Phylogenetic Position of Nemertea Derived from Phylogenomic Data

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Nemertea and Platyhelminthes have traditionally been grouped together because they possess a so-called acoelomate organization, but lateral vessels and rhynchocoel of nemerteans have been regarded as coelomic cavities. Additionally, both taxa show spiral cleavage patterns prompting the placement of Nemertea as sister to coelomate Protostomia, that is, either to Neotrochozoa (Mollusca and Annelida) or to Teloblastica (Neotrochozoa plus Arthropoda). Some workers maintain a sister group relationship of Nemertea and Platyhelminthes as Parenchymia because of an assumed homology of Götte’s and Müller’s larvae of polyclad Platyhelminthes and the pilidium larvae of heteronemerteans. So far, molecular data were only able to significantly reject a sister group relationship to Teloblastica.

Although phylogenomic data are available for Platyhelminthes, Annelida, Mollusca, and Arthropoda, they are lacking for Nemertea. Herein, we present the first analysis specifically addressing nemertean phylogenetic position using phylogenomic data. More specifically, we collected expressed sequence tag data from *Lineus viridis* (O.F. Müller, 1774) and combined it with available data to produce a data set of 9,377 amino acid positions from 60 ribosomal proteins. Maximum likelihood analyses and Bayesian inferences place Nemertea in a clade together with Annelida and Mollusca. Furthermore, hypothesis testing significantly rejected a sister group relationship to either Platyhelminthes or Teloblastica. The Coelomata hypothesis, which groups coelomate taxa together to the exclusion of acoelomate and pseudocoelomate taxa, is not congruent with our results. Thus, the supposed acoelomate organization evolved independently in Nemertea and Platyhelminthes. In Nemertea, evolution of acocelom is most likely due to a secondary reduction of the coelom as it is found in certain species of Mollusca and Annelida. Though looking very similar, the Götte’s and Müller’s larvae of polyclad Platyhelminthes are not homologous to the pilidium larvae of heteronemerteans. Finally, the convergent evolution of segmentation in Annelida and Arthropoda is further substantiated.

Introduction

Nemertea or ribbon worms are primarily benthic marine invertebrates, but a few of the approximately 900 described species inhabit pelagic, freshwater, or terrestrial habitats and some are symbionts of other marine invertebrates (Brusca RC and Brusca GJ 2003). These unsegmented worms range in length from less than 1 cm to several meters and their bodies are quite flexible so that some species can increase their contracted body length several times (Brusca RC and Brusca GJ 2003). Specimens of *Lineus longissimus* (Gunnerus, 1770) are among the longest known invertebrates with a length of over 30 m (Turbeville 1996). Due to their general dorsoventrally flattened, and only moderately cephalized, habitus, the first described nemerteans were regarded as turbellarian flatworms. Only in the 19th century was the taxon Nemertea erected and placed outside Platyhelminthes (Schultze 1851; Minot 1876; Coe 1943; Hyman 1951; Brusca RC and Brusca GJ 1990). The monophyly of Nemertea is well established and primarily based on their unique reversible prosocis, which is surrounded by a rhynchocoel (e.g., Turbeville 1996; Brusca RC and Brusca GJ 2003; Jenner 2004b).

Based on traditional concepts of secondary body cavities, bilaterian taxa have been categorized into 3 levels of organization: acoelomate, pseudocoelomate, and coelomate (Hyman 1951; Jenner 2004b). Both, Nemertea and Platyhelminthes, were regarded as acoelomate and accordingly grouped together (Hyman 1951). Additionally, the acoelomate and pseudocoelomate organization were regarded as ancestral and the coelomate one as the supposed synapomorphy of Coelomata (Hennig 1979; Blair et al. 2002; Philip et al. 2005). However, the lateral vessels of the closed circulatory system and the rhynchocoel of nemerteans have been proven to represent coelomic cavities, which arise by schizocoely (Turbeville and Ruppert 1985; Turbeville 1986). Furthermore, Nemertea and Platyhelminthes show spiral cleavage patterns and thus most authors place them in a clade, which also contains other taxa exhibiting spiral cleavage such as Annelida, Mollusca, and Entoprocta (Brusca RC and Brusca GJ 1990, 2003; Meglitsch and Schram 1991; Ax 1995; Nielsen 1995, 2001; Rouse and Fauchald 1995; Haszprunar 1996; Nielsen et al. 1996; Cavalier-Smith 1998; Carey and Schmidt-Rhaesa 1998; Sundberg et al. 1998; Zrzavy et al. 1998, 2001; Giribet et al. 2000; Sorensen et al. 2000; Peterson and Eernisse 2001; Zrzavy 2003; Jenner 2004b).

The taxon composition of this clade, as well as the specific position of Nemertea within it, is controversial. Discussions based on morphological data center on 3 major topics, which result in 3 distinct hypothesis concerning sister group relationships of Nemertea: Are the Götte’s and Müller’s larvae of polyclad Platyhelminthes and the pilidium larvae of heteronemerteans homologous (e.g., Nielsen 1995)? Are the coelomic cavities of Nemertea homologous with coelomic spaces of other taxa, for example, Annelida (e.g., Turbeville 1986)? Finally, though not directly related to Nemertea, is Arthropoda part of this clade or have they to be placed within Ecdysozoa (e.g., Brusca RC and Brusca GJ 1990; Peterson and Eernisse 2001)?

Mainly based on similarities of larval features such as a reduced hyposphere, the lack of an anus and the shape of the larval ciliary bands a close relationship of Nemertea and Platyhelminthes as Parenchymia is still maintained by some authors (Nielsen 1995, 2001; Nielsen et al. 1996; Sorensen et al. 2000). Additional characters supporting Parenchymia are the mode of development of the adult nervous system and the lack of chitin and chitinase (Nielsen 1995, 2001). These authors interpret coelomic cavities of Nemertea as their autapomorphy.
Several authors proposed a sister group relationship of Nemertea to Neotrochozoa, which comprise Mollusca, Annelida, sipuncula, Echiura, Siboglinidae (also known as Pogonophora and Vestimentifera), and Myzostomida (Zrzavy et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001; Zrzavy 2003; Jenner 2004b). Increasing evidence of both morphological and molecular data shows that the latter 4 taxa are annelid subtaxa and thus as a consequence Neotrochozoa refers to the sister group relationship of Annelida and Mollusca (e.g., Bartolomaeus 1995; McHugh 1997; Hessling 2002; Eernisse et al. 2003; Wanninger et al. 2005; Bleidorn et al. 2007; Struck et al. 2007). Among others, the placement of Nemertea as sister to Neotrochozoa is substantiated by the development of the lateral coelom due to schizocoely from mesoderm germ bands derived from the 4d mesoteloblast (e.g., Turbeville 1986, 2002; Eernisse et al. 1992; Peterson and Eernisse 2001; Jenner 2004b). Additionally, in Neotrochozoa and Nemertea, the 3a and 3b blastomeres give rise to the ectomesoderm, whereas in polyclad Platyhelminthes, the ectomesoderm is derived from the 2b cell (Turbeville 2002). For a detailed discussion of several other synapomorphic characters, see the critical reviews of Jenner (2004b) and Turbeville (2002). This clade comprising Neotrochozoa and Nemertea was further substantiated by combined analyses of 18S and morphological data (e.g., Giribet et al. 2000; Peterson and Eernisse 2001) and christened Eutrochozoa (Peterson and Eernisse 2001). Haszprunar (1996) also included Entoprocta in this clade.

Other authors have suggested that Nemertea are sister to Teloblastica (Brusca RC and Brusca GJ 1990; Meglitsch and Schram 1991; Ax 1995; Rouse and Fauchald 1995; Sundberg et al. 1998). Teloblastica comprise, at a minimum, neotrochozoan taxa plus Arthropoda, which were previously seen as the sister to Annelida. Therefore, preference by workers for either Neotrochozoa or Teloblastica as sister to Nemertea rather depends how they view the relative positions of Annelida and Arthropoda; those supporting Lophotrochozoa/Ecdysozoa (e.g., Halanych 2004) prefer Neotrochozoa, whereas Articulata hypothesis (e.g., Brusca RC and Brusca GJ 2003) is consistent with the Teloblastica hypothesis. (Note Entoprocta [Ax 1995] or Tardigrada [Meglitsch and Schram 1991] have been included into Teloblastica as well.) To ensure consistency of names of taxa assemblages throughout the manuscript, we generally followed Jenner (2004b) even if the cited authors themselves did not use this specific name for a certain taxa assemblage. Furthermore, Platyhelminthes is used herein in the sense of Halanych (2004) excluding Acoelomorpha.

Additional evidence of both morphological and molecular data shows that the latter 4 taxa are annelid subtaxa and thus as a consequence Neotrochozoa refers to the sister group relationship of Annelida and Mollusca (e.g., Bartolomaeus 1995; McHugh 1997; Hessling 2002; Eernisse et al. 2003; Wanninger et al. 2005; Bleidorn et al. 2007; Struck et al. 2007). Among others, the placement of Nemertea as sister to Neotrochozoa is substantiated by the development of the lateral coelom due to schizocoely from mesoderm germ bands derived from the 4d mesoteloblast (e.g., Turbeville 1986, 2002; Eernisse et al. 1992; Peterson and Eernisse 2001; Jenner 2004b). Additionally, in Neotrochozoa and Nemertea, the 3a and 3b blastomeres give rise to the ectomesoderm, whereas in polyclad Platyhelminthes, the ectomesoderm is derived from the 2b cell (Turbeville 2002). For a detailed discussion of several other synapomorphic characters, see the critical reviews of Jenner (2004b) and Turbeville (2002). This clade comprising Neotrochozoa and Nemertea was further substantiated by combined analyses of 18S and morphological data (e.g., Giribet et al. 2000; Peterson and Eernisse 2001) and christened Eutrochozoa (Peterson and Eernisse 2001). Haszprunar (1996) also included Entoprocta in this clade.

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Molecular data so far strongly support only a placement of Nemertea within Lophotrochozoa and thus reject a sister group relationship to Teloblastica (Turbeville et al. 1992; Erber et al. 1998; Balavoine et al. 2002; Mallatt and Winchell 2002; Ruiz-Trillo et al. 2002; Anderson et al. 2004; Halanych 2004; Peterson and Butterfield 2005; Passamaneck and Halanych 2006; Turbeville and Smith 2007; Helmkampf et al. 2008). Depending on the respective analyses and the genes used Nemertea are closely related to different taxa within this clade, for example, Platyzoa (Passamaneck and Halanych 2006) or Mollusca (Erber et al. 1998; Zrzavy et al. 1998; Turbeville and Smith 2007; Helmkampf et al. 2008). However, none of these relationships are substantially supported.

Recent phylogenomic approaches have generally improved the robustness of molecular phylogenetic reconstructions (Philippe et al. 2005; Philippe and Telford 2006; Baurain et al. 2007; Hausdorf et al. 2007), but adequate genomic data are still lacking for Nemertea. Herein, we present analyses using expressed sequence tag (EST) data of the nemertean Lineus viridis (O.F. Müller, 1774) to address specifically these major outstanding issues of the origin of Nemertea. The results based on 9,377 amino acids from 60 ribosomal proteins place Nemertea within Eutrochozoa as sister to Mollusca. Hypotheses testing significantly rejected the Parenchymia hypothesis as well as a sister group relationship to Teloblastica.

Materials and Methods
Isolation of RNA and Library Construction

The nemertean L. viridis was collected at the marine biological station Wadden Sea Station Sylt in List (North Sea Island Sylt, Germany) and frozen using liquid nitrogen. Total RNA was extracted employing TRIzol (Invitrogen, Karlsruhe, Germany). Quality of total RNA was visually checked on agarose gel, and mRNA was subsequently captured using Dynabead (Invitrogen). The cDNA library was constructed at the Max Planck Institute for Molecular Genetics in Berlin by primer extension, size fractioning, and directional cloning applying Invitrogen’s CloneMiner technology, using the vector pDONR222. A total of 4,545 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, Hunohen, Germany) using BigDye chemistry (Applied Biosystems).

EST Processing

EST processing was accomplished at the Center for Integrative Bioinformatics in Vienna. Sequencing chromatograms were first base called and evaluated using the Phred application (Ewing et al. 1998). Vector, adaptor, poly-A, and bacterial sequences were removed employing the software tools Lucy (www.tigr.org), SeqClean (http://compbio.dfci.harvard.edu/tgi/software), and CrossMatch (http://www.phrap.org), respectively. Repetitive elements were subsequently masked with RepeatMasker. Clustering and assembly of the clipped sequences were performed using the TIGCL program package (http://compbio.dfci.harvard.edu/tgi/software) by first performing pairwise comparisons (MGBlast) 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 (http://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 EU302527–EU302586.

Sequence Analyses and Ribosomal Proteins Alignment

Ribosomal protein sequences were extracted from the nemertean EST data using the human ribosomal proteome (retrieved from the Ribosomal Protein Gene Database; http://ribosome.med.miyazaki-u.ac.jp) as search template during local Blast searches (TBLastN algorithm and an e value < e−10 as match criterion). Sixty out of the 79 ribosomal proteins were found in the EST data of *L. viridis*. 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. Additional ribosomal protein data were retrieved from the alignments compiled by Hausdorf et al. (2007). Amino acids were used for the phylogenetic analyses instead of nucleotides for several reasons. First, the greater number of character states in amino acid data (21 compared to 4 for nucleotides) minimizes the potential of convergent evolution (maximal difference 0.528 or 0.447, respectively). Second, protein data lack synonymous substitutions, which are a major source of homoplasy in nucleotide data. Third, amino acid alignments are generally less variable than nucleotide alignments and therefore more suitable for reconstruction of deep nodes.

All ribosomal protein sequences obtained were aligned by the ClustalW algorithm using default parameters (Thompson et al. 1994). The resulting 60 ribosomal protein alignments were inspected and only adjusted manually for obviously misaligned positions using GeneDoc (Nicholas KB and Nicholas HB 1997). Questionably aligned positions were eliminated with GBLOCKS (Castresana 2000). Default parameters were used with allowed gap positions set to “with half” and conversation of the flanking positions set to 70%. The alignment of the concatenated sequences was deposited at http://www.treebase.org (accession number: S1979).

Phylogenetic Analysis

Baurain et al. (2007) showed that Platyhelminthes, Nematoda, and Tardigrada introduce long branch problems, which may mislead the placement of these taxa even in phylogenomic analyses. Therefore, 2 data sets were analyzed. In one data set, nematodes and tardigrades were excluded to circumvent possible artificial misplacements of Platyhelminthes or Platyzoa. For both data sets and all 7 implemented criteria, ProfTest (Abascal et al. 2005) determined rtREV + F as the most appropriate substitution model. Maximum likelihood (ML) analyses were conducted with Treefinder (Jobb et al. 2004; Jobb 2007). 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 of tree topology around an edge (LR-ELW; 1,000 replications).

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<th>Table 1</th>
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<td><strong>Topology Test Results of Nemertean Relationships Using the Expected Likelihood Weight (ELW) Test</strong></td>
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<td>Nemertea, sister to</td>
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<td>Mollusca</td>
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<td>Annelida</td>
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<td>Neotrochozoa (Annelida + Mollusca)</td>
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<td>Platyhelminthes</td>
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<td>Platyzoa (Platyhelminthes + Syndermata)</td>
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<td>Teloblastica (Neotrochozoa + Arthropoda)</td>
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<td>Teloblastica + Tardigrada</td>
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<td>Teloblastica + Entoprocta</td>
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**NOTE.—** Asterisks indicate values for the topologies not included in the 0.95 confidence set (i.e., ELWs of the trees with the highest confidence levels that add up to 0.95); n.a. = not applicable.

To test a priori phylogenetic hypotheses (table 1), we constrained trees and used the “resolve multifurcations” option of Treefinder to obtain the ML tree for a specified hypothesis. Next, we investigated whether the ML trees for these hypotheses are part of the confidence set of trees applying the ELWs method with 50,000 replications (Strimmer and Rambaut 2002).

Bayesian inferences (BIs) based on the site heterogeneous CAT model (Lartillot and Philippe 2004) were performed using PhyloBayes v2.1c (Blanquart and Lartillot 2006). For each data set, 2 independent chains were run simultaneously for 5,000 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 for both data sets. According to the divergence of bipartition frequencies, both chains of each data set reached convergence (maximal difference 0.528 or 0.447, respectively; mean difference 0.012 or 0.011). For each data set, taking every 10th sampled tree a 50% majority rule consensus tree was finally computed using both chains.

Results

Alignment of the concatenated sequences of 60 ribosomal proteins included 9,377 amino acid positions. Depending on the method (ML or BI), as well as on the data set (32 taxa or 30 taxa), the topologies of the best trees are different mainly with respect to the position of Platyhelminthes and Syndermata. Therefore, the results of all analyses are shown (figs. 1 and 2).

All phylogenetic analyses show Nemertea as a eutrochozoan taxon either as sister to monophyletic Mollusca (figs. 1 and 2A) or to monophyletic Annelida sensu Struck et al. (2007) (fig. 2B). Thus, placement of Nemertea within Eutrochozoa is independent from inclusion of the long-branched tardigrades and nematodes in the analyses. However, nodal support is only strong by posterior probabilities (PP: 0.98, fig. 1A; PP: 0.96, fig. 2A). Eutrochozoa are either sister to monophyletic Bryozoa sensu lato (figs. 1B and 2B) or part of a basal polytomy comprising also Platyhelminthes and bryozoan taxa (figs. 1A and 2A). In both BI’s, Platyhelminthes are part of a basal polytomy in the Lophotrochozoan
clade and Syndermata render Ecdysozoa paraphyletic (figs. 1A and 2A). Both ML analyses recover Platyzoa but in the analysis based on 32 taxa Platyzoa are within Ecdysozoa (fig. 1B) and in the one based on 30 taxa sister to Lophotrochozoa (fig. 2B).

For both data sets, hypothesis testing did not differentiate between a sister group relationship of Nemertea to either Mollusca or Annelida but nemerteans as sister to Neotrochozoa was rejected (table 1). A sister group relationship to Neotrochozoa plus Entoprocta was clearly rejected with the data set comprising 32 taxa. The Parenchymia hypothesis as well as a sister group relationship to Teloblastica with or without either Tardigrada or Entoprocta were significantly rejected.

Discussion

Nemerteans as a Eutrochozoan Taxon

Nemertea is a eutrochozoan taxon closely related to Annelida and Mollusca. These results further substantiate previous assertions that the coelomic cavities of Nemertea, Annelida, and Mollusca are of common ancestry and thus homologous (e.g., Turbeville and Ruppert 1985; Turbeville 1986; Eernisse et al. 1992; Peterson and Eernisse 2001; Jenner 2004b). The lateral vessels of the nemertean circulatory system are surrounded by a continuous lining of mesoderm cells, which are connected to each other by adherens and septate junctions and possess, if at all, only rudimentary cilia (Turbeville and Ruppert 1985; Turbeville 1986). In these ultrastructural details, the vessels are similar to peritoneal linings of coelomic cavities, for example, of Annelida (Turbeville 1986; Bartolomaeus 1994; Rieger and Purschke 2005). Furthermore, development of nemertean lateral vessels and coelom formation in annelids both occur by schizocoely (Potswald 1981; Turbeville 1986). The vessels “begin as solid epithelial bands, which secondarily cavitate, resulting in a cell-lined channel” (Turbeville 1986). Finally, the mesodermal bandlets of the eutrochozoan taxa derive from the 4d mesoteloblast and give rise to lateral coelom cavities by schizocoely (Henry and Martindale 1998; Peterson and Eernisse 2001; Jenner 2004b). The lateral vessels of the nemertean circulatory system are surrounded by a continuous lining of mesoderm cells, which are connected to each other by adherens and septate junctions and possess, if at all, only rudimentary cilia (Turbeville and Ruppert 1985; Turbeville 1986). In these ultrastructural details, the vessels are similar to peritoneal linings of coelomic cavities, for example, of Annelida (Turbeville 1986; Bartolomaeus 1994; Rieger and Purschke 2005). Furthermore, development of nemertean lateral vessels and coelom formation in annelids both occur by schizocoely (Potswald 1981; Turbeville 1986). The vessels “begin as solid epithelial bands, which secondarily cavitate, resulting in a cell-lined channel” (Turbeville 1986). Finally, the mesodermal bandlets of the eutrochozoan taxa derive from the 4d mesoteloblast and give rise to lateral coelom cavities by schizocoely (Henry and Martindale 1998; Peterson and Eernisse 2001; Jenner 2004b).

The homology of coelomic cavities of Nemertea, Annelida, and Mollusca has been doubted because in the latter 2 taxa, the mesodermal bands also give rise to body musculature (Bartolomaeus 1994). However, at least parts of the circulatory system of an interstitial nemertean Cephalothrix sp. are similar to a myoepithelium forming portions of the body-wall musculature (Turbeville 2002). Additionally, in 2 other nemerteans, parts of the mesothelium are not
separated from adjacent muscle cells by an intervening extracellular matrix, which is similar in organization to some annelids (Turbeville 2002). Nielsen (1995, 2001) has argued that the lack of associated metanephridia (or otherwise open nephridial connections) as well as the nonsegmental organization suggest that nemertean circulatory system is not homologous to neotrochozoan coelomic cavities. However, the secondary circulatory system of Hirudinea (Annelida), which is derived from coelomic cavities, is of nonsegmental nature and the ciliated funnels of their metanephridia, if present, are not connected to the nephridial ducts. The latter prevents that blood cells are continuously lost to the environment. Overall, the circulatory system of Nemertea is comparable but not homologous to the secondary circulatory system of Hirudinea.

Thus, the traditional delineation of body organization into acoelomate, pseudocoelomate, and coelomate is rather a classification scheme for a morphological character than the basis for phylogenetic clades (Halanych and Passamaneck 2001; Jenner 2004b). Additionally, the significant rejection of Parenchymia indicates that similar body organization evolved independently in Platyhelminthes and Nemertea in contrast to traditional concepts. The phylogenetic position of Nemertea recovered here is consistent with the morphological hypothesis (Turbeville 1986) that their body cavity has been secondarily reduced to the point of assuming an acoelomate-like state. Secondary reductions of the coelom can also be seen, for example, in Hirudinea, several other annelids, Mollusca, and Arthropoda (e.g., Rieger and Purschke 2005). Therefore, Nemertea could also be regarded as coelomate. However, it may be more informative to drop these categories and instead focus more on identifying homologous elements of coelomic cavities. Differing developmental origins of coelomic cavities in the different bilaterian lineages cast doubts on the homology of the coelom across bilaterians (Minelli 1995; Salvini-Plawen and Bartolomaeus 1995; Nielsen 2001). Therefore, the possession of a coelom is a weak character to unite all coelom-p possess ing taxa as Coelomata (Hennig 1979; Blair et al. 2002; Philip et al. 2005). Furthermore, our results are consistent with numerous previous molecular studies that suggest Coelomata, as traditionally recognized, is not a real clade (e.g., Giribet et al. 2000; Peterson and Eernisse 2001; Halanych 2004; Philippe et al. 2005; Passamaneck and Halanych 2006; Baurain et al. 2007; Hausdorf et al. 2007).

In hoplonemerteans and palaeonemerteans juveniles, nonspecialized uniformly ciliated planktonic larva develops directly into adult stages (e.g., Norenburg and Stricker 2002; Maslakova et al. 2004b). This mode of development

Fig. 2.—Nemertea are a eutrochozoan taxon in the analyses with 30 taxa. Nematoda and Tardigrada were excluded from the analyses to circumvent possible artificial misplacements of Platyhelminthes or Platyzoa. Phylogenetic analyses were performed on the basis of 9,377 amino acid positions derived from 60 concatenated ribosomal proteins. Nemertea are highlighted. Dashed lines indicate paraphyletic taxa in that tree. (A) BI. Posterior probabilities are shown at the nodes. Syn. = Syndermata. (B) ML. Approximate bootstrap support values (LR-ELW) are shown at the nodes.
with a so-called planuliform larva is thought to belong to the
ground pattern of Nemertea (Thollesson and Norenburg
2003; Maslakova et al. 2004b). In contrast, all other eutro-
chozoan taxa plus Entoprocta possess a trochophore larva,
which is defined by the presence of a prototroch derived
from trochoblasts (Rouse 1999). Maslakova et al.
(2004a; 2004b) showed that the “planuliform” larva of
Carinoma tremaphoros Thompson 1900 (Palaeonemertea)
possesses a preoral belt of large ciliated cells, which are cell
cleavage arrested and are derived from 4 primary trocho-
blast. Thus, the development of the belt is similar to the
one of prototrochs in Annelida and Mollusca (Maslakova
et al. 2004b). Therefore, though uniformly ciliated the pla-
nuliform larva is likely to be homologous to a trochophore
larva (Maslakova et al. 2004b). Our results further warrant
this conclusion.

A sister group relationship of Nemertea to monophyle-
etic Neotrochozoa is not very likely contrary to previous
combined analyses (Giribet et al. 2000; Peterson and
Eernisse 2001). Nemertea are placed as sister to either
Annelida or Mollusca, though weakly supported. Neither
position is well supported by morphological data, but
Cavalier-Smith (1998) united Annelida and Nemertea as
Vermiforma due to the possession of closed blood vessels,
ciliated larvae without bivalved shells, and 2 ventrolateral
or one primitively paired ventral nerve cord. Unfortunately,
these features are wide spread within Bilateria and circula-
tory systems are not homologous across bilaterians. As
mentioned above, Nemertea use a reduced coelom deriva-
tive for blood transport, whereas Annelida (except for
Gnathobdelliformes and Pharyngobdelliformes, Hirudinea)
use spaces in the extracellular matrix between 2 adjoining
epithelia, a morphology typical of invertebrates in general
(Westheide 1997; Hartenstein and Mandal 2006). Compar-
avively, a close relationship of Nemertea to Mollusca has
been found in several studies using different molecular
markers (intermediate filament: Erber et al. 1998; 18S:
Zrzavy et al. 1998; mtDNA: Turbeville and Smith 2007;
and 7 nuclear genes: Helmkkampf et al. 2008). However,
as in our analyses support for such an association is weak
(PP: 0.98, fig. 1A; LR-ELW: 55, fig. 1B; and PP: 0.96, fig.
2A). Because posterior probabilities are generally higher
than bootstrap values approximated herein by the LR-
ELW method (see figs. 1 and 2 and Huelsenbeck et al.
2002) and are less reliable measurements of support
bootstrap values (e.g., Suzuki et al. 2002; Lewis et al.
2005), the term “significant support” should refer only
to bootstrap support above 95 or to results based on hypo-
thesis tests such as the conducted ELW test with defined
P values. Therefore, the position of Nemertea within Eutro-
chozoa remains uncertain.

To address the position of Nemertea future studies
could combine this data set of ribosomal proteins with data
of both additional genes and taxa. Though phylogenomic
approaches have been proposed to end incongruence
(e.g., Gee 2003; Rokas et al. 2003), recent studies showed
that as for single or few gene analyses increased taxon sam-
pling is also necessary to increase robustness and minimize
systematic errors due to, for example, long branches (e.g.,
Hillis et al. 2003; Soltis et al. 2004; Philippe and Telford
2006; Baurain et al. 2007). Thus, data of additional
nemerteans, mollusks, annelids, and other lophotrochozoan
taxa such as Brachiopoda and Phoronida, which are not
covered yet, might improve the phylogenetic reconstruc-
tions. This could also increase the robustness and nodal
support of the present topologies (figs. 1 and 2) in general,
which is still low at several deep nodes. Especially, the po-

tition of long-branched taxa such as Nematoda, Tardigrada,
and Platyhelminthes (Baurain et al. 2007) can be possibly
determined more accurately.

Nemertea and Platyhelminthes/Platyzoa

A sister group relationship of Nemertea and Platyhel-
minthes can be clearly rejected. Because some studies pro-
pose monophyletic Platyzoa, which also comprises
Platyhelminthes (Cavalier-Smith 1998; Garey and
Schmidt-Rhaesa 1998; Giribet et al. 2000; Passamanbeck
and Halanych 2006), we also tested if Nemertea were sister
to Platyzoa. This can also be clearly rejected (table 1). Ther-


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determined more accurately.
in a phylogenetic context as either plesiomorphic absent or secondary lost and thus apomorphic absent is difficult (e.g., Purschke et al. 2000; Collin and Cipriani 2003; Jenner 2004a; Struck 2006).

Nemertea and Teloblastica

Not surprisingly, a sister group relationship of Nemertea to Teloblastica could be significantly rejected because Arthropoda is not closely related to neotrochozoan taxa (e.g., Halanych 2004; Philippe et al. 2005; Baurain et al. 2007; Hausdorf et al. 2007; Helmkkampf et al. 2008). Additionally, Hausdorf et al. (2007) could significantly reject the Articulata hypothesis (e.g., Nielsen 1995; Brusca RC and Brusca GJ 2003). Furthermore, none of our analyses constraining Teloblastica with or without Entoprocta or Tardigarda recovered Articulata. Arthropoda (even including Tardigarda) were always placed as the most basal taxon within the constrained Teloblastica. Thus, segmentation evolved independently in annelids and arthropods (Seaver and Kaneshige 2006). This is further substantiated by the derived position of Annelida within Lophotrochozoa in our analyses. Furthermore, unsegmented worms such as Sipuncula and Echiura are placed within Annelida showing the variability of the character complex segmentation (McHugh 1997; Peterson and Eernisse 2001; Bleidorn et al. 2003, 2006; Struck et al. 2007).

Acknowledgments

We would like to thank J. von Döhren and T. Bartolomaeus (Free University Berlin) for providing tissue material. We are also grateful to M. Kube and R. Reinhardt (Max Planck Institute for Molecular Genetics, Berlin) for the construction and sequencing of cDNA libraries and to I. Ebersberger, S. Strauss, and A. von Haeseler (Max F. Perutz Laboratories, Center for Integrative Bioinformatics, Vienna) for the processing of our ESTs. B. Hausdorf (University of Hamburg), T. Hankeln, and B. Lieb (both University of Mainz) provided data of ribosomal proteins prior to public release. A. Müller and A. Paululat (both University of Osnabrück) provided access to a Linux server or a MacPro to run PhyloBayes v2.1c, respectively. We thank K. M. Halanych (Auburn University), B. Hausdorf (University of Hamburg), and G. Purschke (University of Osnabrück) for comments on first drafts of the manuscript. This study was funded by the priority program “Deep Metazoan Phylogeny” of the Deutsche Forschungsgemeinschaft (grant STR 683/3-1 to Torsten H. Struck).

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