Spiralian Phylogenomics Supports the Resurrection of Bryozoa Comprising Ectoprocta and Entoprocta

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Phylogenetic analyses based on 79 ribosomal proteins of 38 metazoans, partly derived from 6 new expressed sequence tag projects for Ectoprocta, Entoprocta, Sipuncula, Annelida, and Acanthocephala, indicate the monophyly of Bryozoa comprising Ectoprocta and Entoprocta, 2 taxa that have been separated for more than a century based on seemingly profound morphological differences. Our results also show that bryozoans are more closely related to Neotrochozoa, including molluscs and annelids, than to Syndermata, the latter comprising Rotifera and Acanthocephala. Furthermore, we find evidence for the position of Sipuncula within Annelida. These findings suggest that classical developmental and morphological key characters such as cleavage pattern, coelomic cavities, gut architecture, and body segmentation are subject to greater evolutionary plasticity than traditionally assumed.

Introduction

With the establishment of Lophotrochozoa and Ecdysozoa (Halanych et al. 1995; Aguinaldo et al. 1997), molecular data have substantially changed our view of animal evolution. Recent phylogenomic approaches have generally sustained these hypotheses (Philippe et al. 2005; Philippe and Telford 2006; Baurain et al. 2007), but adequate genomic data are still lacking for many minor phyla whose affinities are still in dispute (Giribet et al. 2000; Halanych 2004). Two of the most enigmatic minor animal phyla are the moss animals, that is, Ectoprocta and Entoprocta. When first discovered, entoprocts (Kamptozoa) were treated together with the ectoproct bryozoans because of their sessile life style and ciliated tentacles. Nitsche (1869) pointed to the differences between the position of the anus and the retractability of the tentacle crowns and proposed the names Entoprocta and Ectoprocta for the 2 main groups of bryozoans. Subsequently, the 2 groups have almost unanimously been treated as separate higher taxa, mainly based on the differences in cleavage patterns and body cavities (Hatschek 1891; Korschelt and Heider 1893; Hennig 1979; Emschermann 1982; Schram 1991; Zrzavý et al. 1998; Ax 1999; Giribet et al. 2000; Sørensen et al. 2000; Brusca and Brusca 2002). So far, all analyses of rDNA sequences have supported the assumption that they do not constitute sister taxa (Mackey et al. 1996; Littlewood et al. 1998; Zrzavý et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001; Passamaneck and Halanych 2006). However, Nielsen (1971, 1985, 2001) and Cavalier-Smith (1998) maintained the monophyly of Bryozoa in the broader sense.

To acquire molecular data sufficient for a resolution of the phylogenetic relationships of ectoprocts and entoprocts, we generated 2,000–4,000 expressed sequence tags (ESTs) from representatives of Ectoprocta, Entoprocta, Sipuncula, Annelida, and Acanthocephala (table 1). The comparison of the 6 analyzed transcriptomes revealed a broad coverage of ribosomal proteins, which are valuable markers for phylogenomic analyses (Veuthey and Bittar 1998; Philippe et al. 2004; Hughes et al. 2006; Marlétaz et al. 2006) because of the rarity of known gene duplications resulting in paralogs and their conservation among eukaryotes. We compiled from our EST projects a data set comprising 79 ribosomal proteins, which we complemented by orthologous sequences of 32 additional taxa obtained from public databases.

Key words: Metazoa, phylogenomics, Bryozoa, Ectoprocta, Entoprocta, Spiralia.

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Materials and Methods

Isolation of RNA and Library Construction

Total RNA of the organisms specified in table 1 was extracted from living or frozen tissue employing TRIzol (Invitrogen, Karlsruhe, Germany) or column-based methods (Qiagen RNeasy Plant Mini Kit). *Flustra* RNA was additionally purified by the RNeasy Mini Kit cleanup procedure (Qiagen, Hilden, Germany), whereas for the purification of *Barentsia* RNA, we applied the NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany). Quality of total RNA was visually checked on agarose gel, and mRNA was subsequently captured by using the polyATract mRNA Isolation System III (Promega, Mannheim, Germany) or Dynabeads (Invitrogen, Karlsruhe, Germany) for *Sipunculus*. All cDNA libraries were constructed at the Max Planck Institute for Molecular Genetics in Berlin by primer extension, size fractioning, and directional cloning applying the Creator SMART cDNA Libraries Kit (Clontech, Heidelberg, Germany) or Invitrogen's CloneMiner technology (*Arenicola* only), using the respective vectors pDNR-LIB or pDONR222. Clones containing cDNA inserts were sequenced from the 5′ end on the automated capillary sequencer systems ABI 3730 XL (Applied Biosystems, Darmstadt, Germany) and MegaBace 4500 (GE Healthcare, München, Germany) using BigDye chemistry (Applied Biosystems). If possible, clones containing ribosomal proteins from the libraries of *Barentsia* and *Sipunculus* were completed by reverse sequencing with polyT- and vector-specific reverse primer to maximize sequence coverage.

EST Processing

EST processing was accomplished at the Center for Integrative Bioinformatics in Vienna. Sequencing

Table 1
List of Investigated Taxa and Data Used in Phylogenetic Analyses

Species	Taxon	Origin	# EST	# RP
Flustra foliacea (Linnaeus 1758)	Ectoprocta	Helgoland, North Sea	4.074	77
Barentsia elongata (Jullien and Calvet 1903)[a]	Entoprocta	Lab culture	2.154	47
Arenicola marina (Linnaeus 1758)	Annelida	Sylt, North Sea	2.199	61
Eurythoe complanata (Pallas 1776)	Annelida	Lab culture	2.257	41
Sipunculus nudus (Linnaeus 1766)	Sipuncula	Roscoff, France	2.329	48
Pomphorhynchus laevis (Müller 1776)	Acanthocephala	Gimbsheim, Germany (from host *Barbus fluviatilis*)	2.207	65

NOTE.—# EST: number of sequenced EST clones; # RP: number of ribosomal proteins retrieved at least partially from the EST data sets. Voucher specimens were deposited at the Zoological Museum, Hamburg.

[a] The data set of *B. elongata* was complemented by 2 sequences derived from 95 ESTs of *Barentsia benedeni* (Foettinger 1886).

chromatograms were first base called and evaluated using the Phred application (Ewing et al. 1998). Vector, adapter, poly-A, and bacterial sequences were removed employing the software tools Lucy (www.tigr.org), SeqClean (compbio.dfci.harvard.edu/tgi/software), and CrossMatch (www.phrap.org). Repetitive elements were subsequently masked with RepeatMasker. Clustering and assembly of the clipped sequences were performed using the TIGCL program package (compbio.dfci.harvard.edu/tgi/software) by first performing pairwise comparisons (MGIBlast) and a subsequent clustering step (CAP3). Low-quality regions were then removed by Lucy. Finally, contigs were tentatively annotated by aligning them pairwise with the 25 best hits retrieved from National Center for Biotechnology Information's nonredundant protein database using the BlastX algorithm (www.ncbi.nlm.nih.gov). Alignment and computation of the resulting match scores on which annotation was based were conducted by GeneWise (Birney et al. 2004) in order to account for frameshift errors. The EST data used in our analyses have been deposited in GenBank under the accession numbers EU139167–EU139243 (*Flustra*), EU116892–EU116936, EU220741 (*Barentsia*), EU116844–EU116891 (*Sipunculus*), EU124931–EU124992 (*Arenicola*), EU124993–EU125033 (*Eurythoe*), and AM849482–AM849546 (*Pomphorhynchus*).

Sequence Analyses and Ribosomal Proteins Alignment

Ribosomal protein sequences were extracted from the newly obtained EST data by their annotation or by using the human ribosomal protein genes retrieved from the Ribosomal Protein Gene Database (ribosome.med.miyazaki-u.ac.jp) as search template during local Blast searches (using the TblastN algorithm and an *e* value $<e^{-10}$ as match criterion). The observed sequences were checked for assembly errors by visual inspection and by comparison with corresponding sequences of related taxa, and translated into amino acid sequences. Orthologous sequences of *Priapulus caudatus*, *Ascaris suum*, *Aplysia californica*, *Idiosepius paradoxus*, *Macrostomum lignano*, *Philodina roseola*, *Flaccisagitta enflata*, and *Strongylocentrotus purpuratus* were obtained from public EST databases using TblastN searches also employing human sequences as query. Additional ribosomal protein data were retrieved from the alignments compiled by Baurain et al. (2007) and provided by H. Philippe (Université de Montréal), and complemented for missing genes. Ribosomal proteins of *Ciona intestinalis*, *Takifugu rubripes*, *Anopheles gambiae*, and, in part, *Apis mellifera* were acquired directly from the Ribosomal Protein Gene Database. Sequences of *Spadella cephaloptera* were provided by F. Marlétaz (Station Marine d'Endoume, Marseille).

All ribosomal protein sequences obtained were aligned by the ClustalW algorithm (Thompson et al. 1994). The resulting 79 ribosomal protein alignments were inspected and adjusted manually. Questionably aligned positions were eliminated with Gblocks (Castresana 2000), applying all less stringent block selection parameters available and thereafter concatenated to a single multiple sequence alignment. This alignments is available at TreeBASE (http://www.treebase.org; accession number S1884).

Phylogenetic Analyses

Maximum Likelihood (ML) analyses were conducted with Treefinder (Jobb et al. 2004; Jobb 2007). The rtRev + G + F model of protein evolution was used for the ML analyses because it was superior to other uniform models for the concatenated data set as well as a mixed model combining separate models as determined by ProtTest (Abascal et al. 2005) for each of the 79 gene partitions according to the Akaike Information Criterion with a correction term for small sample size. Confidence values for the edges of the ML tree were computed by applying expected likelihood weights (ELWs) (Strimmer and Rambaut 2002) to all local rearrangements (LR) of tree topology around an edge (1,000 replications).

To test predefined phylogenetic hypotheses, we used constrained trees and the 'resolve multifurcations' option of Treefinder to obtain the ML tree for a specified hypothesis. Then we investigated whether the ML trees for these hypotheses are part of the confidence set of trees applying the expected likelihood weights method (Strimmer and Rambaut 2002).

Bayesian inference (BI) analyses based on the site-heterogeneous CAT model (Lartillot and Philippe 2004) were performed using PhyloBayes v2.1c (Blanquart and Lartillot 2006). Two independent chains were run simultaneously for 10,000 points each. Chain equilibrium was estimated by plotting the log-likelihood and the alpha parameter as a function of the generation number. The first 1,000 points were consequently discarded as burn-in. According to the

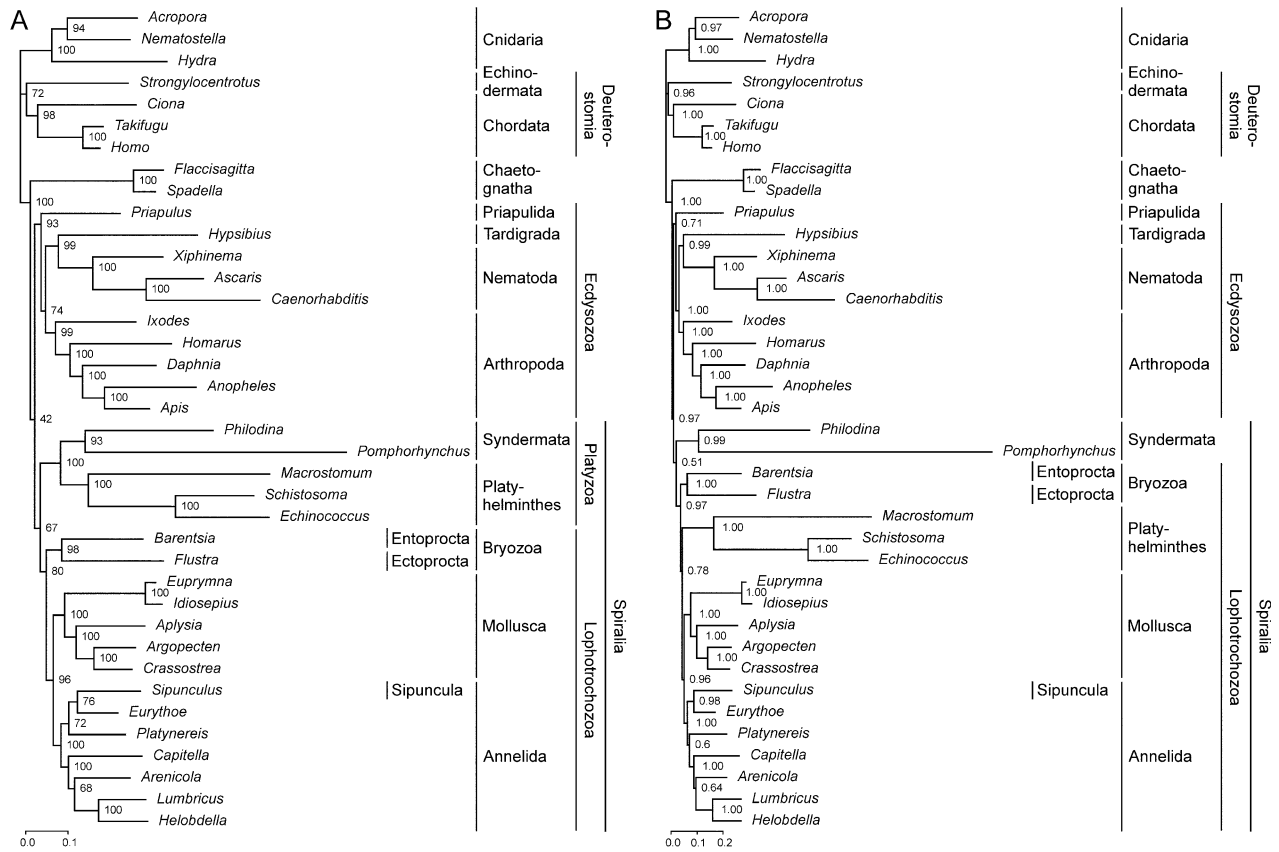

FIG. 1.—Spiralian phylogenomics unites ectoprocts with entoprocts, resurrecting Bryozoa *sensu lato*. Phylogenetic analyses were performed on the basis of 11,428 amino acid positions derived from 79 concatenated ribosomal proteins. (*A*) ML tree. Approximate bootstrap support values (LR-ELW) are shown to the right of the nodes. (*B*) BI reconstruction. Bayesian posterior probabilities are shown to the right of the nodes.

divergence of bipartition frequencies, both chains reached convergence (maximal difference <0.3, mean difference <0.005), supported by the fact that both chains produced the same consensus tree topology. Taking every 10th sampled tree, a 50% majority rule consensus tree was finally computed using both chains.

Results and Discussion

Bryozoa *sensu lato*: A Century-Old Hypothesis Resurrected

Phylogenetic analyses of the concatenated sequences of 79 ribosomal proteins encompassing 11,428 amino acid positions show for the first time Bryozoa as a monophyletic clade comprising Entoprocta and Ectoprocta. The monophyly is supported by strong nodal support values (fig. 1). Therefore, the century-old hypothesis of Bryozoa in the broader sense has to be resurrected.

Ectoprocts have been included in Lophophorata based on similarities of the tentacular apparatus and the radial cleavage they share with phoronids and brachiopods. Lophophorata was traditionally considered the sister or paraphyletic stem group of Deuterostomia (Hennig 1979; Schram 1991; Ax 1995; Brusca and Brusca 2002). However, studies employing rDNA (Halanych et al. 1995; Mackey et al. 1996; Littlewood et al. 1998; Peterson and Eernisse 2001; Mallatt and Winchell 2002; Halanych 2004; Passamaneck and Halanych 2006), *Hox* genes (Passamaneck and Halanych 2004), multiple nuclear genes (Helmkampf et al. forthcoming), and mitochondrial protein sequences (Stechmann and Schlegel 1999; Helfenbein and Boore 2004; Waeschenbach et al. 2006) showed that Ectoprocta as well as Phoronida and Brachiopoda are more closely related to Annelida, Mollusca, and allies than to Deuterostomia or Ecdysozoa. Therefore, Halanych et al. (1995) united them under the name Lophotrochozoa. Some of these studies further demonstrated that Lophophorata is polyphyletic (Halanych et al. 1995; Mackey et al. 1996; Littlewood et al. 1998; Giribet et al. 2000; Halanych 2004; Passamaneck and Halanych 2006; Helmkampf et al. forthcoming). On the basis of our data, the hypotheses that ectoprocts are related to Deuterostomia, that they are sister to all remaining Spiralia (Halanych et al. 1995; Littlewood et al. 1998; Halanych 2004; Passamaneck and Halanych 2006), and that they are sister to all other protostomes except chaetognaths (Giribet et al. 2000) could be rejected by topology tests (table 2, hypotheses 1–3).

Entoprocts exhibit spiral cleavage and trochophora-type larvae, leading to the assumption of closer connections to taxa also possessing these features (Ax 1995, 1999; Zrzavý et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001). Molecular phylogenetic analyses of 18S rDNA generally confirmed the affiliation of entoprocts with taxa having trochophora larvae, but their exact relationships

Table 2
Topology Test Results

Number	Phylogenetic Hypothesis	References	ELW Test
	ML tree (fig. 1*A*)		0.3452*
1	Lophophorata + Deuterostomia	Hennig (1979); Schram (1991); Ax (1995); Sørensen et al. (2000); Brusca and Brusca (2002)	0.0000
2	Ectoprocta sister to other Spiralia	Halanych et al. (1995); Halanych (2004); Passamaneck and Halanych (2006)	0.0007
3	Ectoprocta sister to other Spiralia + Ecdysozoa	Giribet et al. (2000)	0.0006
4	Lacunifera (=Entoprocta + Mollusca)	Bartolomaeus (1993); Haszprunar (1996); Ax (1999); Haszprunar (2000)	0.0037
5	Entoprocta + Annelida (+Sipuncula)	Emschermann (1982)	0.0094
6	Entoprocta + Platyzoa	Halanych (2004); Passamaneck and Halanych (2006)	0.0262
7	Entoprocta + Neotrochozoa	Zrzavý et al. (1998); Giribet et al. (2000); Peterson and Eernisse (2001)	0.0804*
8	Articulata (=Annelida + Arthropoda)	Hennig (1979); Schram (1991); Ax (1999); Sørensen et al. (2000); Nielsen (2001); Brusca and Brusca (2002)	0.0000
9	Annelida monophyly (exclusive Sipuncula)	Schram (1991); Zrzavý et al. (1998); Ax (1999); Giribet et al. (2000); Sørensen et al. (2000); Nielsen (2001); Brusca and Brusca (2002); Passamaneck and Halanych (2006)	0.1000*
10	Sipuncula + Mollusca	Scheltema (1993); Zrzavý et al. (1998)	0.0000
11	Sipuncula sister to (Annelida + Mollusca)	Giribet et al. (2000)	0.0000
12	Eubilateria	Hennig (1979); Ax (1985)	0.0000
13	Chaetognatha + Deuterostomia	Ghirardelli (1981); Sørensen et al. (2000); Brusca and Brusca (2002)	0.0271*
14	Chaetognatha sister to Spiralia	Matus et al. (2006)	0.2221*
15	Chaetognatha + Ecdysozoa	Littlewood et al. (1998); Zrzavý et al. (1998); Peterson and Eernisse (2001)	0.1847*

NOTE.—Numbers refer to the order of appearance in the text. Values for the topologies included in the 0.95 confidence set are indicated by an asterisk (i.e., ELW of the trees with the highest confidence levels that added up to 0.95).

remained controversial (Mackey et al. 1996; Littlewood et al. 1998; Zrzavý et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001). Combined analyses of 18S and 28S rDNA data resulted in a placement within Platyzoa, but also without significant support (Passamaneck and Halanych 2006).

With our data, most alternative hypotheses concerning the phylogenetic position of Entoprocta, in particular a sister group relationship between Entoprocta and Mollusca (Bartolomaeus 1993; Haszprunar 1996, 2000; Ax 1999), a neotenic origin of entoprocts from annelids (Emschermann 1982), and their placement within Platyzoa (Halanych 2004; Passamaneck and Halanych 2006) could be ruled out according to the expected likelihood weights test (table 2, hypotheses 4–6). However, a sister group relationship between Entoprocta and Neotrochozoa, which comprises Mollusca, Sipuncula, and Annelida (Zrzavý et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001), could not be significantly rejected (table 2, hypothesis 7). Nonetheless, our analyses strongly support the monophyly of Bryozoa in the broader sense including Ectoprocta and Entoprocta and thus confirm the morphology-based argumentation of Nielsen (1971, 1985, 2001) and Cavalier-Smith (1998). Morphological data (Funch and Kristensen 1995; Zrzavý et al. 1998; Sørensen et al. 2000) and rDNA sequences (Passamaneck and Halanych 2006) indicate that Entoprocta and Cycliophora are sister groups. Although genomic data for Cycliophora are unfortunately still missing, we suggest to also include Cycliophora in Bryozoa *sensu lato* as has been done by Cavalier-Smith (1998).

Sipuncula as an Annelid Taxon

Both ML (fig. 1*A*) and BI analyses (fig. 1*B*) recovered Neotrochozoa, which comprises Mollusca, Sipuncula, and Annelida, thus confirming studies using morphological and molecular data (Zrzavý et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001). Based on segmentation, Annelida has traditionally been regarded as sister to Arthropoda (Hennig 1979; Schram 1991; Sørensen et al. 2000; Nielsen 2001; Brusca and Brusca 2002), but this so-called Articulata hypothesis is significantly rejected by topology testing (table 2, hypothesis 8).

In accordance with mitochondrial amino acid sequences and gene order data (Boore and Staton 2002; Staton 2003; Jennings and Halanych 2005; Bleidorn et al. 2006), our analyses indicate with strong support that Sipuncula is more closely related to Annelida than to Mollusca (fig. 1). More precisely, these unsegmented worms appear as a subtaxon of Annelida, which has also been suggested in some previous analyses (Peterson and Eernisse 2001; Bleidorn et al. 2006; Struck et al. 2007). However, the monophyly of Annelida excluding Sipuncula (Schram 1991; Zrzavý et al. 1998; Ax 1999; Giribet et al. 2000; Sørensen et al. 2000; Nielsen 2001; Brusca and Brusca 2002; Passamaneck and Halanych 2006) could not be ruled out by topology testing (table 2, hypothesis 9). On the other hand, the alternative hypotheses that Sipuncula forms a monophyletic group with Mollusca (Scheltema 1993; Zrzavý et al. 1998) and that Sipuncula is sister to Annelida plus Mollusca (Giribet et al. 2000) were rejected (table 2, hypotheses 10 and 11).

Spiralia—Syndermata, Platyhelminthes, and Lophotrochozoa

Our analyses strongly support the clade Syndermata, formed by Rotifera and Acanthocephala (fig. 1). This taxon has been established on the basis of morphological evidence (Ahlrichs 1995a, 1995b, 1997) and has been further

supported by analyses of 18S rDNA sequences (Garey et al. 1996; Garey and Schmidt-Rhaesa 1998; Littlewood et al. 1998; Zrzavý et al. 1998; Giribet et al. 2000; Herlyn et al. 2003).

The position of Platyhelminthes differs in our analyses as either being sister to Syndermata (fig. 1*A*) or to Neotrochozoa (fig. 1*B*). The former confirms the Platyzoa hypothesis. Platyzoa comprise Platyhelminthes, Syndermata, Gastrotricha, and Gnathostomulida (Garey and Schmidt-Rhaesa 1998; Cavalier-Smith 1998; Giribet et al. 2000) and has first been hypothesized by Ahlrichs (1995a) based on sperm morphology. Platyzoa was either corroborated (Giribet et al. 2000; Passamaneck and Halanych 2006) or contradicted (Zrzavý et al. 1998; Peterson and Eernisse 2001) by rDNA and total evidence analyses. The lack of a robust resolution of the phylogenetic relationships of Platyhelminthes within Spiralia despite the large available data set is probably due to increased substitution rates in Platyhelminthes and Syndermata causing long-branch attraction artifacts. However, the Eubilateria hypothesis (Hennig 1979; Ax 1985) can clearly be rejected by topology testing (table 2, hypothesis 12). According to this hypothesis, Platyhelminthes, which do not have an anus, are considered to be the sister group of all other Bilateria possessing a 1-way gut and an anus.

Lophotrochozoa is defined as including the last common ancestor of lophophorates, molluscs, and annelids, and its descendants (Halanych et al. 1995). Because Bryozoa is more closely related to Neotrochozoa than to Syndermata in our analyses (fig. 1), syndermatans (and according to the ML analysis also platyhelminths) are not lophotrochozoans, even though to further substantiate this conclusion genomic data of Phoronida and Brachiopoda are necessary.

For the clade including Lophotrochozoa, Platyhelminthes, and Syndermata, some authors have used the name Spiralia (Garey and Schmidt-Rhaesa 1998; Giribet et al. 2000; Helmkampf et al. forthcoming). We follow this usage because spiral quartet cleavage might be an autapomorphy of that taxon (see below).

Chaetognatha Remain Enigmatic

Chaetognatha, or arrow worms, represents the sister group of Spiralia and Ecdysozoa in our analyses (fig. 1). This confirms previous findings based on analyses of 18S rDNA (Giribet et al. 2000), mitochondrial DNA (Helfenbein et al. 2004), and an EST data set (Marlétaz et al. 2006). However, alternative hypotheses, namely a common ancestry with Deuterostomia (Ghirardelli 1981; Brusca and Brusca 2002) or Ecdysozoa (Littlewood et al. 1998; Zrzavý et al. 1998; Peterson and Eernisse 2001) or a sister group relationship to Spiralia (Matus et al. 2006), could not be excluded (table 2, hypotheses 13–15). The phylogenetic position of chaetognaths thus remains elusive.

Implications for Character Evolution

Cleavage pattern was often considered a key character for the reconstruction of metazoan phylogeny. Typical spiral quartet cleavage with mesoderm formation by the 4d mesoteloblast or one of its daughter cells (Sørensen et al. 2000; Nielsen 2001) is known from several lophotrochozoan groups (Mollusca, Annelida, Nemertea, and Entoprocta), Platyhelminthes, and Gnathostomulida. If we map this character state on our tree (fig. 1) considering the close relationship of Syndermata to Gnathostomulida (Ahlrichs 1995a, 1995b, 1997; Cavalier-Smith 1998; Garey and Schmidt-Rhaesa 1998; Giribet et al. 2000; Sørensen et al. 2000; Nielsen 2001), it turns out to be a possible autapomorphy of the clade including Syndermata, Plathyhelminthes, and Lophotrochozoa, for which we accepted the name Spiralia, although it has been secondarily modified several times within this clade (e.g., in Syndermata, Neoophora, Ectoprocta, Brachiopoda, and Cephalopoda). The sister group relation of ectoprocts and entoprocts demonstrates that the transition from spiral to radial cleavage can happen within a clade without any transitional stages being preserved. After all, the different cleavage types were one of the main reasons that the 2 taxa were classified in different major groups for more than a century.

Often coelomic cavities were considered an autapomorphy of a clade Coelomata (Hennig 1979; Blair et al. 2002; Philip et al. 2005). If the coelomic cavities of lophotrochozoans are considered homologous to those of deuterostomes and to the small coelomic cavities present in some ecdysozoans, our trees would indicate a frequent reduction of coelomic cavities in several bilaterian lineages (e.g., in chaetognaths, priapulids, nematodes, platyzoans, and entoprocts). However, the differing developmental origin of coelomic cavities in the different bilaterian lineages cast doubts on the homology of the coelom across bilaterians (Nielsen 2001).

The significant rejection of the Eubilateria hypothesis and the derived position of platyhelminths within Spiralia indicates that the anus has been secondarily reduced in platyhelminths, in which the mouth is the only opening to the intestinal system.

Finally, the significant rejection of Articulata as well as the derived position of Annelida within Spiralia supports the hypothesis that segmentation originated convergently in annelids and arthropods. The placement of unsegmented worms within Annelida, namely Sipuncula (this study; Peterson and Eernisse 2001; Bleidorn et al. 2006; Struck et al. 2007) and Echiura (McHugh 1997; Bleidorn et al. 2003; Struck et al. 2007), further reveals that segmentation has been secondarily lost in annelid subtaxa. Sipunculans possess a U-shaped gut, a feature already established in Cambrian fossils (Huang et al. 2004). The movement of the anus in the anterior direction requires the disorganization of segmentation, a factor that may have eased inhabiting holes in solid substrates.

The results presented herein, therefore, indicate that several of the supposed key characters of animal phylogeny such as cleavage pattern, coelomic cavities, body segmentation, and gut architecture are much more variable during evolution than previously thought.

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