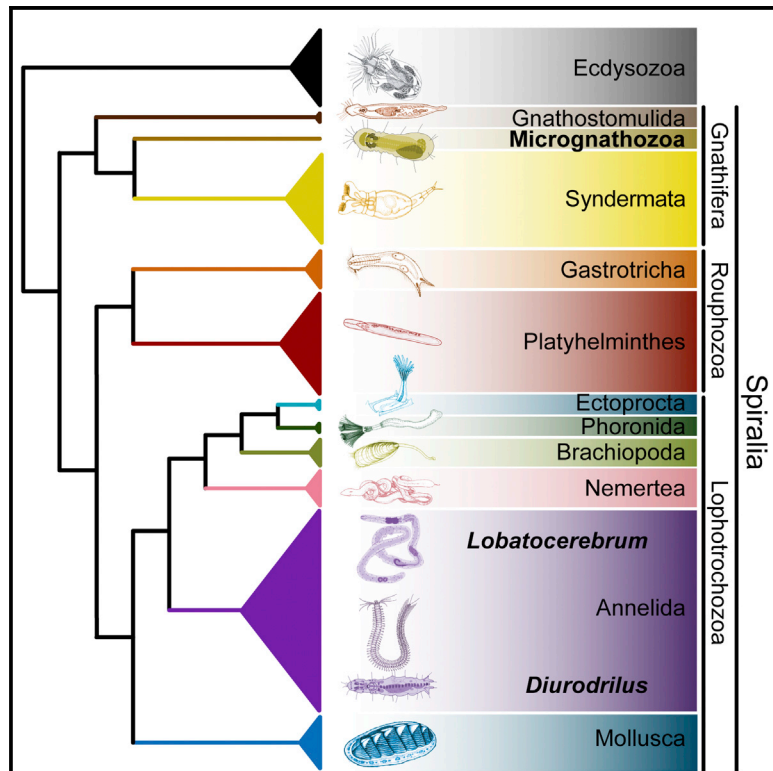# Current Biology

Spiralian Phylogeny Informs the Evolution of Microscopic Lineages

Graphical Abstract

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In Brief

Laumer et al. reconstruct the phylogeny of Spiralia, the animal group including molluscs, annelids, flatworms, and many microscopic worms. The new tree suggests that some previously unsampled, interstitial Problematica originated through miniaturization from large-bodied ancestors but also implies a primarily interstitial origin for many lineages.

Highlights

- *Diurodrilus* and *Lobatocerebrum*, two problematic meiofauna, are miniaturized annelids

- Micrognathozoa, the newest-described animal phylum, is the sister group of Rotifera

- Bayesian mixture models recover strong support for deep spiralian relationships

- Two clades comprising Platyzoa form separate early branches in Spiralia

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Spiralian Phylogeny Informs the Evolution of Microscopic Lineages

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SUMMARY

Despite rapid advances in the study of metazoan evolutionary history [1], phylogenomic analyses have so far neglected a number of microscopic lineages that possess a unique combination of characters and are thus informative for our understanding of morphological evolution. Chief among these lineages are the recently described animal groups Micrognathozoa and Loricifera, as well as the two interstitial "Problematica" *Diurodrilus* and *Lobatocerebrum* [2]. These genera show a certain resemblance to Annelida in their cuticle and gut [3, 4]; however, both lack primary annelid characters such as segmentation and chaetae [5]. Moreover, they show unique features such as an inverted body-wall musculature or a novel pharyngeal organ. This and their ciliated epidermis have led some to propose relationships with other microscopic spiralians, namely Platyhelminthes, Gastrotricha, and in the case of *Diurodrilus*, with Micrognathozoa [6, 7]—lineages that are grouped by some analyses into "Platyzoa," a clade whose status remains uncertain [1, 8–11]. Here, we assess the interrelationships among the meiofaunal and macrofaunal members of Spiralia using 402 orthologs mined from genome and transcriptome assemblies of 90 taxa. *Lobatocerebrum* and *Diurodrilus* are found to be deeply nested members of Annelida, and unequivocal support is found for Micrognathozoa as the sister group of Rotifera. Analyses using site-heterogeneous substitution models further recover a lophophorate clade and position Loricifera + Priapulida as sister group to the remaining Ecdysozoa. Finally, with several meiofaunal lineages branching off early in the diversification of Spiralia, the emerging concept of a microscopic, acoelomate, direct-developing ancestor of Spiralia is reviewed.

RESULTS

Understanding metazoan evolutionary history requires resolving the phylogenetic positions of not only the major animal groups but also of more obscure lineages showing unique character combinations. Examples of such important "Problematica" are Lobatocerebromorpha [3, 12, 13], Diurodrilida [4, 5], Micrognathozoa [6, 7], and Loricifera [14], representing among the smallest animals ever discovered, which have received phylum-level affiliations or remain of uncertain position within Protostomia. We present here the first nuclear protein-coding data from representatives of all four clades, incorporating these and other new and published protein-coding surveys into a 402-ortholog, 90-taxon supermatrix comprising all free-living lineages of Spiralia (Table S1). Phylogenetic analyses of this matrix were performed using maximum likelihood (ML; Figures 1 and S1), with partitioned analyses of the full-size matrix (Figure 1A) and unpartitioned analyses of two submatrices constructed to investigate putative long-branch attraction (LBA) artifacts (Figures 1B and 1C). To further control for other potential systematic artifacts, we also undertook analyses using Bayesian inference (BI) under a site-heterogeneous mixture model (CAT + GTR + $\Gamma 4$; [15]), using a matrix groomed of unstable taxa and sites showing evidence of compositional non-stationarity (Figure 2). Bayesian analyses of the complete matrix were also performed (Figure S2).

The ML and BI analyses differ, at least superficially, in the topology they present for deep spiralian interrelationships. Our ML trees from partitioned analyses of the full matrix (Figure 1A) and from analyses of a slow-evolving subset of the full matrix (Figure 1B) are nearly identical and recapitulate results found in previous large-scale ML investigations of spiralian phylogeny [10, 11], e.g., monophyly of Trochozoa, Platyzoa, and Polyzoa [1, 15]. In contrast, analyses of a fast-evolving subset (Figure 1C) of this matrix do not recover the monophyly of Platyzoa, Polyzoa, or even Ecdysozoa. In general, however, few relevant clades
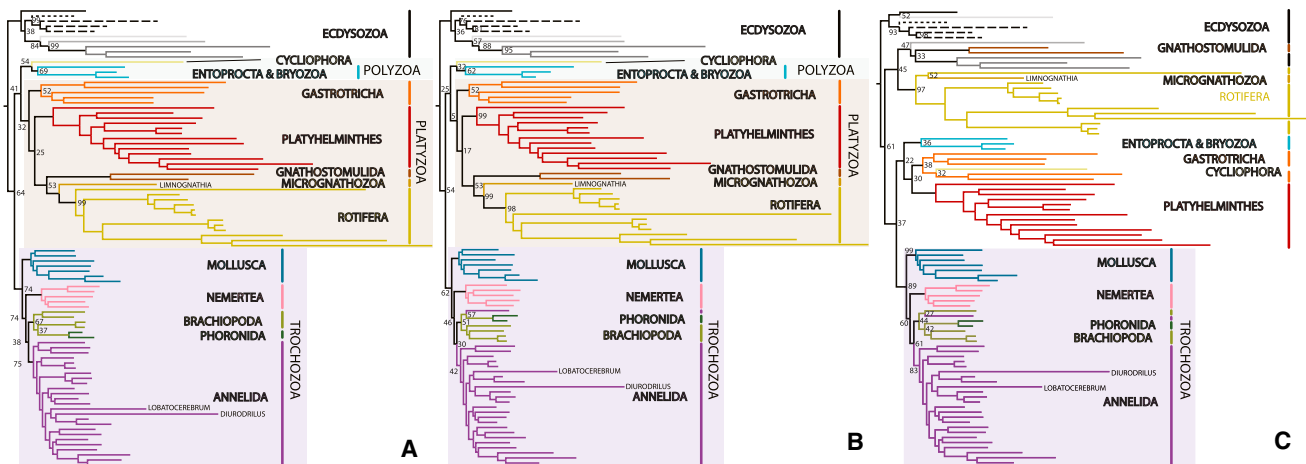
Figure 1. Maximum Likelihood Inference of Spiralian Interrelationships from the Complete Matrix and Two Submatrices Stratified by Evolutionary Rate

Maximum likelihood (ExaML v.3.0.0) phylograms inferred from the 402-gene, 79,954-amino-acid (aa) supermatrix (57.57% missing data).

(A) Partitioned analysis (from PartitionFinderProtein v.1.1.1) inferred from the total matrix.

(B) LG4M + F analysis from a submatrix comprising all but the fastest-evolving quartile of variable sites (20,167 aa).

(C) LG4M + F analysis from a submatrix comprising all but the slowest-evolving quartile of variable sites (20,293 aa).

Nodal values (given only to show support for the monophyly and interrelationships of the labeled major clades, i.e., not depicting support for intra-phylum relationships) reflect frequency of clades in a set of 100 bootstrap trees; clades with full support are not labeled. For labeled terminal taxa and full support values, refer to Figure S1 and the data Dryad accession.

find strong support in any ML analysis, with even several uncontroversially monophyletic taxa (e.g., Annelida, Gastrotricha) failing to see strong support (Figure 1). In contrast, the BI analyses under a site-heterogeneous model (CAT + GTR + Γ4) find strong support for many spiralian clades, including all those that are also supported in the ML analyses, but also for Spiralia, Gnathifera, and Lophotrochozoa, among others (Figure 2). Thus, while the ML trees and BI consensus phylograms topologically differ, there is no evidence of strongly supported incongruence between ML and BI. Most importantly, BI places both *Diurodrilus* and *Lobatocerebrum* as deeply nested members of Annelida (as does ML, although with lesser support). Finally, BI also finds strong support for the non-monophyly of "Platyzoa," with Gnathifera forming the earliest-diverging branch (Figures 2 and S2). Platyzoan non-monophyly is also recovered under ML in our fastest-evolving matrix subset (Figure 1C), but support for basal relationships is poor in this analysis.

The BI analyses of the trimmed (Figure 2) and untrimmed (Figure S2) matrices differ in only few respects. Platyhelminthes + Gastrotricha (called Rouphozoa in [11]) and Lophotrochozoa (in the sense of its original definition by [16] and not the looser common usage introduced by [17]) are supported in the trimmed matrix, but not the untrimmed matrix. Mixture model inference on both matrices, in sharp contrast to our ML analyses, also recovers the monophyly of the lophophorate phyla with high support, with Phoronida (here as in [18]) forming the sister group of Bryozoa. Mollusca was recovered as the sister group to the other Lophotrochozoa (in marked contrast to recent studies [11, 18]), albeit with weak support in the complete matrix (Figure S2). Indeed, the only strongly supported deep topological difference observed between analyses of the trimmed versus complete matrix concerns the position of Nemertea, which forms the sister

taxon of Annelida in the untrimmed matrix (Figure S2), or of the lophophorate clade in the trimmed matrix (Figure 2). Remarkably, in the complete matrix, we see no support for the hypothesis previously suggested by both molecules and morphology [18–20] of a sister-group relationship between Cycliophora and Entoprocta (the latter being instead recovered as sister group to Bryozoa; [21]); here, Cycliophora falls, but with low posterior probability (pp; pp = 0.5), as the sister group of Lophotrochozoa (Figure S2), a result perhaps related to the poor sequencing depth of this transcriptome.

Within Ecdysozoa, we find strong support under BI analysis of the untrimmed matrix (Figure S2) only for Onychophora + Arthropoda and Tardigrada + Nematoda, as found in a recent study focused on Ecdysozoa [22]. However, in the trimmed matrix (Figure 2), support (pp = 0.98) also emerges for a scenario in which the meiofaunal Loricifera fall together with our other scalidophoran representative, Priapulida, as the sister group to other members of Ecdysozoa. Although evidence for Scalidophora itself is poor (pp = 0.78), and we lack a representative of Kinorhyncha, this is the first time molecular data have recovered a clade of Loricifera + Priapulida, two taxa that share many common morphological traits [23].

DISCUSSION

Diurodrilus and *Lobatocerebrum* Are Miniaturized Annelids

The deeply nested positions of *Diurodrilus* and *Lobatocerebrum* within Annelida suggest independent miniaturizations of these lineages from an indirect-developing, macrofaunal annelid ancestor. *Diurodrilus* has traditionally been considered a member of Archiannelida [4, 24], a taxon of morphologically simple
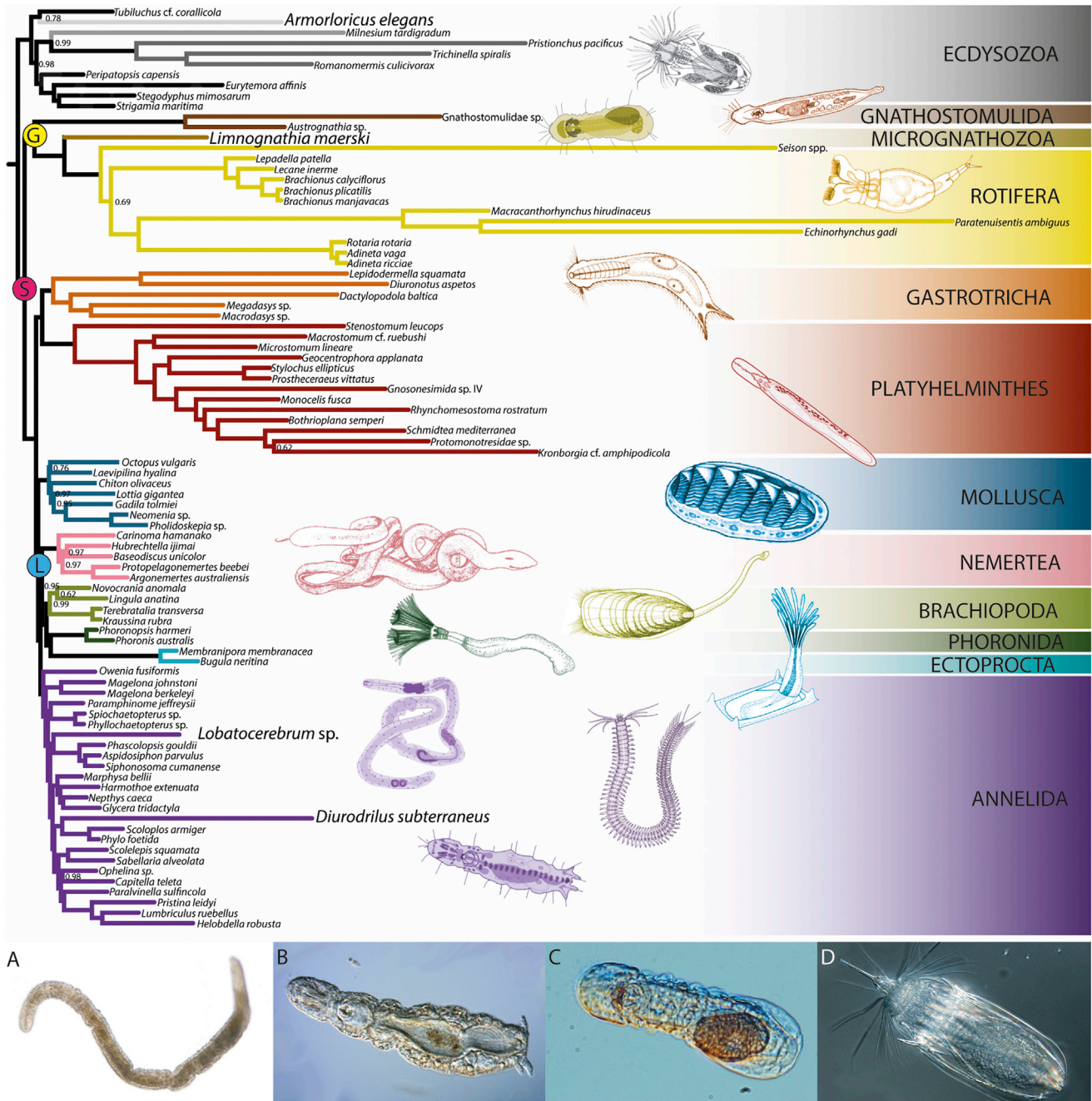
Figure 2. Bayesian Mixture Model Inference of Spiralian Interrelationships, with Special Reference to the Placement of Problematic Interstitial Taxa

Bayesian inference of the interrelationships among taxa of Spiralia in the 88-taxon "trimmed" matrix (72,243 aa, 58.17% missing data), made by PhyloBayes-MPI v.1.4e, under the CAT + GTR + Γ4 mixture model. Nodal support values represent posterior probability; completely supported nodes are unlabeled. G, Gnathifera; L, Lophotrochozoa; S, Spiralia. Inset at lower right: selected images of problematic interstitial meiofauna placed in this phylogenetic analysis.

(A) Adult *Lobatocerebrum* sp. from Bird Island, Bocas del Toro, Panama; differential interference contrast (DIC) optics.

(B) Adult *Diurodrilus* sp. from Bailey's Rock, Nahant, Massachusetts; DIC optics.

(C) Adult *Limnognathia maerski* with egg from Isunngua Spring, Disko Island, Greenland; DIC optics.

(D) Adult of *Armorloricus elegans* from Roscoff, France; DIC optics.

interstitial annelids originally considered "ancestral" to the other annelid taxa [25, 26], other members of which have recently been shown to be non-monophyletic and derived from macrofaunal ancestors [27, 28]. However, for *Diurodrilus*, several authors have also proposed a relationship outside of Annelida, specifically to the recently discovered Micrognathozoa, with which

they share, e.g., characteristic mid-ventral trunk ciliophores and a ventral muscular plate of the pharynx [5–7]. Equally complicated is the case of *Lobatocerebrum*, originally described as "a turbellariomorph member of the annelid line of evolution" [3], i.e., an intermediate between Platyhelminthes and Annelida—a position maintained by Rieger [12] and Hazprunar et al. [2] (who erected for it the phylum Lobatocerebromorpha), which we aimed to test here.

None of these hypotheses are supported in the present study. The precise position in which we recovered *Diurodrilus* within Annelida—as sister taxon to the macrofaunal Orbiniidae—has also been supported by ML analysis of mitogenomic data (although curiously, orbiniids appear more distant in gene order analyses) [29]. Remarkably, in previous rRNA-based phylogenetic studies orbiniids have been recovered as relatives of Parergodrilidae, another meiofaunal annelid lineage [30]. However, *Diurodrilus* shows with its apomorphic pharyngeal organ, adhesive toes, and ventral ciliophores no close resemblance to any known orbiniid, adult, larval or juvenile [29, 31]. Indeed, it represents the most "reduced" annelid to date, both sexes being of microscopic size and lacking all common annelid traits such as segmentation, coelomic cavities, chaetae, and nuchal organ [26]. With respect to *Lobatocerebrum*, we find it strongly supported as the sister group of Sipuncula [32], constituting an intriguing clade of unsegmented annelids; however, there are no other obvious synapomorphies for the two groups.

Lobatocerebrum and *Diurodrilus* share gross anatomical characteristics with many interstitial annelids, most prominent among these being an acoelomate or pseudocoelomate condition (with coincident protonephridia and absence of a vascular system). This organization may be related to small body size and can arise homoplastically as the consequence of diverse processes, such as an enlarged peritoneal lining and/or endoderm, or lack of cavity formation within the mesoderm [26, 33–35]. These different manifestations of an acoelomate condition, as well as the apparent independent origin of *Lobatocerebrum*, *Diurodrilus*, and most other interstitial annelid families [26, 28, 31] indicate that their miniaturizations do not follow a predictable pattern. Accordingly, it cannot easily be explained by the popular theory of progenesis [31], especially considering their lack of specific resemblance to larval or juvenile stages of macrofaunal relatives (e.g., Orbiniidae). Regardless of the mechanism of their reduction, however, our recovered placement of *Diurodrilus* and *Lobatocerebrum* within Annelida contributes to the enormous morphological disparity of this taxon, together with the recent positioning of other aberrant annelids such as Sipuncula, Echiura, Myzostomida, and Pogonophora [27].

Micrognathozoa Is Sister Group to Rotifera within Gnathifera

All our analyses supported monophyletic Gnathifera—a clade composed of protostomes with a special type of cuticular jaws—with Micrognathozoa as the sister group of Rotifera, both constituting the sister group of Gnathostomulida (Figures 1 and 2). Despite the microscopic size and understudied biology of most gnathiferan lineages (e.g., male micrognathozoans having not been observed), this topology has been supported previously with morphological data [6, 36, 37], albeit not using conventional molecular markers [38]. The main synapomorphies of

Rotifera + Micrognathozoa have been uncovered in ultrastructural studies of the epidermis [39] and of the jaw apparatus composed of rod-like structures [37], with Rotifera + Micrognathozoa having some common supporting musculature [7].

"Platyzoa" Is Likely a Systematic Artifact

Our mixture model analyses reject the monophyly of Platyzoa [8], a grouping of mainly interstitial taxa whose only shared characteristics, such as minute size (excepting some secondarily large Platyhelminthes and the acanthocephalan Rotifera; [40]), direct development, external ciliation, and an acoelomate or pseudocoelomate condition, are features also found in many other animals. The poorly supported division between Platyzoa/Polyzoa and Trochozoa, which we recover only under ML (Figure 1), neatly correlates (with the exceptions of *Diurodrilus* and *Lobatocerebrum*) with a division between fast-evolving and slow-evolving spiralians, suggesting the possibility of an LBA artifact [11]. Further, even though under both phylogenetic methods the problematic *Diurodrilus* and *Lobatocerebrum* are recovered as deeply nested annelids, the positions of these taxa within Annelida differ between reconstruction methods, with ML (Figure 1) placing these fast-evolving lineages in close proximity, consistent also with an LBA effect. It is remarkable that even the use of a statistically well-justified partitioning scheme, as provided by the PartitionFinder algorithm [41], groups the fast-evolving interstitial taxa into a clade (Figure 1). Only under the CAT + GTR + Γ4 mixture model do we recover non-monophyly of this long-branched assemblage, consistent with previous observations that such flexible models better fit the substitution-pattern heterogeneity characteristic of such large matrices, thereby rendering them more robust to model misspecification and subsequent LBA [42]. Apparently the relevant substitution process heterogeneities in such data may be occurring not between genes but between sites within genes (at, e.g., the domain level; [43]).

Interestingly, a similar resolution of "Platyzoa" as non-monophyletic has also been proposed in another recent study [11], also using RNA sequencing libraries as a source of phylogenetic evidence (several of which we reanalyze here with distinct assembly and orthology assignment algorithms). However, in this study, such a topology only emerged under consideration of specific gene and taxon subsets, and even then, no single analysis offered strong resampling support for all newly introduced clades (i.e., "Rouphozoa" and "Platytrochozoa"). Indeed, choosing to exclude specific data subsets may at times prove positively misleading: for instance, ML analysis of our fastest-evolving submatrix recovers a topology (albeit with low support) similar to our BI analyses (Figure 1C). This may thus be seen as an argument in favor of a "total evidence" approach to phylogenetics even at this scale of inference; although fast-evolving sites and genes may indeed mislead simple reconstruction methods, they may also retain valuable phylogenetic signal [44].

Was the Spiralian Ancestor a Microscopic, Acoelomate, Direct-Developing Worm?

The colonization of the interstices of marine sediments is among the most successful modes of life employed by metazoans, with nearly every major animal clade having at least some interstitial representatives and some being known exclusively from this habitat [45–47]. Animals that have adapted to such lifestyles,

sometimes known as meiofauna, bear a common set of characteristics, being generally of microscopic size, direct developing, with limited reproductive output and lifespan, and showing, relative to larger metazoans, a simplified, often acoelomate body design. Phylogenetic discussions regarding such meiofauna, including the members of "Platyzoa" [34], interstitial Annelida [3, 31], and other taxa such as the acoelomorph flatworms [48], have centered on the question of whether these morphologically "simple" taxa have originated via miniaturization from a macrofaunal ancestor, or have instead inherited their simple morphology from ancestors with similarly microscopic adults.

In this contribution, we aimed to address these themes within the major metazoan clade Spiralia, by resolving the interrelationships between the meiofaunal and macrofaunal members of this clade, including genome and transcriptome sampling of a range of previously sparsely sampled (Gnathostomulida) or unsampled microscopic taxa (Catenulida, Micrognathozoa, Chaetonotoida, Lobatocerebridae, Diurodrilidae). Under a phylogenetic mixture model (Figure 2), we find uniformly strong support for a topology in which a monophyletic Gnathifera forms the sister group to all other spiralians, with the remaining members of Spiralia split between a clade of, on the one hand, Platyhelminthes and Gastrotricha, and on the other, Lophotrochozoa. A parsimonious reading of this topology posits the common features of these interstitial worms as plesiomorphies, implying an interstitial, direct developing, unsegmented, acoelomate or pseudocoelomate condition for the spiralian ancestor. This further implies multiple independent origins of, e.g., segmentation, coelomic cavities, planktotrophic larvae, and other morphological structures across Bilateria.

However, under the topology recovered here, only two separate reductions in body size (miniaturizations) and transitions to an acoelomate condition—perhaps, though not necessarily, via progenesis—are required to derive Gnathifera and Rouphozoa from a macrofaunal, coelomate spiralian ancestor. If miniaturized taxa such as *Lobatocerebrum* and *Diurodrilus* have separate origins within Annelida, might not Gnathifera and Rouphozoa, clades that evince rather distinct manifestations of the acoelomate condition [17], therefore also be the remaining survivors of two ancient miniaturization events [13, 48, 49]? The principle of parsimony casts doubt on this scenario, as it posits the existence and independent extinction of two separate macrofaunal lineages related to both branches of "Platyzoa," a suggestion for which there is no fossil evidence, despite the widespread availability of exceptionally preserved Cambrian fossils of most other soft-bodied macrofaunal bilaterian lineages. This being recognized, there are continued arguments from comparative developmental genetic studies (reviewed by [50]) for homology across Bilateria in traits seemingly specific to macrofaunal animals, most recently extending to larval apical organs [51], a complex, tripartite forebrain [52], and collagenous midline supportive structures [53]. Unfortunately, the interpretation of such studies remains biased by the absence of data on the expression and function of developmental genes during the embryogenesis of gnathiferans, platyhelminths, and gastrotrichs.

Comparisons to outgroup taxa are critical to understanding the nature of the ancestor of Spiralia and earlier branches (Protostomia, and Bilateria). Ecdysozoa, one of two possible outgroups to Spiralia [1], encompasses substantial body plan diversity, and the relationships within this clade remain incompletely understood. However, it is possibly suggestive in this context that in this analysis as well as others [22], the members of Scalidophora, a clade of primarily interstitial, largely acoelomate or pseudocoelomate animals, are supported as sister taxon to other ecdysozoans. The precise placement of two other extant vermiform taxa—the enigmatic chaetognaths, representing a likely distinct branch of protostomes in their own right [54], and the acoelomorph flatworms (with or without *Xenoturbella*), representing either early-branching bilaterians or deuterostomes of uncertain precise placement [1]—may also provide some additional signal required to test the homology of the traits common to the "platyzoan" taxa. With the continued availability of genomic and genome-informed datasets from representatives of problematic taxa such as those presented here, we are approaching a clearer picture of the relationships, limits, and shared derived characteristics of not only these microscopic groups but also the most familiar branches of the metazoan tree. The evidence presented here has yielded the first well-resolved spiralian phylogeny inclusive of all free-living groups and hence provides clear hypotheses for future investigations to test, not least among which is the supposition that the ancestor of Spiralia was most probably a meiofaunal animal, as this is the predominant lifestyle of the two earliest-branching lineages within this diverse clade.

ACCESSION NUMBERS

See Table S1 for a full list of SRA accession numbers for previously unreported data.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.06.068.

AUTHOR CONTRIBUTIONS

C.E.L., K.W., and G.G. conceived the study. K.W., N.B., A.K., M.V.S. R.C.N., R.M.K., and A.H. collected specimens for RNA sequencing. A.H, C.E.L., C.W.D., K.W., and F.G. produced and sequenced cDNA libraries. A.K., A.H., C.E.L., N.B., and K.W. produced and improved all figures.

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REFERENCES

1. Dunn, C.W., Giribet, G., Edgecombe, G.D., and Hejnol, A. (2014). Animal phylogeny and its evolutionary implications. Annu. Rev. Ecol. Evol. Syst. *45*, 371–395.

2. Haszprunar, G., Rieger, R.M., and Schuchert, P. (1991). Extant "Problematica" within or near the Metazoa. In The Early evolution of Metazoa and the Significance of Problematic Taxa, A.M. Simonetta, and S. Conway Morris, eds. (Cambridge: Oxford University Press), pp. 99–105.

3. Rieger, R.M. (1980). A new group of interstitial worms, Lobatocerebridae nov. fam. (Annelida) and its significance for metazoan phylogeny. Zoomorphologie *95*, 41–84.

4. Kristensen, R.M., and Niilonen, T. (1982). Structural studies on *Diurodrilus* Remane (Diurodrilidae fam.n.), with description of *Diurodrilus westheidei* sp.n. from the Arctic interstitial meiobenthos, W. Greenland. Zool. Scr. *11*, 1–12.

5. Worsaae, K., and Rouse, G.W. (2008). Is *Diurodrilus* an annelid? J. Morphol. *269*, 1426–1455.

6. Kristensen, R.M., and Funch, P. (2000). Micrognathozoa: a new class with complicated jaws like those of Rotifera and Gnathostomulida. J. Morphol. *246*, 1–49.

7. Bekkouche, N., Kristensen, R.M., Hejnol, A., Sørensen, M.V., and Worsaae, K. (2014). Detailed reconstruction of the musculature in *Limnognathia maerski* (Micrognathozoa) and comparison with other Gnathifera. Front. Zool. *11*, 71.

8. Cavalier-Smith, T. (1998). A revised six-kingdom system of life. Biol. Rev. Camb. Philos. Soc. *73*, 203–266.

9. Giribet, G., Distel, D.L., Polz, M., Sterrer, W., and Wheeler, W.C. (2000). Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. Syst. Biol. *49*, 539–562.

10. Hejnol, A., Obst, M., Stamatakis, A., Ott, M., Rouse, G.W., Edgecombe, G.D., Martinez, P., Baguñà, J., Bailly, X., Jondelius, U., et al. (2009). Assessing the root of bilaterian animals with scalable phylogenomic methods. Proc. R. Soc. B Biol. Sci. *276*, 4261–4270.

11. Struck, T.H., Wey-Fabrizius, A.R., Golombek, A., Hering, L., Weigert, A., Bleidorn, C., Klebow, S., Iakovenko, N., Hausdorf, B., Petersen, M., et al. (2014). Platyzoan paraphyly based on phylogenomic data supports a noncoelomate ancestry of spiralia. Mol. Biol. Evol. *31*, 1833–1849.

12. Rieger, R.M. (1988). Comparative ultrastructure and the Lobatocerebridae: keys to understand the phylogenetic relationship of Annelida and the acoelomates. In Microfauna Marina, *Volume 4*, P. Ax, ed. (Gustav Fischer Verlag), pp. 373–382.

13. Rieger, R.M. (1991). Neue Organisationstypen aus der Sandlückenraumfauna: Die Lobatocerebriden und *Jennaria pulchra*. Verh. Dtsch. Zool. Ges. *84*, 247–259.

14. Kristensen, R.M. (1983). Loricifera, a new phylum with Aschelminthes characters from the meiobenthos. J. Zoolog. Syst. Evol. Res. *21*, 163–180.

15. Dunn, C.W., Hejnol, A., Matus, D.Q., Pang, K., Browne, W.E., Smith, S.A., Seaver, E., Rouse, G.W., Obst, M., Edgecombe, G.D., et al. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. Nature *452*, 745–749.

16. Halanych, K.M., Bacheller, J.D., Aguinaldo, A.M., Liva, S.M., Hillis, D.M., and Lake, J.A. (1995). Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. Science *267*, 1641–1643.

17. Aguinaldo, A.M.A., Turbeville, J.M., Linford, L.S., Rivera, M.C., Garey, J.R., Raff, R.A., and Lake, J.A. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. Nature *387*, 489–493.

18. Nesnidal, M.P., Helmkampf, M., Meyer, A., Witek, A., Bruchhaus, I., Ebersberger, I., Hankeln, T., Lieb, B., Struck, T.H., and Hausdorf, B. (2013). New phylogenomic data support the monophyly of Lophophorata and an Ectoproct-Phoronid clade and indicate that Polyzoa and Kryptrochozoa are caused by systematic bias. BMC Evol. Biol. *13*, 253.

19. Funch, P., and Kristensen, R.M. (1995). Cycliophora is a new phylum with affinities to Entoprocta and Ectoprocta. Nature *378*, 711–714.

20. Giribet, G., Dunn, C.W., Edgecombe, G.D., Hejnol, A., Martindale, M.Q., and Rouse, G.W. (2009). Assembling the spiralian tree of life. In Animal Evolution: Genes, Genomes, Fossils and Trees, M.J. Telford, and D.T.J. Littlewood, eds. (Oxford: Oxford University Press), pp. 52–64.

21. Nielsen, C. (2012). Animal Evolution - Interrelationships of the Living Phyla (Oxford: Oxford University Press).

22. Borner, J., Rehm, P., Schill, R.O., Ebersberger, I., and Burmester, T. (2014). A transcriptome approach to ecdysozoan phylogeny. Mol. Phylogenet. Evol. *80*, 79–87.

23. Lemburg, C. (1995). Ultrastructure of the sense organs and receptor cells of the neck and lorica of Halicryptus spinulosus larva (Priapulida). In Microfauna Marina, *Volume 10*, P. Ax, ed. (Gustav Fischer Verlag), pp. 7–30.

24. Remane, A. (1925). Diagnosen neuer Archianneliden (zugleich 3. Beitrag zur Fauna der Kieler Bucht). Zool. Anz. *65*, 15–17.

25. Hermans, C.O. (1969). The systematic position of the Archiannelida. Syst. Biol. *18*, 85–102.

26. Worsaae, K., and Kristensen, R.M. (2005). Evolution of interstitial Polychaeta (Annelida). Hydrobiologia *179*, 319–340.

27. Weigert, A., Helm, C., Meyer, M., Nickel, B., Arendt, D., Hausdorf, B., Santos, S.R., Halanych, K.M., Purschke, G., Bleidorn, C., and Struck, T.H. (2014). Illuminating the base of the annelid tree using transcriptomics. Mol. Biol. Evol. *31*, 1391–1401.

28. Andrade, S., Novo, M., Kawauchi, G., Pleijel, F., Giribet, G., and Rouse, G. Articulating the "archiannelids": a phylogenomic approach to annelid relationships with emphasis on meiofaunal taxa. Mol. Biol. Evol. Published online July 30, 2015. http://dx.doi.org/10.1093/molbev/msv157.

29. Golombek, A., Tobergte, S., Nesnidal, M.P., Purschke, G., and Struck, T.H. (2013). Mitochondrial genomes to the rescue—Diurodrilidae in the myzostomid trap. Mol. Phylogenet. Evol. *68*, 312–326.

30. Bleidorn, C. (2005). Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. Zool. J. Linn. Soc. *144*, 59–73.

31. Westheide, W. (1987). Progenesis as a principle in meiofauna evolution. J. Nat. Hist. *21*, 843–854.

32. Boyle, M.J., and Rice, M.E. (2014). Sipuncula: an emerging model of spiralian development and evolution. Int. J. Dev. Biol. *58*, 485–499.

33. Fransen, M.E. (1980). Ultrastructure of coelomic organization in annelids. Zoomorphologie *95*, 235–249.

34. Rieger, R.M. (1985). The phylogenetic status of the acoelomate organization within the Bilateria: a histological perspective. In The Origins and Relationships of Lower Invertebrates Systematics Association Special Volumes, S. Conway-Morris, J.D. George, R. Gibson, and H.M. Platt, eds. (Oxford: Oxford University Press), pp. 101–122.

35. Koch, M., Quast, B., and Bartolomaeus, T. (2014). Coeloms and nephridia in annelids and arthropods. In Deep Metazoan Phylogeny: the Backbone of the Tree of Life; New Insights from Analyses of Molecules, Morphology, and Theory of Data Analysis, J.W. Waegele, and T. Bartolomaeus, eds. (Berlin: De Gruyter), pp. 173–284.

36. Sørensen, M.V. (2002). Phylogeny and jaw evolution in Gnathostomulida, with a cladistic analysis of the genera. Zool. Scr. *31*, 461–480.

37. Sørensen, M.V. (2003). Further structures in the jaw apparatus of *Limnognathia maerski* (Micrognathozoa), with notes on the phylogeny of the Gnathifera. J. Morphol. *255*, 131–145.

38. Giribet, G., Sørensen, M.V., Funch, P., Kristensen, R.M., and Sterrer, W. (2004). Investigations into the phylogenetic position of Micrognathozoa using four molecular loci. Cladistics *20*, 1–13.

39. Ahlrichs, W.H. (1997). Epidermal ultrastructure of *Seison nebaliae* and *Seison annulatus*, and a comparison of epidermal structures within the Gnathifera. Zoomorphology *117*, 41–48.

40. Laumer, C.E., Hejnol, A., and Giribet, G. (2015). Nuclear genomic signals of the 'microturbellarian' roots of platyhelminth evolutionary innovation. eLife *4*, 05503.

41. Lanfear, R., Calcott, B., Ho, S.Y.W., and Guindon, S. (2012). Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. *29*, 1695–1701.

42. Lartillot, N., Brinkmann, H., and Philippe, H. (2007). Suppression of long-branch attraction artefacts in the animal phylogeny using a site-heterogeneous model. BMC Evol. Biol. *7* (1), S4.

43. Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G., et al. (2014). Phylogenomics resolves the timing and pattern of insect evolution. Science *346*, 763–767.

44. Gatesy, J., and Baker, R.H. (2005). Hidden likelihood support in genomic data: can forty-five wrongs make a right? Syst. Biol. *54*, 483–492.

45. Swedmark, B. (1964). The interstitial fauna of marine sand. Biol. Rev. Camb. Philos. Soc. *39*, 1–42.

46. Higgins, R.P., and Thiel, H. (1988). Introduction to the Study of Meiofauna (Washington, D.C.: Smithsonian Institution Press).

47. Curini-Galletti, M., Artois, T., Delogu, V., De Smet, W.H., Fontaneto, D., Jondelius, U., Leasi, F., Martínez, A., Meyer-Wachsmuth, I., Nilsson, K.S., et al. (2012). Patterns of diversity in soft-bodied meiofauna: dispersal ability and body size matter. PLoS ONE *7*, e33801.

48. Tyler, S. (2001). The early worm–origins and relationships of the lower flatworms. In Interrelationships of the Platyhelminthes Systematics Association Special, *Volume D*, T.J. Littlewood, and R.A. Bray, eds. (London: Taylor and Francis), pp. 3–12.

49. Rieger, R.M. (1994). The biphasic life cycle—a central theme of metazoan evolution. Am. Zool. *34*, 484–491.

50. Hejnol, A., and Martindale, M.Q. (2008). Acoel development supports a simple planula-like urbilaterian. Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 1493–1501.

51. Marlow, H., Tosches, M.A., Tomer, R., Steinmetz, P.R., Lauri, A., Larsson, T., and Arendt, D. (2014). Larval body patterning and apical organs are conserved in animal evolution. BMC Biol. *12*, 7.

52. Strausfeld, N.J., and Hirth, F. (2013). Deep homology of arthropod central complex and vertebrate basal ganglia. Science *340*, 157–161.

53. Lauri, A., Brunet, T., Handberg-Thorsager, M., Fischer, A.H.L., Simakov, O., Steinmetz, P.R.H., Tomer, R., Keller, P.J., and Arendt, D. (2014). Development of the annelid axochord: insights into notochord evolution. Science *345*, 1365–1368.

54. Marlétaz, F., Martin, E., Perez, Y., Papillon, D., Caubit, X., Lowe, C.J., Freeman, B., Fasano, L., Dossat, C., Wincker, P., et al. (2006). Chaetognath phylogenomics: a protostome with deuterostome-like development. Curr. Biol. *16*, R577–R578.