
*wide-lipped I171-36a
*wide-lipped I171-36b
*wide-lipped I174-43a — sp. 1 — genus A
extra-wide-lipped T879-A8
extra-wide-lipped T879-A10
extra-wide-lipped D176-A1
extra-wide-lipped D176-A5
extra-wide-lipped D176-A2 — sp. 1 — genus B
extra-wide-lipped T1013-A8
extra-wide-lipped T1011
extra-wide-lipped D177-A28 — sp. 2
Tergivelum sp. 1 I163-17
Tergivelum sp. 1 I165-24
Tergivelum sp. 1 I168-28 — sp. 1
Tergivelum baldwinae T1094
Tergivelum baldwinae T1076-2
Tergivelum baldwinae T1076-1
Tergivelum baldwinae T1078-1 — *Tergivelum*
narrow-lipped T886-A4
narrow-lipped T1012-A8
narrow-lipped D80-A2 — genus C
plain-collared T438
plain-collared D98-pc66 — sp. 1
*plain-collared I174-43b — sp. 2 — genus D

no proboscis skeleton — Torquaratoridae

'Ptychoderid sp. Tampa'
Balanoglossus carnosus
Balanoglossus clavigerus
Ptychodera flava a
Ptychodera flava b
Glossobalanus berkeleyi
Ptychodera bahamensis
Glossobalanus minutus — Ptychoderidae
'Tornaria larva'
Glandiceps hacksi — Spengelidae
Saccoglossus kowalevski
Saccoglossus bromophenolosus
Saccoglossus pusillus
Saxipendium coronatum a
Saxipendium coronatum b
Saxipendium sp. 1 D176-A11
Harrimania planktophilus
Protoglossus koehleri — Harrimaniidae
Rhabdopleura normani — Pterobranchia
Cephalodiscus hodgsoni
Cephalodiscus nigrenscens
Cephalodiscus densus
Cephalodiscus gracilis — Pterobranchia
Parastichopus californicus
Aspidodiadema jacobi
Asterias forbesii
Ophioderma brevisinum
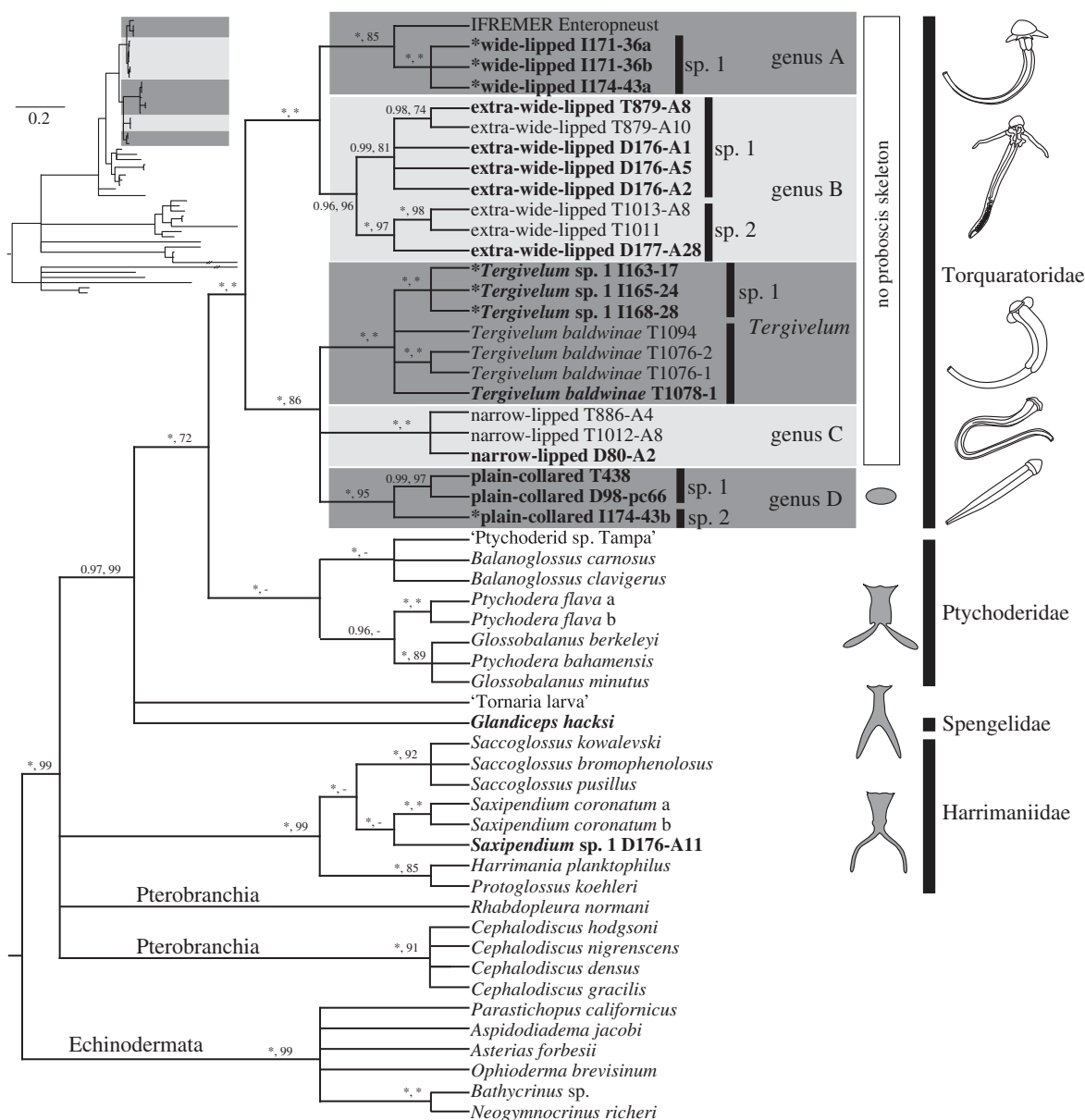*Bathycrinus* sp.
Neogymnocrinus richeri — Echinodermata

Figure 4. Phylogenetic analysis of phylum Hemichordata based on rDNA; shaded parts of the tree indicate a clade of deep-sea enteropneusts comprising the family Torquaratoridae. Ninety per cent majority rule consensus tree from the Bayesian analysis (50 million generations) of complete 18S and partial 16S concatenated, but unlinked nucleotide sequences. Support is indicated for each branch as posterior probabilities/bootstraps from the parsimony analysis (equally weighted, heuristic search, tree bisection and reconnection branch swapping, 1000 random addition replicates): asterisks on branches indicate 1.0 or 100% support, respectively; unsupported branches were collapsed. Phylogram given in upper left to show relative branch lengths. Drawings show major morphological differences between generic-level clades and the shape of the proboscis skeleton. Asterisks in front of names indicate collection from the Mid-Atlantic Ridge, while bold species names indicate new sequences. IFREMER enteropneust, 'Tornaria larva' and 'Ptychoderid sp. Tampa' are from Cannon *et al.* [5]. Scale bar, 0.2.

burrowing and instead live on the sea floor surface (figure 1). Because most torquaratorids have minimal musculature, are gelatinous and are extremely fragile (possible adaptations to limited high-quality food in the deep sea and/or use of the water column to move about [28]), it seems unlikely that they are capable of burrowing. Torquaratorids appear to be adapted to feeding on the sea floor surface and these adaptations are particularly obvious in the case of the extra-wide-lipped species (figure 1g,h) that were found almost exclusively on deep-sea lava formations that have only a fine layer of sediment dusting the surface. While these pillow lava formations are near intermittent sediment ponds, animals were not observed feeding in the ponds, but were instead consistently observed on the rocks themselves. On the other hand, broad-collared and wide-lipped species were observed either on mud or hard substrate, but the same species was not observed on both substrates. This partitioning of species between habitats suggests that these intermediate and more common morphologies (wide-lipped and broad-collared) allow animals to survive on either type of habitat.

As with most deep-sea animal distributions, it is not possible to define complete geographical ranges because so little area of the deep-sea floor has been surveyed and the exploration sites are not chosen with systematic coverage in mind. On the Mid-Atlantic Ridge, the zone around the Charlie Gibbs Fracture Zone has been shown to be associated with a faunal divide between

species north of the sub-polar front, where there is high seasonal surface productivity, and more oligotrophic conditions in the south [29]. Genus A sp. 1 and *Tergivelum* sp. 1 occur both north and south of the sub-polar front; however, the former is more common in the southern oligotrophic conditions and is very rare north of the front, while *Tergivelum* sp. 1 is common below more productive and cooler waters north of the front, and occurs in low numbers in the south. The two most commonly observed Pacific torquaratorids were also the most widespread (*Te. baldwinae* and genus B sp. 1; figure 2); however, genus B sp. 2 was also widespread with only eight observations. Genetics confirmed the identity of specimens from the northern and southern reaches of the ranges for both genus B species (electronic supplementary material, table S1). Finding this rare species across a wide geographical range suggests that even biogeographic ranges reported for our rarely observed species are good indicators of how widespread the individual species are within the explored area. Similarly, the even division of the number of observations on hard and soft substrate within the Pacific is probably a factor of the large proportion of time spent exploring areas dominated by hard substrate and may not be a good representation of the typical habitat of deep-sea torquaratorids.

Tergivelum is a morphologically distinct genus because of the 'back veils', dark pigmentation, proboscis and collar shapes, and thus identification of the genus from still images or video is straightforward. In addition to the observations reported here (figure 2), members of the genus have been reported from the western Pacific [6], both the eastern and western continental margin of Australia [30], and the NE Atlantic directly west of Brest, France (P. Tyler 2011, personal communication). Specimens were not available from the northern portion of the observed Pacific range, so identity could not be genetically confirmed as *Te. baldwinae* for the northern observations. However, based on the lack of genetic divergence found within the two other species with similar biogeographic ranges in the Pacific basin (genus B sp. 1 and 2), we would not expect two *Tergivelum* species in the north Pacific. Genetic assessment will be crucial for identification of the diversity within the genus as specimens are eventually collected from the rest of their range.

The four torquaratoid species observed only off central California are the most likely to have significantly wider biogeographic ranges than reported here. MBARI's location within this area means the area is more thoroughly explored than most deep-sea areas and thus we are more likely to have observed animals here than in other parts of their ranges. The number of observations we have accumulated (table 1), coupled with assessment of the genetic diversity within the group, provides a baseline of ecological information and an evolutionary framework for further study of deep-sea enteropneust biology.

The mature eggs of genus D sp. 1 (1.5 mm) are some of the largest invertebrate eggs, surpassed only by various cephalopods whose eggs reach nearly 42 mm in their longest dimension [31]. Large egg size could indicate that these species are direct developers, like many harrimaniid species, which also have large eggs (up to 1.2 mm). Large eggs in torquaratorids contrast with the small eggs of most ptychoderids and spengelids, which pass through a tornaria larva stage. Alternatively, it is tempting to speculate that the exceptionally large eggs of torquaratorids might develop into the exceptionally large planctosphaera larvae (up to 28 mm [32]), which have never been traced to an adult stage and are widespread [32–34]. Hadfield & Young [32] tantalizingly suggested that these enigmatic larvae are part of the life history of an abyssal enteropneust. Appropriate fixation and sequencing of a planctosphaera would almost certainly solve this long-standing mystery now that enteropneust diversity is well represented in public databases.

(b) Evolutionary history

Phylogenetic analyses of the hemichordates as a whole did not recover the monophyly of the pterobranchs, contrasting slightly with two previous analyses that found weak support for monophyly of the group [5,35]. Resolution of pterobranch relationships requires further sampling of spengelids and pterobranchs, and does not pertain directly to the question at hand—that of the relationships of the deep-living enteropneusts to previously known enteropneusts.

Our analyses found Torquaratoridae sister to Ptychoderidae, not nested within it, as was recently suggested [5]; thus the family is now firmly established within Enteropneusta. The continued inability to collect tissue from the type genus and species for Torquaratoridae is problematic, but it is likely that when finally sequenced *To. bullocki* will fall within the Torquaratoridae as defined here based on the reduced proboscis skeleton and stomochord, broad collar and proboscis, and deep-living, epibenthic habit. *Torquarator*, *Tergivelum* and the four as yet undescribed genera (A–D in figure 4) show the wide range of morphological diversity within the family. This diversity is fairly unusual within the Enteropneusta, where fine details of internal anatomy are typically required to distinguish species and sometimes even higher taxa [9]. The rediagnosis and phylogenetic framework provided here make it possible to now describe six new species and three new genera for which high-quality specimens are available.

Our phylogenetic analyses further suggested that torquaratorids and ptychoderids do not represent the ancestral hemichordate form, as suggested by preliminary findings based on central nervous system development [36]. This has implications for the evolutionary history and origins of the clade. Many marine invertebrate higher taxa (families and above) originated in shallow water and later spread to deep water, a trend consistently found throughout the Palaeozoic [37]. These findings were based on examination of the fossil records for well-preserved taxa (crinoids, corals, brachiopods, echinoids, sponges, molluscs and bryozoans) compared with poorly preserved ones, which were expected to have random patterns owing to inconsistent preservation. The phylogenetic position of the torquaratorid enteropneusts leaves little doubt that they conform to this trend and lends support to this idea from a poorly preserved group. Torquaratorids' oldest reliable fossil suggests their presence in the deep sea since the Early Triassic [38]. The phylogenetic position of the torquaratorid enteropneusts suggests they are an example of a deep-water clade that has abandoned its shallow-water beginnings to occur exclusively in the deep sea.

Our findings show that we have probably only scratched the surface of deep-sea enteropneust diversity.

Prior to the present addition of at least 13 undescribed species (seven torquaratorids and one harrimanid from MBARI observations, three torquaratorids from *ISIS* ROV observations in the Atlantic and two torquaratorids from *Little Hercules* observations in the Celebes Sea), only 89 enteropneust species were described. This work shows the value of exploration and direct observation in order to understand the diversity, ecology and evolutionary processes of the deep ocean, particularly the demersal communities that have been missed by historical observing and sampling methods.

REFERENCES

1 Lowe, C. J. *et al.* 2006 Dorsoventral patterning in hemichordates: insights into early chordate evolution. *PLoS Biol.* **4**, 1603–1619.

2 Brown, F. D., Prendergast, A. & Swalla, B. J. 2008 Man is but a worm: chordate origins. *Genesis* **46**, 605–613. (doi:10.1002/dvg.20471)

3 Nomaksteinsky, M., Röttinger, E., Dufour, H., Chettouh, Z., Lowe, C. J., Martindale, M. & Brunet, J. 2009 Centralization of the deuterostome nervous system predates chordates. *Curr. Biol.* **19**, 1264–1269. (doi:10.1016/j.cub.2009.05.063)

4 Cameron, C. B. 2005 A phylogeny of the hemichordates based on morphological characters. *Can. J. Zool.* **83**, 196–215. (doi:10.1139/z04-190)

5 Cannon, J. T., Rychel, A. L., Eccleston, H., Halanych, K. L. & Swalla, B. J. 2009 Molecular phylogeny of Hemichordata, with updated status of deep-sea enteropneusts. *Mol. Phylogenet. Evol.* **52**, 17–24. (doi:10.1016/j.ympev.2009.03.027)

6 Holland, N. D., Clague, D. A., Gordon, D. P., Gebruk, A., Pawson, D. L. & Vecchione, M. 2005 'Lophenteropneust' hypothesis refuted by collection and photos of new deep-sea hemichordates. *Nature* **434**, 374–376. (doi:10.1038/nature03382)

7 Holland, N. D., Jones, W. J., Ellena, J., Ruhl, H. A. & Smith, K. L. J. 2009 A new deep-sea species of epibenthic acorn worm (Hemichordata, Enteropneusta). *Zoosystema* **31**, 333–346. (doi:10.5252/z2009n2a6)

8 Cameron, C. B., Deland, C. & Bullock, T. H. 2010 A revision of the genus *Saccoglossus* (Hemichordata: Enteropneusta: Harrimaniidae) with taxonomic descriptions of five new species from the Eastern Pacific. *Zootaxa* **2483**, 1–22.

9 Deland, C., Cameron, C. B., Rao, K. P., Ritter, W. E. & Bullock, T. H. 2010 A taxonomic revision of the family Harrimaniidae (Hemichordata: Enteropneusta) with descriptions of seven species from the Eastern Pacific. *Zootaxa* **2408**, 1–30.

10 Spengel, J. W. 1893 *Enteropneusten des Golfes von Neapel. Fauna und Flora des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Herausgegeben von der Zoologischen Station zu Neapel.* Berlin, Germany: Friedländer.

11 Bourne, D. W. & Heezen, B. C. 1965 A wandering enteropneust from the abyssal Pacific, and the distribution of 'spiral tracks' on the sea floor. *Science* **150**, 60–63. (doi:10.1126/science.150.3692.60)

12 Smith, K. L. J., Holland, N. D. & Ruhl, H. A. 2005 Enteropneust production of spiral fecal trails on the deep-sea floor observed with time-lapse photography. *Deep Sea Res. I* **52**, 1228–1240. (doi:10.1016/j.dsr.2005.02.004)

13 Woodwick, K. H. & Sensenbaugh, T. 1985 *Saxipendium coronatum*, new genus, new species (Hemichrodata: Enteropneusta): the unusual spaghetti worms of the Galapagos hydrothermal vents. *Proc. Biol. Soc. Wash.* **98**, 351–365.

14 Jones, D., Alt, C., Gebruk, A., Rogacheva, A. & Gooday, A. 2010 ROV video transects. In *RSS James Cook cruise 048. ECOMAR ecosystem of the mid-Atlantic ridge at the sub-polar front and Charlie Gibbs fracture zone* (eds I. G. Priede & P. M. Bagley), pp. 65–80. Aberdeen, UK: Oceanlab, University of Aberdeen.

15 Schlining, B. & Jacobsen Stout, N. 2006 MBARI's Video Annotation Reference System. In *Institute of Electrical and Electronics Engineers Oceans Conference.* Boston, MA: Proceedings of the Marine Technology Society. (doi:10.1109/OCEANS.2006.306879)

16 Osborn, K. J. & Rouse, G. W. 2011 Phylogenetics of Acrocirridae and Flabelligeridae (Cirratuliformia, Annelida). *Zool. Scripta* **40**, 204–219.

17 Hillis, D. M. & Dixon, M. T. 1991 Ribosomal DNA: molecular evolution and phylogenetic inference. *Q. Rev. Biol.* **66**, 410–453.

18 Halanych, K. M., Bacheller, J. D., Aguinaldo, A. M. A., Liva, S. M., Hillis, D. M. & Lake, J. A. 1995 Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science* **267**, 1641–1643. (doi:10.1126/science.7886451)

19 Posada, D. 2008 jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**, 1253–1256. (doi:10.1093/molbev/msn083)

20 Miller, J. E. & Pawson, D. L. 1990 Swimming sea cucumbers (Echinodermata: Holothuroidea): a survey, with analysis of swimming behavior in four bathyal species. *Smithsonian Contr. Mar. Sci.* **35**, 1–18.

21 Heezen, B. C. & Hollister, C. D. 1971 *The face of the deep.* New York, NY: Oxford University Press.

22 Ikeda, I. 1908 On the swimming habit of a Japanese enteropneust, *Glandiceps hacksi* Marion. *Annot. Zool. Jpn.* **6**, 255–257.

23 Spengel, J. W. 1909 Pelagisches Vorkommen von Enteropneusten. *Zoolgischer Anzeiger* **34**, 54–59.

24 Yoshimatu, S. & Nishikawa, T. 1999 Swimming swarms of a usually benthic enteropneust. *Zool. Sci.* **16**(Suppl.), 39.

25 Gaillard, C. 1991 Recent organism traces and ichnofacies on the deep-sea floor off New Caledonia, Southwestern Pacific. *Palaios* **6**, 302–315. (doi:10.2307/3514910)

26 Billett, D. S. M., Lampitt, R. S., Rice, A. L. & Mantoura, R. F. C. 1983 Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature* **302**, 520–522. (doi:10.1038/302520a0)

27 Beaulieu, S. E. & Smith, K. L. 1998 Phytodetritus entering the benthic boundary layer and aggregated on the sea floor in the abyssal NE Pacific: macro- and microscopic composition. *Deep Sea Res. II* **45**, 781–815. (doi:10.1016/S0967-0645(98)00003-4)

28 Kozlow, T. 2007 *The silent deep: the discovery, ecology, and conservation of the deep sea.* Chicago, IL: University of Chicago Press.

29 Gebruk, A., Budaeva, N. A. & King, N. J. 2010 Bathyal benthic fauna of the Mid-Atlantic Ridge between the Azores and the Reykjanes Ridge. *J. Mar. Biol. Assoc. UK* **90**, 1–14. (doi:10.1017/S0025315409991111)

30 Anderson, T. J., Przeslawski, R. & Tran, M. 2011 Distribution, abundance and trail characteristics of acorn worms at Australian continental margins. *Deep Sea Res. II* **58**, 970–978. (doi:10.1016/j.dsr.2011.06.005)

31 Allcock, A. L., Hochberg, F. G. & Stranks, T. N. 2003 Re-evaluation of *Graneledone setebos* (Cephalopoda: Octopodidae) and allocation to the genus *Megaleledone*. *J. Mar. Biol. Assoc. UK* **83**, 319–328. (doi:10.1017/S0025315403007148h)

32 Hadfield, M. G. & Young, R. E. 1983 Planctosphaera (Hemichordata: Enteropneusta) in the Pacific Ocean. *Mar. Biol.* **73**, 151–153. (doi:10.1007/BF00406882)

33 Scheltema, R. S. 1970 Two new records of *Planctosphaera* larvae (Hemichordata: Planctosphaeroidea). *Mar. Biol.* **7**, 47–48. (doi:10.1007/BF00346807)

34 Hart, M. W., Miller, R. L. & Madin, L. P. 1994 Form and feeding mechanism of a living *Planctosphaera pelagica* (phylum Hemichordata). *Mar. Biol.* **120**, 521–533. (doi:10.1007/BF00350072)

35 Cameron, C. B., Garey, J. R. & Swalla, B. J. 2000 Evolution of the chordate body plan: new insights from phylogenetic analyses of deuterostome phyla. *Proc. Natl. Acad. Sci. USA* **97**, 4469–4474. (doi:10.1073/pnas.97.9.4469)

36 Luttrell, S., Bengtsson, B. C. & Swalla, B. J. 2010 Central nervous system development and regeneration in hemichordates. *Integr. Comp. Biol.* **50**(Suppl. 1), e105.

37 Jablonski, D. 2005 Evolutionary innovations in the fossil record: the intersection of ecology, development, and macroevolution. *J. Exp. Zool.* **304B**, 504–519. (doi:10.1002/jez.b.21075)

38 Twitchett, R. J. 1996 The resting trace of an acorn-worm (class: Enteropneusta) from the lower Triassic. *J. Paleontol.* **70**, 128–131.

Table S1. Specimen and associated sequence identification.

	18S	*16S*	voucher	locality
Torquaratoridae				
Genus A sp. 1 (Wide-lipped I171-36a)	**JN886757**	**JN886740**	**NHMUK 2011.5**	48° 43' N, 28° 39' W
Genus A sp. 1 (Wide-lipped I171-36b)	**JN886758**	**JN886741**	**NHMUK 2011.6**	48° 43' N, 28° 39' W
Genus A sp. 1 (Wide-lipped I174-43a)	**JN886759**	**JN886742**	**NHMUK 2011.9**	48° 43' N, 28° 39' W
Genus B sp. 1 (Extra wide-lipped T879-A8)	**JN886760**	EU520500	SIO-BIC H11 [1]	45° 08' N, 130° 08' W
Genus B sp. 1 (Extra wide-lipped T879-A10)	EU520513	EU520501	SIO-BIC H12 [1]	45° 08' N, 130° 08' W
Genus B sp. 1 (Extra wide-lipped D176-A1)	**JN886761**	**JN886744**	**SIO-BIC H15**	36° 50' N, 125° 34' W
Genus B sp. 1 (Extra wide-lipped D176-A5)	**JN886762**	**JN886745**	**SIO-BIC H16**	36° 50' N, 125° 34' W
Genus B sp. 1 (Extra wide-lipped D176-A2)	**JN886763**	**JN886746**	**SIO-BIC H17**	36° 50' N, 125° 34' W
Genus B sp. 2 (Extra wide-lipped T1013-A8)	EU520514	EU520502	SIO-BIC H13 [1]	45° 27' N, 130° 30' W
Genus B sp. 2 (Extra wide-lipped T1011)	EU520515	EU520503	SIO-BIC H14 [1]	45° 25' N, 130° 27' W
Genus B sp. 2 (Extra wide-lipped D177-A28)	**JN886764**	**JN886747**	**SIO-BIC H18**	36° 51' N, 125° 37' W
IFREMER Enteropneust [2]	EU728438	EU728431	na	13° N on the EPR [2]
Genus C sp. 1 (Narrow-lipped T886-A4)	EU520511	EU520499	SIO-BIC H10 [1]	42° 41' N, 126° 47' W
Genus C sp. 1 (Narrow-lipped T1012-A8)	EU520510*	EU520498	SIO-BIC H9 [1]	45° 30' N, 130° 40' W
Genus C sp. 1 (Narrow-lipped D80-A2)	**JN886768**	**JN886751**	**SIO-BIC H19**	45° 02' N, 130° 10' W
Genus D sp. 1 (Plain-collared T438)	**JN886765**	**JN886748**	**SIO-BIC H20**	36° 34' N, 122° 29' W
Genus D sp. 1 (Plain-collared D98-pc66)	**JN886767**	**JN886750**	**na**	36° 37' N, 122° 26' W
Genus D sp. 2 (Plain-collared I174-43b)	**JN886766**	**JN886749**	**NHMUK 2011.7**	48° 43' N, 28° 39' W
Tergivelum baldwinae (T1094)	EU520509	EU520497	SIO-BIC H8 [1]	35° 08-10' N, 123° 01' W
Tergivelum baldwinae (T10762)	EU520508*	EU520496	MNHN E23 [1]	35° 08-10' N, 123° 01' W
Tergivelum baldwinae (T10761)	EU520506*	EU520494	SIO-BIC H6 [1]	35° 08-10' N, 123° 01' W
Tergivelum baldwinae (T10781)	**JN886772**	EU520495	MNHN E24 [1]	35° 08-10' N, 123° 01' W
Tergivelum sp. 1 (I163-17)	**JN886769**	**JN886752**	**NHMUK 2011.2**	53° 59' N, 36° 11' W
Tergivelum sp. 1 (I165-24)	**JN886770**	**JN886753**	**NHMUK 2011.3**	54° 1' N, 34° 9' W
Tergivelum sp. 1 (I168-28)	**JN886771**	**JN886754**	**NHMUK 2011.4**	54° 1' N, 34° 10' W
Ptychoderidae				
Balanoglossus carnosus	D14359	na		
Balanoglossus clavigerus	na	EU728425		
Glossobalanus berkeleyi	EU728435	EU728426		
Glossobalanus minutus	AF119089	na		
Ptychodera bahamensis	AF236802	na		
Ptychodera flava a	AF278681	EU728428		
Ptychodera flava b [2]	EU728436	EU728429		
"Ptychoderid sp. Tampa" [2]	AF278685	EU728427		
Unknown Family				
"Tornaria larva" [2]	EU728438	EU728430		
Spengelidae				
Glandiceps hacksi	**JN886773**	**JN886755**	**HUM-HEMI-001**[3]	34° 18' N, 132° 59' E
Harrimaniidae				
Harrimania planktophilus	AF236799	EU728421		
Protoglossus koehleri	EU728432	EU728420		
Saccoglossus bromophenolosus	AF236801	na		
Saccoglossus kowalevskii	L28054	na		
Saccoglossus pusillus	AF236800	EU728422		
Saxipendium coronatum [2]	EU728433	EU728423		
Saxipendium coronatum [1]	EU520505*	EU520493		
Saxipendium sp. 1 (D176-A11)	**JN886774**	**JN886756**	**SIO-BIC H21**	36° 51' N, 125° 33' W
Pterobranchia				
Cephalodiscus densus	EU728439	na		
Cephalodiscus gracilis	AF236798	na		
Cephalodiscus hodgsoni	EU728441	na		
Cephalodiscus nigrescens	EU728440	na		
Rhabdopleura normani	U15664 *	na		
Echinodermata				
Asteriodea, *Asterias forbesii*	DQ060776	DQ297073		
Crinoidea, *Bathycrinus* sp.	AY275891	na		
Crinoidea, *Neogymnocrinus richeri*	AY275895	na		
Echinoidea, *Aspidodiadema jacobi*	DQ073780	DQ073734		
Holothuroidea, *Parastichopus californicus*	DQ777084	DQ777096		

*partial sequence

[1]Holland et al. 2009

[2]Cannon et al. 2009

[3]Sample is a paragenophore, while all other vouchers noted here are hologenophores (Pleijel, F. et al. 2008. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* 48, 369-371).

Figure S1. Saxipendium sp. 1 in situ. The animal is twisted such that the ventral collar and proboscis are visible and the posterior region lies in coils. Fecal material surrounds the animal.
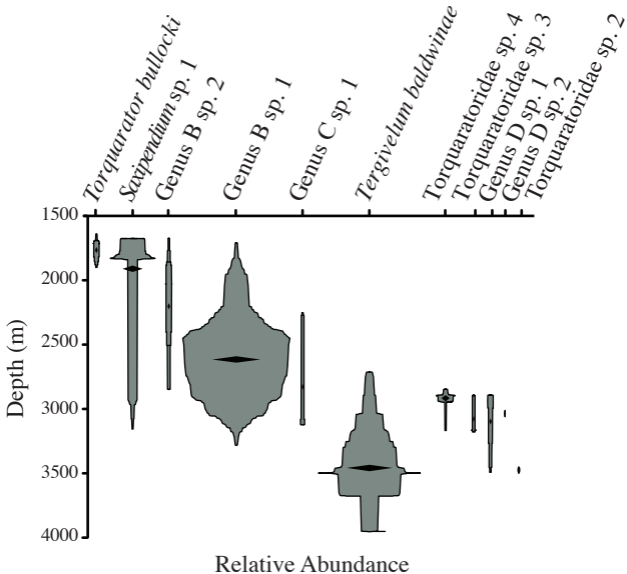
Figure S2. Depth of observations for each NE Pacific species showing relative abundance at each depth. Black diamonds indicate mean depth of observation. Species are ordered from shallow to deep by shallowest observation.