Report

Phylogenomic Resolution of the Hemichordate and Echinoderm Clade

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Summary

Ambulacraria, comprising Hemichordata and Echinodermata [1], is closely related to Chordata, making it integral to understanding chordate origins and polarizing chordate molecular and morphological characters [2–4]. Unfortunately, relationships within Hemichordata and Echinodermata have remained unresolved [1, 5–10], compromising our ability to extrapolate findings from the most closely related molecular and developmental models outside of Chordata (e.g., the acorn worms *Saccoglossus kowalevskii* and *Ptychodera flava* and the sea urchin *Strongylocentrotus purpuratus*). To resolve long-standing phylogenetic issues within Ambulacraria, we sequenced transcriptomes for 14 hemichordates as well as 8 echinoderms and complemented these with existing data for a total of 33 ambulacrarian operational taxonomic units (OTUs). Examination of leaf stability values revealed rhabdopleurid pterobranchs and the enteropneust *Stereobalanus canadensis* were unstable in placement; therefore, analyses were also run without these taxa. Analyses of 185 genes resulted in reciprocal monophyly of Enteropneusta and Pterobranchia, placed the deep-sea family Torquaratoridae within Ptychoderidae, and confirmed the position of ophiuroid brittle stars as sister to asteroid sea stars (the Asterozoa hypothesis). These results are consistent with earlier perspectives concerning plesiomorphies of Ambulacraria, including pharyngeal gill slits, a single axocoel, and paired hydrocoels and somatocoels [1, 4, 11]. The resolved ambulacrarian phylogeny will help clarify the early evolution of chordate characteristics and has implications for our understanding of major fossil groups, including graptolites and somasteroideans.

Results and Discussion

Over the last decade, phylogenomic studies have radically shifted our understanding of deuterostome evolutionary history. Although debates surround chordate interrelationships [2, 4, 12] as well as the positions of the controversial flatworm-like Xenoturbellida [2, 13] and Acoelomorpha [13], Ambulacraria (Hemichordata + Echinodermata) has been consistently recovered [2, 4, 13]. Relationships within these two phyla, however, have remained unresolved, and numbers of sampled hemichordate taxa in phylogenomic studies to date have been limited. Within Hemichordata, there is dispute as to whether colonial, tube-dwelling pterobranchs are sister to, or nested within, vermiform enteropneusts [1, 5–7, 14]. Placement of pterobranchs has major implications for understanding origins and early evolution of graptolite hemichordates, which have an extensive fossil record [15]. Furthermore, there are conflicting hypotheses regarding the position of ophiuroid brittle stars within echinoderms. The Cryptosyringida hypothesis places ophiuroids as sister to Echinozoa (Echinoidea + Holothuroidea) [9, 10], whereas the Asterozoa hypothesis places ophiuroids and asteroids in a clade sister to Echinozoa [8]. Asterozoa has recently been supported by two phylogenomic studies addressing echinoderm [16] and ophiuroid [17] relationships. These two hypotheses offer differing interpretations of larval and adult morphological evolution, as well as whether somasteroid fossils are direct ancestors of Asterozoa. Resolving these relationships is integral to our ability to infer plesiomorphic states for echinoderm and hemichordate characters, as well as the ancestral conditions of Ambulacraria and Deuterostomia overall.

In order to directly address ambulacrarian interrelationships, we combined existing data with novel transcriptomes for all hemichordate families and all echinoderm classes except Echinoidea (for which data were already available). Summary information for taxa and sequence data used herein are provided in Tables S1 and S2 available online. We generated four partitioned data matrices (Table 1) to assess effects of missing data on the resultant topology. The initial matrix was the largest and included 299 ortholog groups (OGs), 107,081 amino acids, and all 33 operational taxonomic units (OTUs) (abbreviated hereafter as the 299/33 matrix) with 65.59% missing data. We used PhyloTreePruner [19] to remove potentially paralogous groups (see Supplemental Experimental Procedures), reducing the number of orthology groups to 185 and the number of amino acid positions to 70,446. Two data matrices were based on this 185 OG set: the 185/33 matrix including all 33 OTUs with 58.45% missing data, and the 185/31 matrix, for which *Stereobalanus canadensis* (Enteropneusta, Harrimaniidae) and *Rhabdopleura* (Pterobranchia), which had poor leaf stability and taxonomic instability index values (lsi/tii = 0.90/613.9 and lsi/tii = 0.83/828.2, respectively; Table S3), were removed. This latter matrix had 55.91% missing data. Lastly, to reduce the proportion of missing data, we used MARE [18] to generate a strictly filtered alignment of 162 OGs, 61,597 amino acids, and the 20 most deeply sequenced taxa (Table 1; Experimental Procedures). This matrix had only 35.29% missing data. Maximum-likelihood (ML) analyses were conducted for all data sets, and Bayesian inference was conducted on the 185/31 data set.

Branching patterns of major ambulacrarian clades obtained from all data sets were the same except for the positions of the

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Table 1. Data Sets Used to Infer Ambulacrarian Phylogeny

Filtering Method	Number of Genes	Number of Taxa	Amino Acid Positions	% Missing Data	Information Content
No paralogy screening	299	33	107,081	62.59	0.34
PhyloTreePruner	185	33	70,446	58.45	0.383
PhyloTreePruner, unstable taxa pruned	185	31	70,446	55.91	0.408
MARE	162	20	61,546	35.29	0.593

Information Content was calculated using MARE [18].

unstable *Stereobalanus canadensis* and *Rhabdopleura* (Figures 1, S1, and S2). Because we sought to directly address relationships within Ambulacraria, and because monophyly for extant hemichordates and echinoderms is well established (reviewed in [4, 5, 20]), we rooted trees so that hemichordates and echinoderms were reciprocally monophyletic.

The assumption that Echinodermata and Hemichordata are monophyletic clades was supported with bootstrap support (BS) of 100% in all analyses. When all taxa were included (185/33), Pterobranchia and Enteropneusta were recovered as reciprocally monophyletic, albeit with poor support (Figure S2). However, when unstable *Rhabdopleura* and *Stereobalanus canadensis* were removed, bootstrap values for Pterobranchia and Enteropneusta increased to 100 (Figure 1). Shimodaira-Hasegawa (SH) tests conducted on the 185/31 data set strongly rejected the alternative hypothesis of paraphyletic Enteropneusta or Pterobranchia + Harrimaniidae (Table 2). Furthermore, there was no indication of systematic error that may have influenced SH tests. In ML analyses of the 299/33 data set (Figure S1A), *Rhabdopleura* was recovered as sister to Enteropneusta, with *Cephalodiscus* sister to *Rhabdopleura* + Enteropnuesta. Despite combining three 454 libraries and an Illumina library into a chimeric OTU, *Rhabdopleura* was still only represented by 8% of genes in the 185/33 data matrix. Presumably, poor coverage resulted in instability of this taxon, limiting confidence in *Rhabdopleura*'s placement. The 162/20 data set (Figure S1B), which includes only the most deeply sampled taxa, leaves pterobranchs (represented by *Cephalodiscus gracilis*) as sister to Enteropneusta.

Exclusion of Pterobranchia from Enteropneusta is robustly supported in all of our analyses. This result is consistent with results obtained from morphological cladistic analysis [14] and recent topologies obtained using nuclear 18S rDNA and mitochondrial 16S rDNA data, albeit with weak support [7]. Although microRNA patterns were suggested to support enteropneust monophyly in a previous study [21], that study examined only three hemichordates, and microRNAs have been shown to be unreliable for phylogenetic purposes [22]. The ancestral hemichordate has been hypothesized to have been a solitary, ptychoderid-like worm with gill slits and indirect development [3–5]. Our results suggest that ptychoderids have a more derived placement, but because gill slits are shared with chordates, this character is likely ancestral for Hemichordata, Ambulacraria, and Deuterostomia. Given that the last common ancestor of hemichordates lived more than 520 million years ago, there are likely morphological differences between present-day groups and the hemichordate ancestor. Morphological, developmental, and paleontological evidence, particularly from data-limited pterobranchs, will be needed to further address these questions [4–6].

Within Enteropneusta, Harrimaniidae is the most species-rich family (considering currently described species) and includes the developmental model hemichordate *Saccoglossus kowalevskii*. Here, Harrimaniidae (excluding *Stereobalanus canadensis*) was recovered as monophyletic (BS = 100), sister to the remaining enteropneusts. When *Stereobalanus canadensis* was included, it was the earliest branching lineage of Hemichordata (299/33, Figure S1A) or Enteropneusta (185/33, Figure S2). This species has a remarkably long-branched 18S rDNA sequence [6, 7], has a noticeably long branch in analyses herein, and is morphologically distinct among enteropneusts, possessing four short gonad regions directly posterior to the collar and gill pores fused into a common slit. Due to the low coverage obtained here for this worm (5% of OGs) and its poor leaf stability (Table S3), additional sequence information or other sources of data will likely be required to place this unusual species with confidence.

In contrast to analyses based on 18S rDNA [7, 23], we found the recently described deep-sea Torquaratoridae nested within Ptychoderidae. An alternative tree topology in which Ptychoderidae was constrained as monophyletic to the exclusion of torquaratorid taxa was significantly rejected by SH tests (Table 2). Torquaratoridae and Ptychoderidae both have hepatic caecae and genital wings, but torquaratorids lack gill bar synapticules, a character previously thought to be an apomorphy of Ptychoderidae. Torquaratoridae also have a reduced or absent proboscis skeleton, and the stomochord is either absent or disconnected from the buccal cavity of the collar. The position of torquaratorids within Ptychoderidae suggests that these represent secondary losses. Some torquaratorid taxa have shown unusual reproductive structures or strategies, such as externalized ovaries in *Allapasus aurantiacus* [24] or brooding in *Coleodesmium karaensis* [25]. These features appear to have been derived from ptychoderid-like forms with indirect development via tornaria larvae. Given that the Cambrian fossil enteropneust *Spartobranchus tenuis* [26] is probably allied to torquaratorids [27], which are derived acorn worms, enteropneust origins reach into the Lower Cambrian. The assertion for harrimaniid affinities of this fossil was informed by the presumed relationship between pterobranchs and harrimaniids, which is refuted here. Thus, tubes of *Spartobranchus* are unlikely to represent the precursor to the pterobranch periderm [27], as suggested by [26].

Each of the five recognized echinoderm classes was recovered as monophyletic with 100% bootstrap support, with Crinoidea as sister to Eleutherozoa (BS = 100). Within Eleutherozoa, we found strong support for Asterozoa (Ophiuroidea + Asteroidea) (BS = 100) as sister to Echinozoa (Holothuroidea + Echinoidea). Additionally, Cryptosyringida (Ophiuroidea, [Holothuroidea + Echinoidea]) was rejected by SH tests (Table 2). Other phylogenomic studies recently recovered Asterozoa with strong support [16, 17], and our analyses corroborate these results. The Asterozoa hypothesis suggests that pluteus larvae found in ophiuroids and echinoids evolved via convergent evolution. Pluteus larvae are distinguished from the generalized echinoderm dipleurula larva by elongated ciliated arms that are supported by calcite skeletal elements derived from the mesoderm. These larval skeletal elements may have
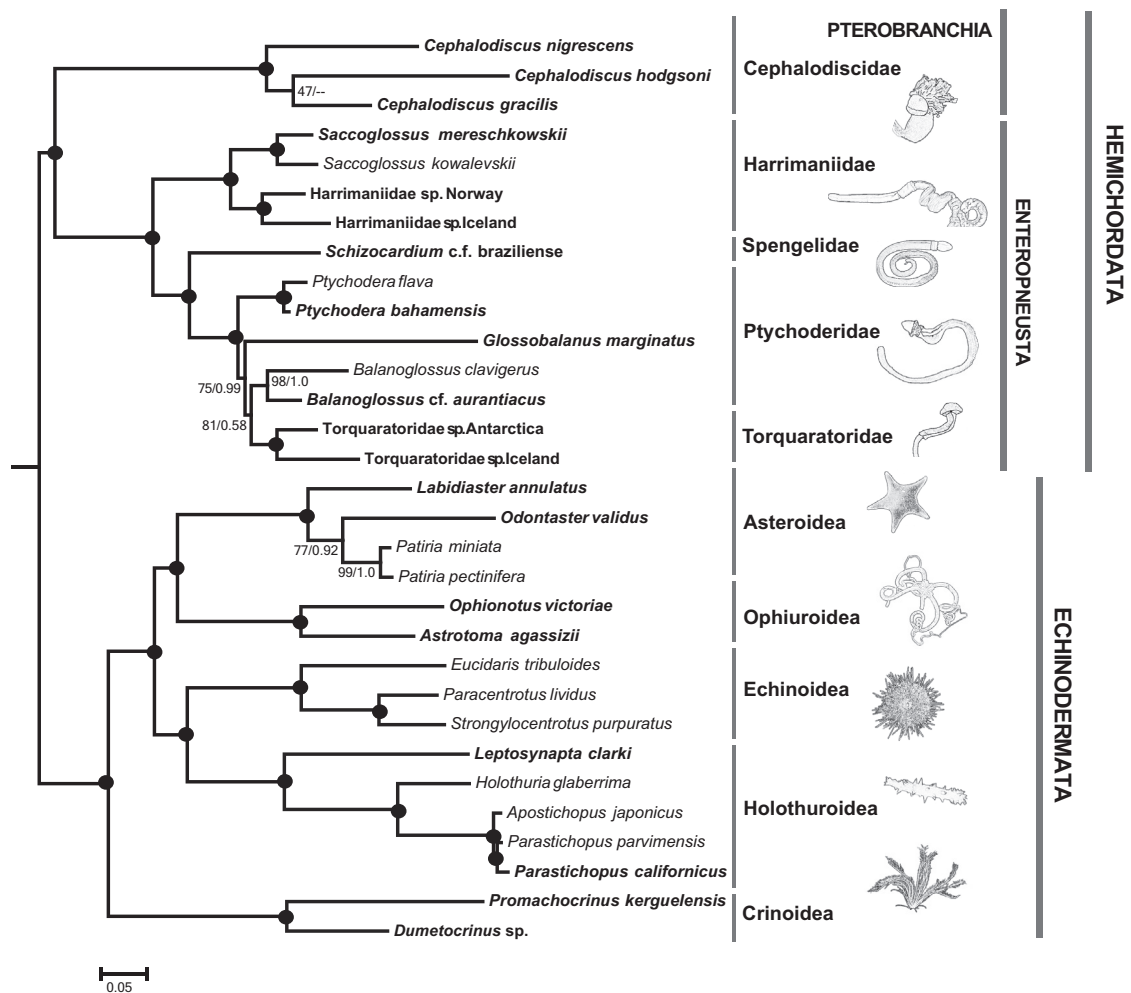
Figure 1. Ambulacrarian Phylogeny Based on the 185/31 Data Set with Unstable Taxa Removed

Maximum-likelihood tree is shown with bootstrap values and Bayesian posterior probabilities indicated at the nodes. Filled circles (●) indicate 100% bootstrap support and 1.0 posterior probability. Bold names in the tree at left indicate novel data collected herein.

evolved independently via co-option of genes involved in adult skeletal development [28, 29]. The primary adult character uniting Cryptosyringida, radial elements enclosing the water vascular system and radial nerve, may not be apomorphic, even within echinoids [10]. Stellate body-plan organization, saccate gut, and Ambulacraria ossicle structure are morphological synapomorphies for Asterozoa [8]. Somasteroids have been hypothesized to be ancestral to both asteroids and ophiuroids, or a distinct group of Asterozoa. Support for Asterozoa lends credence to placement of somasteroids near or spanning the asteroid/ophiuroid split [30].

Based on our summarized results (Figure 2), the last common ancestor of Ambulacraria had an axial complex and pharyngeal gill slits homologous to chordate gill slits [1, 3, 4, 6].

Interestingly, the presence of planktotrophic dipleurula larvae can be interpreted as having been lost twice (in Harrimaniidae and Pterobranchia), as the likelihood of independent acquisition of dipleurula (in Spengelidae + Ptychoderidae and Echinodermata) is low. However, uncertainty regarding the position of *Rhabdopleura* and *Stereobalanus* (whose development is unknown) may prompt reevaluation of this issue in the future. Importantly, results here provide a better understanding of the evolutionary origins of model species (the acorn worms *Saccoglossus kowalevskii* and *Ptychodera flava* and the sea urchin *Strongylocentrotus purpuratus*). These findings should allow developmental features of both hemichordates [3–5, 31, 32] and echinoderms [33] that have been homologized to early chordate embryos to be more thoroughly explored in a

Table 2. Results of Hypothesis Testing by Shimodaira-Hasegawa Tests

Hypothesis	Likelihood	D(LH)	Standard Deviation	Significantly Worse (5%)
Pterobranchia + Harrimaniidae	−782314.9336	−1374.267009	69.610663	yes
Cryptosyringida	−781019.6053	−78.938677	23.038683	yes
Monophyletic Ptychoderidae (excluding Torquaratoridae)	−781200.8691	−260.202494	40.262256	yes

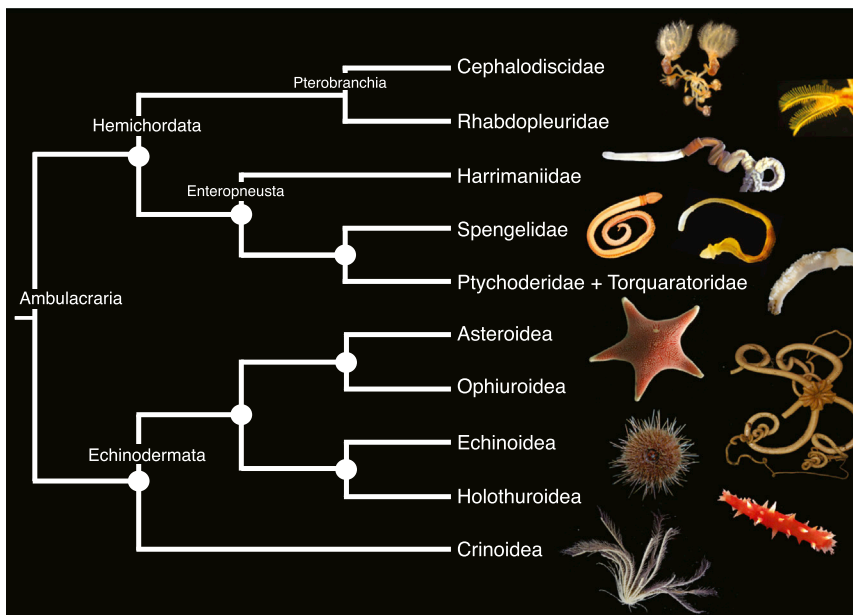Likelihood value for the best tree was −780940.6666. D(LH), difference in likelihood score.

Figure 2. Summary of Phylogenomic Results for Ambulacraria

Filled circles (●) indicate 100% bootstrap support and 1.0 posterior probability. Colonial Pterobranchia are sister group to the Enteropneusta in the Hemichordata (top clade), which is additionally supported by [7]. Asteroidea and Ophiuroidea are sister group to the Echinozoa (Echinoidea + Holothuroidea) in the Echinodermata.

Single-OG trees were then constructed for each OG using RAxML v7.3.8 [41] with the PROTGAMMALGF model. Individual gene trees were manually evaluated for sequence contamination as described in Supplemental Experimental Procedures. After manual screening of alignments and individual OG trees, 299 alignments remained, constituting the basis for the 299/33 data set. To screen for potential paralogs, we used PhyloTreePruner [19] (details in Supplemental Experimental Procedures). Alignments passing screening via PhyloTreePruner constituted the 185/33 and 185/31 data sets. To test the effect of low-coverage taxa and missing data on our phylogenetic reconstruction, we employed MARE [18] to generate the 165/20 data set (weighting of information content parameter $\alpha = 3$).

comparative developmental context. Furthermore, they also identify taxa (e.g., pterobranchs) that can help us unravel the evolution of deuterostome characters.

Experimental Procedures

Taxon Sampling

We sampled transcriptomic data from representatives of all recognized hemichordate families and all echinoderm classes except Echinoidea, for which data were already available (Tables S1 and S2). At least two species of each taxonomic group were sampled, except for Spengelidae. Samples from which novel transcriptomic data were obtained were collected in various locations, transported live to the laboratory, transferred to RNAlater, frozen at −80°C, or kept in ethanol at −20°C.

Sequencing and Assembly

Total RNA was extracted from fresh or preserved samples, and cDNA libraries were prepared using the SMART cDNA Library Construction Kit (Clontech; see Supplemental Experimental Procedures for further details). Samples were sequenced with 454 FLX, 454 Titanium, or Illumina HiSeq 2000. Generated sequence data were augmented with publicly available data. Taxa sequenced by 454 or Sanger methods were assembled and processed using the EST2uni pipeline [34], while those sequenced by Illumina were digitally normalized using khmer [35] and assembled using Trinity [36]. Contigs (for Illumina libraries) or contigs + high-quality singletons (for 454 and Sanger libraries) were translated using TransDecoder (http://sourceforge.net/projects/transdecoder/). Table S2 provides the number of unigenes (contigs for Illumina libraries and contigs + singletons for 454 and Sanger libraries) obtained for each taxon.

Identification of putative ortholog groups (OGs) was conducted with HaMStR [37] using the "model organisms" reference taxon set. After orthology determination, sequences from two 454 libraries from *Ophionotus victoriae* and four libraries from *Rhabdopleura* species were combined into chimeric OTUs in order to reduce the amount of missing data per taxon.

Orthology groups were filtered following the approach of Kocot et al. [38] First, only sequences greater than 100 aa in length, and OGs with at least 15 ambulacrarian taxa and including at least one pterobranch sequence, were retained for further analyses. Sequences less than 100 aa in length were deleted because they are likely to be incorrectly aligned. To remove mistranslated sequence ends, we trimmed amino acid sequences when stop codons (marked by X) were present in either the first or last 20 characters. Each OG was aligned with MAFFT [39] and then trimmed with Aliscore and Alicut [40] to remove columns with ambiguous alignment.

Next, individual alignments were manually evaluated for partially mistranslated sequences, which were deleted or trimmed as appropriate.

Phylogenetic Analyses

Maximum-likelihood phylogenetic analyses of all data sets were conducted using RAxML v7.7.6 [41] using a PROTGAMMALGF model for each individual OG partition. This model was the most appropriate for the majority of genes and was among the best-fitting models for nearly all OGs as assessed by ProtTest [42]. Previous work [38] and preliminary analyses on these data sets indicated that employing a single model across all partitions recovers highly similar trees while proving considerably less computationally expensive than specifying individual models for all partitions. Nodal support was assessed with 1,000 replicates of nonparametric bootstrapping. Bootstrapped trees from the 185/33 data set were used to calculate leaf stability and taxonomic instability indices of each OTU using the RogueNaRok server (http://rnr.h-its.org). Competing hypotheses of ambulacrarian phylogeny were evaluated using the SH test [43] as implemented in RAxML with the PROTGAMMALGF model for each OG partition. Bayesian inference analyses were conducted using PhyloBayes 2.3 [44] with the CAT model, which accounts for site-specific rate heterogeneity, with two independent chains run for 11,000 cycles each and 1,100 cycles discarded as burn-in. Topology and posterior consensus support was generated using the bpcomp program within PhyloBayes; convergence of the two chains was indicated by "maxdiff" values below 0.2. All phylogenetic analyses were conducted on the Auburn University CASIC HPC supercomputer.

Accession Numbers

Sequence Read Archive (SRA) accession numbers for all data are given in Table S2. The four data matrices are available from the Dryad Digital Repository (http://datadryad.org) with the DOI http://dx.doi.org/10.5061/dryad.20s7c.

Supplemental Information

Supplemental Information includes two figures, three tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.10.016.

Author Contributions

J.T.C. and K.M.H. conceived the project. J.T.C., K.M.K., B.J.S., and K.M.H. collected organisms. J.T.C. and K.M.K. prepared transcriptomic libraries. J.T.C., K.M.K., D.S.W., D.A.W., K.M.H., and S.R.S. contributed to

References

1. Halanych, K.M. (1995). The phylogenetic position of the pterobranch hemichordates based on 18S rDNA sequence data. Mol. Phylogenet. Evol. *4*, 72–76.

2. Bourlat, S.J., Juliusdottir, T., Lowe, C.J., Freeman, R., Aronowicz, J., Kirschner, M., Lander, E.S., Thorndyke, M., Nakano, H., Kohn, A.B., et al. (2006). Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. Nature *444*, 85–88.

3. Brown, F.D., Prendergast, A., and Swalla, B.J. (2008). Man is but a worm: chordate origins. Genesis *46*, 605–613.

4. Swalla, B.J., and Smith, A.B. (2008). Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 1557–1568.

5. Cameron, C.B., Garey, J.R., and 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.

6. Cannon, J.T., Rychel, A.L., Eccleston, H., Halanych, K.M., and Swalla, B.J. (2009). Molecular phylogeny of hemichordata, with updated status of deep-sea enteropneusts. Mol. Phylogenet. Evol. *52*, 17–24.

7. Cannon, J.T., Swalla, B.J., and Halanych, K.M. (2013). Hemichordate molecular phylogeny reveals a novel cold-water clade of harrimaniid acorn worms. Biol. Bull. *225*, 194–204.

8. Janies, D. (2001). Phylogenetic relationships of extant echinoderm classes. Can. J. Zool. *79*, 1232–1250.

9. Pisani, D., Feuda, R., Peterson, K.J., and Smith, A.B. (2012). Resolving phylogenetic signal from noise when divergence is rapid: a new look at the old problem of echinoderm class relationships. Mol. Phylogenet. Evol. *62*, 27–34.

10. Littlewood, D.T.J., Smith, A.B., Clough, K.A., and Emson, R.H. (1997). The interrelationships of the echinoderm classes: morphological and molecular evidence. Biol. J. Linn. Soc. Lond. *61*, 409–438.

11. Smith, A.B. (2008). Deuterostomes in a twist: the origins of a radical new body plan. Evol. Dev. *10*, 493–503.

12. Delsuc, F., Brinkmann, H., Chourrout, D., and Philippe, H. (2006). Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature *439*, 965–968.

13. Philippe, H., Brinkmann, H., Copley, R.R., Moroz, L.L., Nakano, H., Poustka, A.J., Wallberg, A., Peterson, K.J., and Telford, M.J. (2011). Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. Nature *470*, 255–258.

14. Cameron, C.B. (2005). A phylogeny of the hemichordates based on morphological characters. Can. J. Zool. *83*, 196–215.

15. Mitchell, C.E., Melchin, M.J., Cameron, C.B., and Maletz, J. (2013). Phylogenetic analysis reveals that *Rhabdopleura* is an extant graptolite. Lethaia *46*, 34–56.

16. Telford, M.J., Lowe, C.J., Cameron, C.B., Ortega-Martinez, O., Aronowicz, J., Oliveri, P., and Copley, R.R. (2014). Phylogenomic analysis of echinoderm class relationships supports Asterozoa. Proc. Biol. Sci. *281*, 20140479.

17. O'Hara, T.D., Hugall, A.F., Thuy, B., and Moussalli, A. (2014). Phylogenomic resolution of the class ophiuroidea unlocks a global microfossil record. Curr. Biol. *24*, 1874–1879.

18. Meyer, B., Meusemann, K., and Misof, B. (2010). MARE v0.1.2-rc. http://mare.zfmk.de/.

19. Kocot, K.M., Citarella, M.R., Moroz, L.L., and Halanych, K.M. (2013). PhyloTreePruner: A Phylogenetic Tree-Based Approach for Selection of Orthologous Sequences for Phylogenomics. Evol. Bioinform. Online *9*, 429–435.

20. Kocot, K.M., Cannon, J.C., and Halanych, K.M. (2010). Elucidating animal phylogeny. In Key Transitions in Animal Evolution, R. DeSalle and B. Schierwater, eds. (Enfield: CRC Press), pp. 15–33.

21. Peterson, K.J., Su, Y.-H., Arnone, M.I., Swalla, B., and King, B.L. (2013). MicroRNAs support the monophyly of enteropneust hemichordates. J. Exp. Zoolog. B Mol. Dev. Evol. *320*, 368–374.

22. Thomson, R.C., Plachetzki, D.C., Mahler, D.L., and Moore, B.R. (2014). A critical appraisal of the use of microRNA data in phylogenetics. Proc. Natl. Acad. Sci. USA *111*, E3659–E3668.

23. Osborn, K.J., Kuhnz, L.A., Priede, I.G., Urata, M., Gebruk, A.V., and Holland, N.D. (2012). Diversification of acorn worms (Hemichordata, Enteropneusta) revealed in the deep sea. Proc. Biol. Sci. *279*, 1646–1654.

24. Holland, N.D., Kuhnz, L.A., and Osborn, K.J. (2012). Morphology of a new deep-sea acorn worm (class Enteropneusta, phylum Hemichordata): a part-time demersal drifter with externalized ovaries. J. Morphol. *273*, 661–671.

25. Osborn, K.J., Gebruk, A.V., Rogacheva, A., and Holland, N.D. (2013). An externally brooding acorn worm (Hemichordata, Enteropneusta, Torquaratoridae) from the Russian arctic. Biol. Bull. *225*, 113–123.

26. Caron, J.-B., Morris, S.C., and Cameron, C.B. (2013). Tubicolous enteropneusts from the Cambrian period. Nature *495*, 503–506.

27. Halanych, K.M., Cannon, J.T., Mahon, A.R., Swalla, B.J., and Smith, C.R. (2013). Modern Antarctic acorn worms form tubes. Nat. Commun. *4*, 2738.

28. McCauley, B.S., Wright, E.P., Exner, C., Kitazawa, C., and Hinman, V.F. (2012). Development of an embryonic skeletogenic mesenchyme lineage in a sea cucumber reveals the trajectory of change for the evolution of novel structures in echinoderms. Evodevo *3*, 17.

29. Byrne, M., Nakajima, Y., Chee, F.C., and Burke, R.D. (2007). Apical organs in echinoderm larvae: insights into larval evolution in the Ambulacraria. Evol. Dev. *9*, 432–445.

30. Mooi, R., and David, B. (2000). What a new model of skeletal homologies tells us about asteroid evolution. Am. Zool. *40*, 326–339.

31. Lowe, C.J. (2008). Molecular genetic insights into deuterostome evolution from the direct-developing hemichordate *Saccoglossus kowalevskii*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 1569–1578.

32. Nomaksteinsky, M., Röttinger, E., Dufour, H.D., Chettouh, Z., Lowe, C.J., Martindale, M.Q., and Brunet, J.-F. (2009). Centralization of the deuterostome nervous system predates chordates. Curr. Biol. *19*, 1264–1269.

33. Morris, V.B. (2012). Early development of coelomic structures in an echinoderm larva and a similarity with coelomic structures in a chordate embryo. Dev. Genes Evol. *222*, 313–323.

34. Forment, J., Gilabert, F., Robles, A., Conejero, V., Nuez, F., and Blanca, J.M. (2008). EST2uni: an open, parallel tool for automated EST analysis and database creation, with a data mining web interface and microarray expression data integration. BMC Bioinformatics *9*, 5.

35. Brown, C.T., Howe, A., Zhang, Q., Pyrkosz, A.B., and Brom, T.H. (2012). A reference-free algorithm for computational normalization of shotgun sequencing data. ArXiv, arXiv:1203.4802. http://arxiv.org/abs/1203.4802.

36. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. *29*, 644–652.

37. Ebersberger, I., Strauss, S., and von Haeseler, A. (2009). HaMStR: profile hidden markov model based search for orthologs in ESTs. BMC Evol. Biol. *9*, 157.

38. Kocot, K.M., Cannon, J.T., Todt, C., Citarella, M.R., Kohn, A.B., Meyer, A., Santos, S.R., Schander, C., Moroz, L.L., Lieb, B., and Halanych, K.M. (2011). Phylogenomics reveals deep molluscan relationships. Nature *477*, 452–456.

39. Katoh, K., Kuma, K., Toh, H., and Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. *33*, 511–518.

40. Misof, B., and Misof, K. (2009). A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. Syst. Biol. *58*, 21–34.

41. Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics *22*, 2688–2690.

42. Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2011). ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics *27*, 1164–1165.

43. Shimodaira, H. (2002). An approximately unbiased test of phylogenetic tree selection. Syst. Biol. *51*, 492–508.

44. Lartillot, N., Rodrigue, N., Stubbs, D., and Richer, J. (2013). PhyloBayes MPI: phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment. Syst. Biol. *62*, 611–615.