Blackwell Publishing

Cladistics 22 (2006) 1–23

Cladistics

10.1111/j.1096-0031.2006.00128.x

A molecular phylogeny of annelids

Vincent Rousset[a]*†, Fredrik Pleijel[b], Greg W. Rouse[c]†, Christer Erséus[d]‡ and Mark E. Siddall[e]

[a]*Laboratory of Molecular Systematics, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden and Natural History Museum, University of Oslo, Department of Zoology, PO Box 1172 Blindern, NO-0318 Oslo, Norway;* [b]*Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Göteborg University, SE-452 96 Strömstad, Sweden, and Muséum national d'Histoire naturelle, Département Systématique et Evolution, CNRS UMR 7138, "Systématique, Adaptation, Evolution", 43 rue Cuvier, 75231 Paris Cedex 05, France;* [c]*South Australian Museum, North Terrace, Adelaide, SA 5000, and Environmental Biology, Adelaide University, Adelaide SA 5005, Australia;* [d]*Swedish Museum of Natural History, Department of Invertebrate Zoology, Stockholm;* [e]*American Museum of Natural History, Division of Invertebrate Zoology, Central Park West at 79th Street, New York, New York 10024, USA*

Accepted 28 June 2006

Abstract

We present parsimony analyses of annelids based on the largest taxon sample and most extensive molecular data set yet assembled, with two nuclear ribosomal genes (18S rDNA and the D1 region of 28S rDNA), one nuclear protein coding-gene (Histone H3) and one mitochondrial ribosomal gene (16S rDNA) from 217 terminal taxa. Of these, 267 sequences are newly sequenced, and the remaining were obtained from GenBank. The included taxa are based on the criteria that the taxon must have 18S rDNA or at least two other loci. Our analyses show that 68% of annelid family ranked taxa represented by more than one taxon in our study are supported by a jackknife value > 50%. In spite of the size of our data set, the phylogenetic signal in the deepest part of the tree remains weak and the majority of the currently recognized major polychaete clades (except Amphinomida and Aphroditiformia) could not be recovered. Terbelliformia is monophyletic (with the exclusion of Pectinariidae, for which only 18S data were available), whereas members of taxa such as Phyllodocida, Cirratuliformia, Sabellida and Scolecida are scattered over the trees. Clitellata is monophyletic, although Dinophilidae should possibly be included, and Clitellata has a sister group within the polychaetes. One major problem is the current lack of knowledge on the closest relatives to annelids and the position of the annelid root. We suggest that the poor resolution in the basal parts of the trees presented here may be due to lack of signal connected to incomplete data sets both in terms of terminal and gene sampling, rapid radiation events and/or uneven evolutionary rates and long-branch attraction.

Annelids are segmented worms that are found worldwide in most habitats, except the aerial and the most arid ones. Earthworms and leeches are the most familiar members of this group; however, most annelid diversity lies within the largely marine polychaetes. Until recently, Annelida was split into three major groups, each given class rank: Polychaeta (bristleworms), Oligochaeta (earthworms, etc.) and Hirudinea (leeches). However, in recent years it has become well recognized that Hirudinea is nested within Oligochaeta and that giving both these taxa the rank of class renders the latter group paraphyletic. These assignments have a long history and it may be some time before Class Oligochaeta and Class Hirudinea are eliminated. Comprehensive phylogenetic studies using molecular sequence data and morphology provide strong support that the "oligochaete" group Lumbriculida is the sister group to the ectoparasitic clade comprised of Hirudinida, Acanthobdellida and Branchiobdellida (Martin, 2001; Siddall et al., 2001; Erséus and Källersjö, 2004). The whole group including

*Corresponding author:
E-mail address: vrousset@ucsd.edu

†Present address: Marine Biology Research Division, Scripps Institution of Oceanography, UCSD La Jolla, CA 92093-0202, USA

‡Present address: Göteborg University, Department of Zoology, PO Box 463, SE-405 30 Göteborg, Sweden

both the traditional oligochaetes and Hirudinida should therefore be referred to either as Oligochaeta (Siddall et al., 2001), or Clitellata (Martin, 2001; Erséus and Källersjö, 2004). There are arguments for using either name but we use the name Clitellata here because it provides a connection to the actual synapomorphy shared by leeches and oligochaetes. We apply the vernacular name "oligochaetes" for Clitellata to the exclusion of Hirudinida, Acanthobdellida and Branchiobdellida.

Even though the systematics of annelids has been the object of a growing interest in the last 10 years, most questions regarding annelid large-scale relationships remain unanswered (for reviews see McHugh, 2000, 2005). For instance, the interrelationships and the status of the higher polychaete clades are unsettled and under debate. The most recent comprehensive systematization of polychaetes proposed by Rouse and Fauchald (1997) suggested that polychaetes comprise two major clades, Scolecida and Palpata. The most inclusive taxa within Palpata were Canalipalpata and the Aciculata (the latter largely corresponding to what previously was referred to as errant polychaetes). So far, none of the more inclusive polychaete taxa recovered by Rouse and Fauchald (1997) or earlier authors, except for Terebelliformia (Hall et al., 2004; Rousset et al., 2004), have been convincingly supported by molecular studies. This incongruence between morphological and molecular data is difficult to evaluate. Numerous recent molecular analyses focusing on broad scale analyses of polychaete relationships suffer from a weak phylogenetic signal resulting in a poor resolution of basal nodes (for instance, Rota et al., 2001; Bleidorn et al., 2003b; Struck and Purschke, 2005). Some have suggested that a rapid annelid radiation may explain the lack of resolution of basal annelid nodes in phylogenetic analyses of 18S rDNA sequences (e.g., Martin, 2001; Rota et al., 2001; Struck et al., 2002a; Bleidorn et al., 2003a,b), but those earlier difficulties may have arisen from low or uneven taxon sampling (see McHugh, 2005 for a review of those molecular studies). As suggested by Siddall et al. (2001) and McHugh (2005), the hypothesis of a rapid radiation of annelids as a cause of the poor resolution could only be supported if analysis of multiple independent gene sequences with a comprehensive taxon sampling also yields poor resolution in basal nodes of the annelid tree.

At a general level, the monophyly of Annelida is not well supported by anatomical features proposed to date (Rouse and Fauchald, 1997). Three character systems that usually are discussed include segmentation, chaetae and nuchal organs (see Rouse and Pleijel, 2001 and Purschke, 2002), although none of these provide unequivocal evidence. This lack of morphological support has resulted in a number of recent studies focussing on the monophyly and delineation of annelids (Westheide et al., 1999; McHugh, 2000; Halanych et al., 2002). The earliest molecular study dealing with the status and delineation of Annelida was that of Winnepenninckx et al. (1995). They used 18S rDNA sequences to examine relationships among protostome worms such as Annelida, Echiura, Nemertea, Pogonophora and Vestimentifera. However, the only two included annelids (*Lanice* and *Eisenia*) in their study did not form a clade. McHugh (1997) and Kojima (1998), using the nuclear gene elongation factor-1α, found that Clitellata and Pogonophora clustered among various polychaetes, and the former study also found that Echiura was nested among polychaetes. Their taxon sampling was such that the possibility of a number of other protostome taxa also being included in Annelida was not assessed. Brown et al. (1999) then studied relationships within Annelida using data from three genes and a broader taxon sample from among annelids and other protostomes, and also found clitellates, pogonophorans and even sipunculans nested among annelids. Martin (2001) analyzed available sequences of 18S rDNA with the primary aim of assessing the placement of Clitellata. He could not recover a monophyletic Annelida without also including taxa referred to Mollusca and Sipuncula. Subsequent studies including larger numbers of protostomes continue to show taxa from Arthropoda, Brachiopoda, Mollusca, Platyhelminthes, Sipuncula and Phoronida nested among annelid taxa (Rota et al., 2001; Bleidorn et al., 2003b; Hall et al., 2004; Jördens et al., 2004; Struck and Purschke, 2005). Thus, large-scale molecular studies have not been encouraging. Morphological studies also are essential as there are critical gaps in our knowledge about basic anatomy of many groups (see Rouse and Pleijel, 2001).

The placement of the "root" on the annelid tree is a related issue that deserves special attention. Clitellata and simple-bodied polychaetes such as Questidae and Paraonidae were suggested to be basal annelids in the morphological analyses of Rouse and Fauchald (1997). However, conclusions regarding morphological homologies between annelids and putative close relatives such as mollusks are notoriously difficult to draw. To date, molecular analyses have not settled on the root placement, and no consensus is in sight. This problem, of course, does not only relate to sister group relationships of annelids, but also hinders our identifying many of the major clades within annelids; we are actually working with an unrooted tree (Rouse and Pleijel, 2003). The position of Clitellata serves as an example. While the monophyly of Clitellata is well supported by morphological and molecular data (see Erséus and Källersjö, 2004 and references within) and the placement of Hirudinea as a clade well inside that group is now clear (Siddall et al., 2004), the sister group to Clitellata itself remains elusive. Hypothetical evolutionary scenarios have been forwarded as evidence that the clitellates have a derived position within a paraphyletic polychaete grade (Nielsen, 1995; Westheide, 1997; Giangrande and

Gambi, 1998; Purschke, 1999, 2003 Purschke et al., 2000). Furthermore, as with the problem of rooting the whole of the Annelida, molecular analyses of various genes such as elongation factor-1α (McHugh, 1997; Kojima, 1998), histone H3, U2 snRNA and 28S rDNA (Brown et al., 1999), 18S rDNA (Erséus et al., 2000; Martin, 2001; Rota et al., 2001; Struck et al., 2002a; Bleidorn et al., 2003b; Hall et al., 2004), 18S rDNA, 28S rDNA and COI (Jördens et al., 2004) and 18S rDNA and COI (Struck and Purschke, 2005) are inconsistent regarding which polychaete taxon is sister group to Clitellata.

In order to (1) find the sister group of clitellates, (2) evaluate the interrelationships and the status of the higher polychaete clades, and (3) assess the monophyly of annelids and to find the root of the annelid tree, we here present analyses of the largest taxon sample and most extensive molecular data set yet assembled to assess annelid relationships, with two nuclear ribosomal genes (18S rDNA and the D1 region of 28S rDNA), one nuclear protein coding gene (Histone H3) and one mitochondrial ribosomal gene (16S rDNA) from 217 terminals (Table 1). Of these, 267 sequences in total are newly sequenced, and the remaining part obtained from GenBank. Analyses were conducted with two suites of outgroup taxa, one more restricted than the other.

Materials and methods

Taxon sampling

Terminal taxa were chosen to examine the relationships of Annelida and putatively related taxa. Clitellate taxa were selected, based on the results of Erséus and Källersjö (2004), in order to obtain an optimal estimate of the root of this clade. Both "complete" (i.e., 217 taxa) and "restricted" (i.e., omitting six outgroup taxa) data sets comprised 211 species that uncontroversially are considered as members of Annelida. Outgroup taxa generally not considered to be annelids that were included in the "complete" data set were three arthropods, one brachiopod, five mollusks, two nemerteans and three sipunculids, though the root was actually placed with the centipede arthropod *Hanseniella*. The "restricted" analysis excluded the three arthropods, the brachiopod and two of the five mollusks and the nemertean *Micrura* was used as the root. Four loci were employed in these analyses: the nuclear small and large ribosomal subunits (18S rDNA and D1 region of 28S rDNA, respectively), nuclear protein-coding gene (histone H3), and the mitochondrial ribosomal gene (16S rDNA). The included taxa are based on the criteria that the taxon must have 18S rDNA or at least two other loci. Of the included data, 56 sequences for 18S rDNA, 78 sequences for 28S rDNA, 56 sequences for Histone H3 and 77 sequences for 16S rDNA were newly acquired from ethanol preserved material; remaining data were obtained from GenBank. All taxa included in this study, sampling localities and GenBank accession numbers for new sequences, as well as sequences reported in other studies, are listed in Table 1. All data were handled in a relational database created in FileMaker Pro using the taxonomic binomen as the primary key so as to prevent chimaeric concatenations of loci in the final matrix. Taxonomic representation across loci was 94% for 18S rDNA, 61% for 28S rDNA, 41% for Histone H3 and 53% for 16S rDNA; on the whole the data set was 62% complete.

DNA extraction, amplification and sequencing

DNeasy Tissue Kit (Qiagen Inc., Valencia, California) was used for tissue lysis and DNA purification. Polymerase chain reaction (PCR) amplification of nuclear 18S rDNA and D1 region of 28S rDNA, mitochondrial 16S rDNA and Histone H3 gene fragments was accomplished with the primers in Table 2. The 18S rDNA gene was PCR amplified in three overlapping fragments of about 950, 900 and 850 bp each, using primer pairs 1F-5R, 3F-18Sbi and 18Sa2.0-9R, respectively (see Table 2). Amplifications of the D1 region of 28S, 16S and Histone H3 yielded fragments of approximately 320, 450 and 327 bp, respectively. For a few taxa where the universal primers 16Sar-L and 16Sbr-H did not work well, the primer pair 16SAnnF and 16SAnnR was used. Loci were amplified using Ready-To-Go™ PCR Beads (Amersham Pharmacia Biotech, Piscataway, New Jersey). Each 25 mL reaction contained 1 μL of 10 μM of primer pair mix, 1 μL of template and 23 μL of water. Reactions mixtures were heated to 94 °C for 90 s, followed by 35 cycles of 40 s at 94 °C, 40 s at a specific annealing temperature and 45 s at 72 °C, and then a final extension of 7 min at 72 °C on Eppendorf Mastercyclers. Annealing temperature was set to 49 °C for the 18S primer pairs 1F-5R and 18Sa2.0-9R, 52 °C for the 18S primer pair 3F-18Sbi and for the 28S primer pair C1′-C2, 45 °C for the 16S primer pair 16Sar-L and 16Sbr-H, 60 °C for the 16S primer pair 16SAnnF and 16SAnnR and 53 °C for Histone H3 primer pair H3af and H3ar. The QIAquick PCR Purification Kit protocol (Qiagen) was employed to purify amplification products.

Amplification products were sequenced in both directions. Each sequencing reaction mixture, including 1 μL BigDye™ (Applied Biosystems, Perkin-Elmer Corporation, Foster City, CA), 1 μL of 1 μM primer and 3 μL of DNA template, ran for 40 cycles of 96 °C (15 s), 50 °C (30 s) and 60 °C (4 min). Sequences were purified by ethanol precipitation to remove unincorporated primers and dyes. Products were re-suspended in 6 μL formamide and electrophoresed in an ABI Prism™ 3730 sequencer (Applied Biosystems).

Table 1
Terminals used in the phylogenetic analyses, with accession codes to GenBank (GB) and localities. GenBank numbers in bold indicate new sequences.

Higher taxa		Species	Source	18S rDNA	28S rDNA	Histone H3	16S rDNA
ANNELIDA							
Clitellata							
	Capilloventridae	*Capilloventer australis* Erséus, 1983	Victoria, Australia	AY365455	**AY340384**		**AY340448**
	Enchytraeidae	*Fridericia tuberosa* Rota, 1995	Tuscany, Italy	AF209453	**AY340394**		**AY340457**
	Haplotaxidae	*Haplotaxis* cf. *gordioides* (Hartmann, 1821)	California, USA	AY365456	**AY340398**		**AY340461**
	Lumbricidae	*Eisenia andrei* Bouché, 1972	Västergötland, Sweden	AY365460	**AY340390**	**DQ779728**	**AY340454**
	Lumbricidae	*Lumbricus terrestris* Linnaeus, 1758	GB	AJ272183	AF185172	AF185262	
	Lumbriculidae	*Eclipidrilus frigidus* Eisen, 1881	GB	AY040692			
	Lumbriculidae	*Rhynchelmis tetratheca* (Michaelsen, 1920)	Södermanland, Sweden	AY365464	**AY340414**		**AY340477**
	Megascolecidae	*Pontodrilus litoralis* (Grube, 1855)	Florida, USA	AY365462	**AY340410**		**AY340473**
	Phreodrilidae	*Antarctodrilus proboscidea* (Brinkhurst & Fulton, 1979)	Victoria, Australia	AY365465	**AY340383**	**DQ779716**	**AY340447**
	Propappidae	*Propappus volki* Michaelsen, 1916	Blekinge, Sweden	AY365457	**AY340412**		**AY340475**
	Tubificidae	*Heronidrilus gravidus* Erséus, 1990	Belize	**AY340433**	**AY340399**	**DQ779736**	**AY340462**
	Tubificidae	*Heterochaeta costata* Claparède, 1863	Bohuslän, Sweden	**AY340432**	**AY340397**		**AY340460**
	Tubificidae	*Tubificoides amplivasatus* (Erséus, 1975)	Bohuslän, Sweden	**AY340445**	**AY340421**		**AY340483**
Hirudinida	Erpobdellidae	*Erpobdella octoculata* (Linnaeus, 1758)	Paimpont Foret, France	AF099949		**DQ779729**	
Hirudinida	Glossiphoniidae	*Glossiphonia complanata* (Linnaeus, 1758)	Paimpont Foret, France	AF115982		**DQ779733**	
Hirudinida	Glossiphoniidae	*Helobdella stagnalis* (Linnaeus, 1758)	Västergötland, Sweden	AF115986	**AY340402**	**DQ779735**	**AY340465**
Hirudinida	Hirudinidae	*Hirudo medicinalis* Linnaeus, 1758	Paimpont Foret, France	AY786464		**DQ779738**	AF315058
Hirudinida	Piscicolidae	*Piscicola geometra* (Linnaeus, 1758)	GB	AF115995			
Polychaeta							
Incertae sedis							
	Aelosomatidae	*Aeolosoma hemprichi* Ehrenberg, 1828	Cultures in Bologna, Italy/GB	AHE310500			**DQ779600**
	Aelosomatidae	*Aeolosoma viride* Stephenson, 1911	Cultures in Bologna, Italy	**DQ779638**			
	Hrabeiella	*Hrabeiella periglandulata* Pizl & Chalupsky, 1984	GB	AJ310501			
	Parergodrilidae	*Parergodrilus heideri* Reisinger, 1925	GB	AJ310504			
	Parergodrilidae	*Stygocapitella subterranea* Knoellner, 1934	GB	AF412810			
Palpata							
Aciculata							
Eunicida							
	Dinophilidae	*Dinophilus gyrociliatus* O. Schmidt, 1857	GB	AF412805			AF380116

	Dinophilidae	*Trilobodrilus axi* Westheide, 1967	GB	AF412806			
	Dinophilidae	*Trilobodrilus heideri* Remane, 1925	GB	AF412807			
	Dorvilleidae	*Dorvillea bermudensis* (Åkesson & Rice, 1992)	GB	AF412802			
	Dorvilleidae	*Dorvillea erucaeformis* (Malmgren, 1865)	Trondheim, Norway	**DQ779647**	**DQ779685**		**DQ779611**
	Dorvilleidae	*Parapodrilus psammophilus* (Westheide, 1965)	GB	AF412800			
	Dorvilleidae	*Pettiboneia urciensis* (Campoy & San Martin, 1980)	GB	AF412801			
	Dorvilleidae	*Protodorvillea kefersteini* (McIntosh, 1869)	Bohuslän, Sweden	**DQ779670**	**DQ779708**	**DQ779759**	**DQ779634**
	Dorvilleidae	*Schistomeringos rudolphi* (Chiaje, 1828)	GB	AF412804			
	Eunicidae	*Eunice australis* Quatrefages, 1866	GB		AF185169	AF185255	
	Eunicidae	*Eunice pennata* (O.F. Müller, 1776)	Trondheim, Norway/GB	AY040684	**AY340391**	**DQ779731**	AF321418
	Eunicidae	*Eunice vittata* (Delle Chiaje, 1828)	GB	AF412790			
	Eunicidae	*Lysidice ninetta* Audouin & Milne Edwards, 1834	GB	AF412793			
	Eunicidae	*Marphysa bellii* (Audouin & Milne-Edwards, 1834)	Banyuls, France	**DQ779659**	**DQ779697**	**DQ779743**	**DQ779623**
	Eunicidae	*Nematonereis* sp.	New Caledonia	**DQ779660**	**DQ779698**	**DQ779745**	**DQ779625**
	Lumbrineridae	*Lumbrineris latreilli* Audouin & Milne-Edwards, 1834	GB	AF519238	AF185168	AF185253	
	Lumbrineridae	*Lumbrineris magnidentata* Winsnes, 1981	Bohuslän, Sweden	**DQ779657**	**Q779695a**	**DQ779740**	**DQ779621a**
	Onuphidae	*Aponuphis bilineata* (Baird, 1870)	GB	AF412795			
	Onuphidae	*Hyalinoecia tubicola* O.F. Müller, 1776	Bohuslän, Sweden	**DQ779654**	**DQ779692**		**DQ779618**
Amphinomida							
	Amphinomidae	*Eurythoe complanata* (Pallas, 1766)	GB	AY364851			
	Amphinomidae	*Hermodice carunculata* (Pallas, 1766)	GB	AY495948			
	Amphinomidae	*Hermodice* sp.	One Tree Island, Australia	**DQ779653p**	**DQ779691**		**DQ779617**
	Amphinomidae	*Hipponoe gaudichaudi* (Audouin & Milne-Edwards, 1830)	GB	AY577888			AY577881
	Amphinomidae	*Paramphinome jeffreysii* (McIntosh, 1868)	Trondheim, Norway	**DQ779664**	**DQ779702**		**DQ779629**
	Euphrosinidae	*Euphrosine* sp.	Trondheim, Norway	**DQ779649**	**DQ779687**	**DQ779732**	**DQ779613**

Table 1
Continued

Higher taxa		Species	Source	18S rDNA	28S rDNA	Histone H3	16S rDNA
Phyllodocida							
Incertae sedis							
	Paralacydoniidae	*Paralacydonia paradoxa* (Fauvel, 1913)	Banyuls, France	**DQ779663**	**DQ779701**	**DQ779751**	**DQ779628**
	Phyllodocidae	*Eteone longa* (Fabricius, 1780)	GB	AF448155			
	Phyllodocidae	*Eteone picta* Quatrefages, 1865	Brittany, France	**DQ779648**	**DQ779686**	**DQ779730**	**DQ779612**
	Phyllodocidae	*Eulalia viridis* (Linnaeus, 1767)	Bohuslän, Sweden	**AY340428**	**AY340392**		**AY340455**
	Phyllodocidae	*Notophyllum foliosum* (M. Sars, 1835)	Bohuslän, Sweden	**DQ779662**	**DQ779700**	**DQ779748**	**DQ779627**
	Phyllodocidae	*Phyllodoce* sp.	GB	AB106249	AY583705	AY583719	
Aphroditiformia	Polynoidae	*Harmothoe imbricata* (Linnaeus, 1767)	Bohuslän, Sweden	**AY340434**	**AY340434**		**AY340463**
	Polynoidae	*Lepidonotus squamatus* (Linnaeus, 1758)	Bohuslän, Sweden	**DQ779656**	**DQ779694**	**DQ779739**	**DQ779620**
	Polynoidae	*Paralepidonotus ampulliferus* (Grube, 1878)	GB	AF519237	AF185164	AF185247	
	Sigalionidae	*Sigalion bandaensis* (Mackie & Chambers, 1990)	GB	AB106254	AF185165	AF185248	
	Sigalionidae	*Sthenelais boa* (Johnston, 1833)	Brittany, France	**DQ779672**	**DQ779711**	**DQ779767**	**DQ779635**
Glyceriformia							
	Glyceridae	*Glycera alba* (O.F. Müller, 1776)	Bohuslän, Sweden	**DQ779651**	**DQ779689**		**DQ779615**
	Goniadidae	*Goniada maculata* Öersted, 1843	Bohuslän, Sweden	**DQ779652**	**DQ779690**		**DQ779616**
Nereidiformia							
	Hesionidae	*Hesione* sp.	Lifou, New Caledonia	**DQ442617**	**DQ442619**	**DQ779737**	**DQ442615**
	Hesionidae	*Hesiospina aurantiaca* (Sars, 1862)	Madang, Papua New Guinea	**AY340435**	**AY340401**		**AY340464**
	Hesionidae	*Nereimyra punctata* (O.F. Müller, 1788)	Bohuslän, Sweden	**DQ779661**	**DQ779699**	**DQ779746**	**DQ779626**
	Nephtyidae	*Nephtys australiensis* Fauchald, 1965	GB		AF185166	AF185250	
	Nephtyidae	*Nephtys hombergi* Savigny, 1818	GB	U50970	X80649		
	Nereididae	*Ceratocephale loveni* Malmgren, 1867	Bohuslän, Sweden	**DQ442616**	**DQ442618**		**DQ442614**
	Nereididae	*Ceratonereis longiceratophora* Hartmann-Schröder, 1985	GB	AB106251	AY859731	AF185251	
	Nereididae	*Nereis pelagica* Linnaeus, 1758	Bohuslän, Sweden	**AY340438**	**AY340407**		**AY340470**
	Pilargidae	*Ancistrosyllis* sp.	GB	AF474280			
	Pilargidae	*Sigambra* sp.	Japan	**AY340444**	**AY340419**		**AY340481**

	Syllidae	*Amblyosyllis* sp.	GB	AF474284			
	Syllidae	*Branchiosyllis* sp.	GB	AF474283			
	Syllidae	*Epigamia magnus* Berkeley, 1923	GB	AF474309			AF474263
	Syllidae	*Epigamia noroi* (Imajima & Hartman, 1964)	GB	AF474310			AF474264
	Syllidae	*Eusyllis blomstrandi* Malmgren, 1867	GB	AF474281			
	Syllidae	*Exogone naidina* Öersted, 1845	GB	AF474290			
	Syllidae	*Grubeosyllis limbata* (Claparède, 1868)	GB	AF474289			
	Syllidae	*Myrianida pinnigera* (Montagu, 1808)	GB	AF474298			AF474252
	Syllidae	*Odontosyllis gibba* Claparède, 1863	GB	AF474282			
	Syllidae	*Opisthodonta morena* Langerhans, 1879	GB	AF474285			
	Syllidae	*Parapionosyllis* sp.	GB	AF474287			
	Syllidae	*Pionosyllis pulligera* (Krohn, 1852)	GB	AF474286			
	Syllidae	*Proceraea aurantiaca* Claparède, 1868	GB	AF474324			AF474278
	Syllidae	*Proceraea hanssoni* Nygren, 2004	GB	AF474321			AF474275
	Syllidae	*Proceraea paraurantiaca* Nygren, 2003	GB	AF474323			AF474277
	Syllidae	*Proceraea rubroproventriculata* Nygren & Gidholm, 2001	GB	AF474322			AF474276
	Syllidae	*Sphaerosyllis hystrix* Claparède, 1863	GB	AF474288			
	Syllidae	*Typosyllis armillaris* (Müller, 1771)	GB	AF474292			
	Syllidae	*Virchowia clavata* Langerhans, 1879	GB	AF474314			AF474268
Canalipalpata							
Incertae sedis							
	Polygordiidae	*Polygordius lacteus* Schneider, 1868	Brittany, France	**DQ779669**	**DQ779707**	**DQ779757**	**DQ779633**
	Protodrilidae	*Protodrilus purpureus* (Schneider, 1868)	Elba, Italy/GB	AJ310506	**AY340474**	**DQ779760**	**AY340474**
	Saccocirridae	*Saccocirrus* sp.	Banyuls, France	**AY340441**	**AY340415**		**AY340478**
Sabellida							
	Oweniidae	*Myriochele* sp.	Japan	**AY340437**	**AY340405**		**AY340468**
	Oweniidae	*Owenia* sp./*fusiformis*	Japan/GB	AY611447	AF185152	**DQ779750**	
	Sabellariidae	*Gunnarea capensis* Schmarda, 1861	GB	AY577892			
	Sabellariidae	*Idanthyrsus pennatus* (Peters, 1855)	GB		AF185149	AF185231	
	Sabellariidae	*Phragmatopoma* sp.	GB	AY611448	AY611435		
	Sabellariidae	*Sabellaria alveolata* (Linnaeus, 1767)	Brittany, France	AY340442	AY340416	**DQ779763**	**AY340479**
	Sabellidae	*Amphicorina mobilis* (Rouse, 1990)	GB	AY611449	AY611436		
	Sabellidae	*Amphiglena terebro* Rouse, 1993	GB		AF185150	AF185232	
	Sabellidae	*Myxicola* sp.	GB	AY611450	AY611438		
	Sabellidae	*Pseudopotamilla reniformis* (Bruguière, 1789)	GB	AY611451	AY611437		
	Sabellidae	*Sabella penicillus* Linnaeus, 1767	Brittany, France/GB	U67144	AY340420	**DQ779762**	**AY340482**
	Sabellidae	*Sabella spallanzanii* (Gmelin, 1791)	GB	AY436350			

Table 1
Continued

Higher taxa		Species	Source	18S rDNA	28S rDNA	Histone H3	16S rDNA
	Serpulidae	*Chitinopoma serrula* (Stimpson, 1854)	Iceland	**DQ779643**	**DQ779681**	**DQ779722**	
	Serpulidae	*Ficopomatus enigmaticus* (Fauvel, 1923)	GB	AY577889			
	Serpulidae	*Galeolaria caespitosa* Lamarck, 1818	GB	AB106257	AF185151	AF185233	
	Serpulidae	*Hydroides norvegica* Gunnerus, 1768	GB	AY611452	AY611439		
	Serpulidae	*Protula* sp.	Great Barrier Reef, Australia	AY611453	AY611440	**DQ779761**	
	Serpulidae	*Serpula vermicularis* (Linnaeus, 1767)	GB	AY395721			
	Serpulidae	*Spirorbis spirorbis* (Linnaeus, 1758)	GB	AY577887	**DQ779709**		
	Siboglinidae	*Lamellibrachia barhami* Webb, 1969	GB	AF168742			AF315047
	Siboglinidae	*Osedax frankpressi* Rouse *et al.*, 2004	GB	AY586485			AY586471
	Siboglinidae	*Osedax rubiplumus* Rouse *et al.*, 2004	GB	AY586484			AY586470
	Siboglinidae	*Polybrachia* sp.	GB	AF168739			AF315037
	Siboglinidae	*Ridgeia piscesae* Jones, 1985	GB	AF168744	AY344665		AF315054
	Siboglinidae	*Riftia pachyptila* Jones, 1980	GB	AF168745	AY210470		AF315049
	Siboglinidae	*Sclerolinum brattstromi* Webb, 1964	GB	AF315061			AF315046
	Siboglinidae	*Siboglinum fiordicum* Webb, 1963	W. Norway/GB	AF315060	AY340418	**DQ779765**	AF315039
Spionida							
	Apistobranchidae	*Apistobranchus* sp.	Iceland	**DQ779640**	**DQ779675**		**DQ779603**
	Apistobranchidae	*Apistobranchus typicus* (Webster & Benedict, 1887)	GB	AF448150			
	Chaetopteridae	*Chaetopterus sarsi* Boeck in M. Sars, 1851	Trondheim, Norway	**DQ779642**	**DQ779680**		**DQ779607**
	Chaetopteridae	*Chaetopterus variopedatus* (Renier, 1804)	GB	U67324	AY145399	U96764	
	Chaetopteridae	*Phyllochaetopterus* sp. 1	Sydney, Australia	**DQ779666**	**DQ779704**	**DQ779753**	
	Chaetopteridae	*Phyllochaetopterus* sp. 2	Sydney, Australia	**DQ779665**	**DQ779703**	**DQ779752**	
	Chaetopteridae	*Telepsavus* sp.	GB	AF448165			
	Magelonidae	*Magelona* sp.	Banyuls, France/GB	AY611454	AY611441	**DQ779742**	**DQ779622**
	Spionidae	*Aonides oxycephala* (M. Sars, 1872)	GB	AF448149			
	Spionidae	*Laonice* sp.	Bohuslän, Sweden	**DQ779655**	**DQ779693**		**DQ779619**
	Spionidae	*Malacoceros* sp.	GB		AF185162	AF185245	
	Spionidae	*Poecilochaetus* sp.	Banyuls, France	**DQ779667**	**DQ779705**	**DQ779754**	**DQ779630**
	Spionidae	*Polydora ciliata* (Johnston, 1838)	GB	U50971			
	Spionidae	*Polydora giardi* Mesnil, 1896	Trondheim, Norway	AY611455	AY611442	**DQ779756**	**DQ779632**
	Spionidae	*Pygospio elegans* Claparède, 1863	GB	U67143			
	Spionidae	*Scolelepis squamata* (O.F. Müller, 1789)	GB	AF448164			
Terebellida							
Cirratuliformia							
	Acrocirridae	*Macrochaeta clavicornis* (M. Sars, 1835)	Bohuslän, Sweden	**DQ779658**	**DQ779696**	**DQ779741**	
	Cirratulidae	*Aphelochaeta marioni* (de Saint Joseph, 1894)	Iceland	**DQ779639**	**DQ779674**	**DQ779717**	**DQ779602**
	Cirratulidae	*Caulleriella parva* Gillandt, 1979	GB	AF448151			
	Cirratulidae	*Caulleriella* sp.	Iceland		**DQ779679**		**DQ779606**
	Cirratulidae	*Cirratulus cirratus* (O.F. Müller, 1776)	Iceland	**DQ779645**	**DQ779683**	**DQ779724**	**DQ779609**
	Cirratulidae	*Cirriformia tentaculata* (Montagu, 1808)	GB	AY611456	AY611443		
	Cirratulidae	*Dodecaceria concharum* Öersted, 1843	GB	AY577891	AY612631		
	Cirratulidae	*Dodecaceria* sp.	Bohuslän, Sweden/GB	AY340427	AY340389	AF185237	**AY340453**
	Ctenodrilidae	*Ctenodrilus serratus* (Schmidt, 1857)	Massachusetts, USA	**AY340426**	**AY340388**	**DQ779727**	**AY340452**
	Fauveliopsidae	*Fauveliopsis* sp.	Banyuls, France/GB	AY340429	AY340393	AF185243	**AY340456**
	Flabelligeridae	*Diplocirrus glaucus* (Malmgren, 1867)	GB	AY611457	AY611444		

	Flabelligeridae	*Flabelligera affinis* M. Sars, 1829	Iceland	**DQ779650**	**DQ779688**		**DQ779614**
	Flabelligeridae	*Poeobius meseres* Heath, 1930	Monterey Bay, USA	**DQ779668**	**DQ779706**	**DQ779755**	**DQ779631**
	Sternaspidae	*Sternaspis scutata* (Ranzani, 1817)	Banyuls, France	**DQ779671**	**DQ779710**	**DQ779766**	AY532353
Terebelliformia							
	Alvinellidae	*Paralvinella grasslei* Desbruyères & Laubier, 1982	GB	AY577886			
	Alvinellidae	*Paralvinella palmiformis* Desbruyères & Laubier, 1986	GB	AF168747			
	Ampharetidae	*Ampharete acutifrons* (Grube, 1860)	Iceland		**DQ779673**	**DQ779715**	**DQ779601**
	Ampharetidae	*Anobothrus gracilis* (Malmgren, 1866)	GB	AY611458	AF501670		
	Ampharetidae	*Melinna cristata* (M. Sars, 1851)	Bohuslän, Sweden	AY611459	AY611445	**DQ779744**	**DQ779624**
	Pectinariidae	*Pectinaria dodeka* Hutchings & Peart, 2002	GB	AB106263			
	Pectinariidae	*Pectinaria granulata* (Linnaeus, 1767)	GB	AY577890			
	Pectinariidae	*Pectinaria regalis* Verrill, 1901	GB	AY040698			
	Terebellidae	*Amaeana trilobata* (M. Sars, 1863)	GB		AF342695	AF342702	
	Terebellidae	*Amphitritides gracilis* (Grube, 1860)	GB	AF508115			
	Terebellidae	*Amphitritides harpa* Hutchings & Glasby, 1988	GB	AB106260	AF185158	AF185241	
	Terebellidae	*Artacama proboscidea* Malmgren, 1866	Bohuslän, Sweden	AY344666	AY344667		
	Terebellidae	*Eupolymnia nesidensis* (Delle Chiaje, 1828)	GB	AY611460			
	Terebellidae	*Lanice conchilega* Pallas, 1766	Brittany, France/GB	X79873	AY340403		
	Terebellidae	*Loimia medusa* (Savingy, 1818)	GB	AY040690			
	Terebellidae	*Lysilla pacifica* Hessle, 1917	GB	AB106259	AF342696	AF342703	
	Terebellidae	*Pista australis* Hutchings & Glasby, 1988	GB		AF185159	AF185242	
	Terebellidae	*Pista* sp.	GB	AB106261			
	Terebellidae	*Rhinothelepus lobatus* Hutchings, 1974	GB		AF342694	AF342701	
	Terebellidae	*Thelepus cincinnatus* (Fabricius, 1780)	Trondheim, Norway/GB	AY611462	**DQ779712**	**DQ779769**	**DQ779636**
	Trichobranchidae	*Artacamella tribranchiata* Hutchings & Peart, 2000	South Australia		**DQ779677**	**DQ779720**	**DQ779605**
	Trichobranchidae	*Terebellides stroemi* M. Sars, 1835	GB	AY577893	X80658		AY577884
Scolecida							
	Arenicolidae	*Arenicola marina* (Linnaeus, 1758)	Brittany, France	AJ310502	**AY340382**	**DQ779718**	**AY340446**
	Arenicolidae	*Branchiomaldane vincenti* (Langerhans, 1881)	GB	AF508117			AY569690
	Capitellidae	*Barantolla lepte* Hutchings, 1974	GB	AB106265			
	Capitellidae	*Capitella capitata* (Fabricius, 1780)	GB	AF508118			
	Capitellidae	*Dasybranchus caducus* (Grube, 1846)	GB	AF448153			
	Capitellidae	*Notomastus latericeus* M. Sars, 1851	Bohuslän, Sweden/GB	AY040697	**AY340406**	**DQ779747**	**AY340469**
	Cossuridae	*Cossura* sp.	Iceland	**DQ779646**	**DQ779684**	**DQ779726**	**DQ779610**
	Maldanidae	*Clymenura clypeata* (de Saint Joseph, 1894)	Brittany, France	AY340423	**AY340385**	**DQ779725**	**AY340449**
	Maldanidae	*Euclymene trinalis* Hutchings, 1974	GB		AF185170	AF185256	
	Opheliidae	*Armandia bilobata* Hartmann-Schröder, 1986	South Australia	**DQ779641**	**DQ779676**	**DQ779719**	**DQ779604**
	Opheliidae	*Lobochesis bibranchia* Hutchings & Murray, 1984	GB	AB106266			
	Opheliidae	*Ophelia bicornis* Savigny, 1818	GB	AF508122			
	Opheliidae	*Ophelia rathkei* McIntosh, 1908	GB	AF448157	X80651		

Table 1
Continued

Higher taxa		Species	Source	18S rDNA	28S rDNA	Histone H3	16S rDNA
	Opheliidae	*Ophelina acuminata* Öersted, 1843	Bohuslän, Sweden	**AY340439**	**AY340408**	**DQ779749**	**AY340471**
	Opheliidae	*Polyophthalmus pictus* (Dujardin, 1839)	GB	AF448161	AF185171	AF185259	
	Orbiniidae	*Orbinia bioreti* (Fauvel, 1919)	GB	AF448158			AY532334
	Orbiniidae	*Orbinia latreillii* Audouin & Milne-Edwards, 1833	GB	AY532355			AY532335
	Orbiniidae	*Proscoloplos cygnochaetus* Day, 1954	GB	AF448162			AY532340
	Orbiniidae	*Protoaricia oerstedii* (Claparède, 1864)	GB	AF508123			AY532347
	Orbiniidae	*Protoariciella uncinata* Hartmann-Schröder, 1962	GB	AF508124			
	Orbiniidae	*Scoloplos armiger* (O.F. Müller, 1776)	Bohuslän, Sweden	**AY340443**	**AY340417**		**AY340480**
	Orbiniidae	*Scoloplos johnstonei* Day, 1934	GB	AF508126			AY532332
	Paraonidae	*Cirrophorus lyra* (Southern, 1914)	Bohuslän, Sweden	**AY340424**	**AY340386**		**AY340450**
	Questidae	*Questa paucibranchiata* Giere & Erséus, 1998	Bahamas/GB	AF209464	**AY340413**		**AY340476**
	Scalibregmatidae	*Hyboscolex* sp.	GB	AB106268	AY583707	AY583720	
	Scalibregmatidae	*Lipobranchius jeffreysii* (McIntosh, 1869)	GB	AF508120			
	Scalibregmatidae	*Polyphysia crassa* (Öersted, 1843).	Bohuslän, Sweden	**AY340440**	**AY340409**	**DQ779758**	**AY340472**
	Scalibregmatidae	*Scalibregma inflatum* Rathke, 1843	Bohuslän, Sweden	AF448163	AY612624	**DQ779764**	AY532331
	Scalibregmatidae	*Travisia forbesii* Johnston, 1840	Normandie, France		**DQ779713**	**DQ779770**	**DQ779637**
ARTHROPODA							
Myriapoda	Symphyla	*Hanseniella* sp.	GB	AY210823	AY210821	AF110856	AF370864
Chelicerata	Xiphosura	*Limulus polyphemus* Linnaeus, 1758	GB	U91490	X90468	AF370813	AF373606
Crustacea	Branchiopoda	*Triops longicaudatus/australiensis*	GB	AF144219	AY157606	AF110870	AY115610
BRACHIOPODA							
Articulata		*Terebratulina retusa* (Linnaeus, 1758)	Bohuslän, Sweden/GB	U08324	**AY340422**	**DQ779768**	AF334238
ECHIURA							
		Bonellia sp./*viridis*	Great Barrier Reef, Australia/GB	AF123307	**DQ779678**	**DQ779721**	
		Urechis caupo Fisher & MacGinitie, 1928	GB	AF342805	AF342804	X58895	
MOLLUSCA							
Aplacophora		*Chaetoderma nitidilum* Lovén, 1845	Bohuslän, Sweden/GB	AY377658	**AY340387**	AY377763	**AY340451**
Polyplacophora		*Chiton olivaceus* Spengler, 1797	GB	**DQ779644**	**DQ779682**	**DQ779723**	**DQ779608**
Gastropoda		*Gibbula cineraria* (Linnaeus, 1758)	W. Norway	**AY340430**	**AY340395**		**AY340458**
		Haliotis tuberculata Linnaeus, 1758	GB	AF120511	AF327553	AY377775	AY377622
Cephalopoda		*Vampyroteuthis infernalis* Chun, 1903	GB	AY557459	AJ310260	AY557408	AY545101
NEMERTEA							
		Micrura fasciolata/alaskensis	Bohuslän, Sweden/GB	**AY340436**	**AY340404**	AJ436981	**AY340467**
		Tubulanus annulatus (Montagu, 1804)	GB	AY210452	AY210473		AF103756
SIPUNCULA							
		Antillesoma antillarum (Grube & Öersted, 1858)	GB	AF519259		AF519311	
		Cloeosiphon aspergillus (Quatrefages, 1865)	GB	AF519263		AF519316	
		Golfingia elongata (Keferstein, 1862)	Brittany, France	**AY340431**	**AY340396**	**DQ779734**	**AY340459**

Table 2
PCR primers used in amplification and sequencing

Name	Sequence 5′–3′	Source
28S		
C1′	ACCCGCTGAATTTAAGCAT	(Lê et al., 1993)
C2	*TGAACTCTCTCTTCAAAGTTCTTTTC*	(Lê et al., 1993)
16S		
ArL	CGCCTGTTTATCAAAAACAT	(Palumbi et al., 1991)
BrH	*CCGGTCTGACTCAGATCACGT*	(Palumbi et al., 1991)
AnnF	GCGGTATCCTGACCGTRCWAAGGTA	(Sjölin et al., 2005)
AnnR	*TCCTAAGCCAACATCGAGGTGCCAA*	(Sjölin et al., 2005)
18S		
1F	TACCTGGTTGATCCTGCCAGTAG	(Giribet et al., 1996)
5R	*CTTGGCAAATGCTTTCGC*	(Giribet et al., 1996)
3F	GTTCGATTCCGGAGAGGGA	(Giribet et al., 1996)
18Sbi	*GAGTCTCGTTCGTTATCGGA*	(Giribet et al., 1999)
18Sa2.0	ATGGTTGCAAAGCTGAAAC	(Giribet et al., 1999)
9R	*GATCCTTCCGCAGGTTCACCTAC*	(Giribet et al., 1996)
Histone H3		
H3af	ATGGCTCGTACCAAGCAGACVGC	(Colgan et al., 1998)
H3ar	ATATCCTTRGGCATRATRGTGAC	(Colgan et al., 1998)

Italics: reverse primers.

DNA sequence editing and alignment

Sequences of complementary strands were edited and reconciled using Sequencher™ 4.1.4 (Gene Codes, Inc., Ann Arbor, MI). Ribosomal RNA loci were aligned with ClustalX (Thompson et al., 1994, 1997) using its default settings for gap opening and gap extension. Histone H3 sequences were aligned by contig in BioEdit (Hall, 1999) as there was no requirement for insertions or deletions in the data. Alignments are deposited in TREEBASE (http://www.treebase.org) or are available from VR.

Phylogenetic analyses

The "complete" data set was run first with PAUP 4.0b10 (Swofford, 2002), default settings but specifying 100 random taxon addition sequences. However, each replicate took more than 24 h to complete and after the third replicate, the program crashed. Analyses then were conducted for both "complete" (i.e., including all taxa) and "restricted" (i.e., omitting six of the 16 outgroups) data sets in TNT (Goloboff et al., 2003) using sectorial searches with RSS and CSS (Goloboff, 1999), with tree drifting and tree fusing (Goloboff, 1999) turned on, setting the initial level to 60 and requiring that the global optimum be found at least twice. Resulting trees were input to TNT individually for traditional TBR branch swapping with maxtrees set to 10 000. Jackknife support values (jac) also were calculated with TNT.

Results

The combined data set included 3665 nucleotide positions for 217 terminals in the "complete" data set and for 211 terminals in the "restricted" one. For the former, the alignment comprised 2720 variable sites, of which 2190 were parsimony informative. For the "complete" data set, TNT returned 144 equally parsimonious trees of length 33 548 with a retention index of 0.48. In Fig. 1, we present a strict consensus of the 144 trees. Owing to the peculiar positions of some putative "outgroup taxa" nested among the polychaetes, we reran the analysis excluding the arthropods, the brachiopod and two of five molluscs, *Chiton* and *Vampyroteuthis*. Analysis of these "restricted" data sets resulted in 20 equally parsimonious trees with a length of 30 605 steps and a retention index of 0.49. The 20 trees differed only in the resolution within Sabellidae and a clade that included the spionids *Malacoceros* sp., *Polydora ciliata*, *P. giardi*, *Pygospio elegans* and *Scolelepis squamata*. A strict consensus of the 20 trees is given in Fig. 2.

In the following, the first number indicated in parentheses corresponds to the jac value obtained from the analysis of the "complete" data set and the second to the jac value from the "restricted" data set. Consistent results from both analyses include the monophyly of Aeolosomatidae (100/100), Alvinellidae (95/94), Amphinomida (99/100), Amphinomidae (88/78), Aphroditiformia (100/100), Apistobranchidae (100/100), Arenicolidae (94/96), Capitellidae (98/100), Chaetopteridae (92/92), Dinophilidae (100/99), Flabeligeridae (98/96), Lumbrineridae (100/100), Maldanidae (100/100), Nephtyidae (100/97), Nereididae (98/100), Onuphidae (81/80), Opheliidae (99/100), Oweniidae (100/100), Parergodrilidae (100/100), Pectinariidae (100/100), Phyllodocidae (100/100), Pilargidae (100/100), Sabellariidae (95/96), Scalibregmatidae (96/98), Serpulidae (100/100), Siboglinidae (30/43),

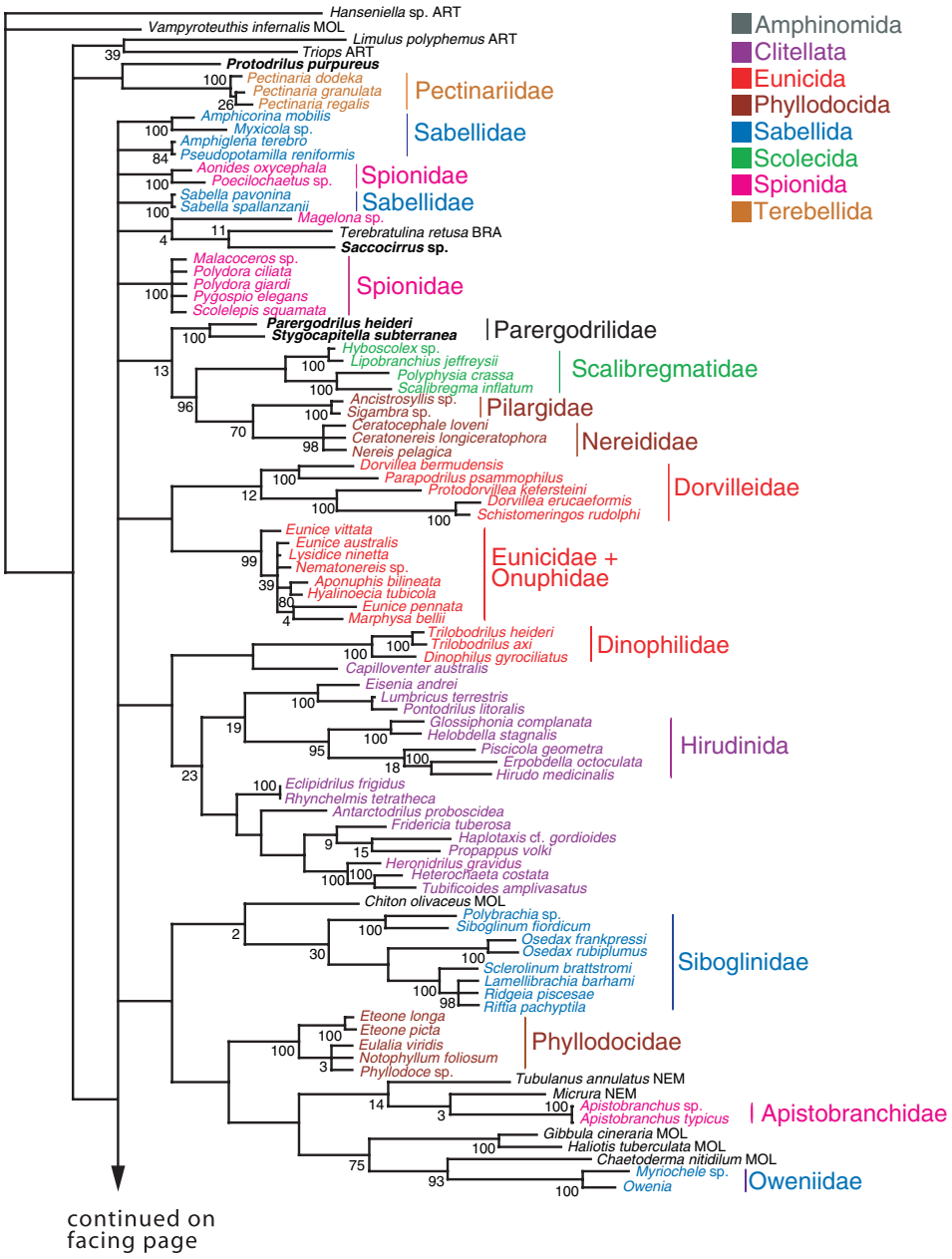

Fig. 1. Strict consensus tree from 144 trees obtained from the analysis of the "complete" data set. Numerals are jac values. More inclusive taxa (usually families) are provided after the species names, and traditional major annelid clades are indicated in color as specified in the upper right corner. Taxa in bold are polychaetes *incertae sedis*. Abbreviations following the names of the outgroup taxa refer to their phylum: ART, Arthropoda; BRA, Brachiopoda; ECH, Echiura; NEM, Nermertea; MOL, Mollusca; SIP, Sipuncula.

Sigalionidae (100/100) and Syllidae (96/97). Other well supported groups in the two analyses were *Chaetoderma* and Owenidae (93/90), Amphinomida and Chaetopteridae (86/86), Sabellariidae and Aphroditiformia (75/85), Arenicolidae and Maldanidae (89/92), members of Eunicidae and Onuphidae (99/100), and Goniadidae as sister to Acrocirridae and Flabeligeridae (97/98). Both analyses also agree on the non-monophyly of Clitellata owing to the sister group relationship between Dinophilidae (Polychaeta) and the basalmost clitellate, *Capilloventer australis* (Clitellata) (0/11), and on the non-monophyly of Ampharetidae, Cirratulidae, Dorvilleidae, Eunicidae, Hesionidae, Orbiniidae, Polynoidae, Terebellidae, Trichobranchidae, all the major polychaete clades except Amphinomida, and Polychaeta and Annelida. Main disagreements between the two analyses include a better resolution in the basal part of the strict consensus tree of the "restricted" data set (Fig. 2) than in the strict consensus tree (Fig. 1) of the "complete" data set. This difference is mainly due to a higher

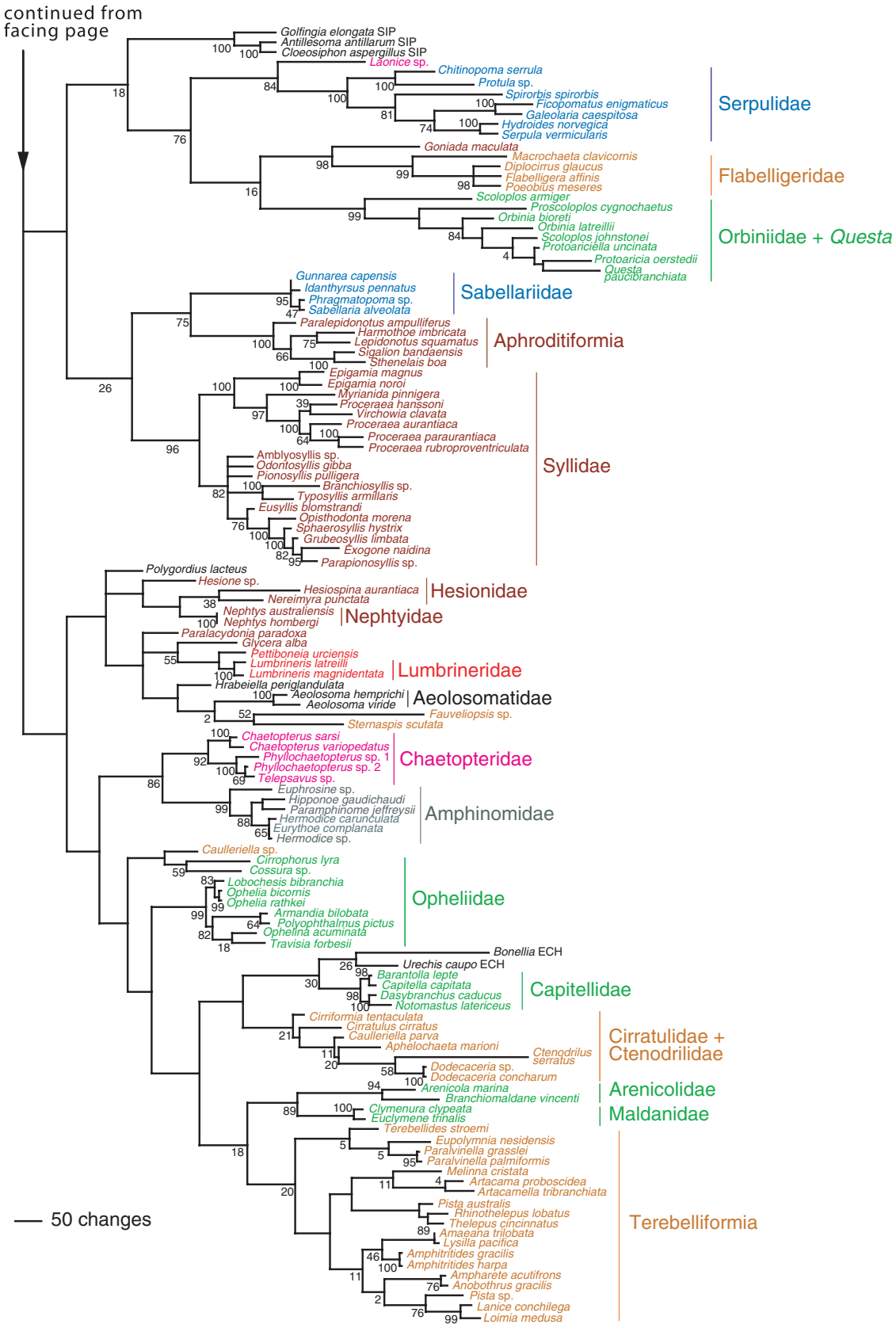

Fig. 1. *Continued*

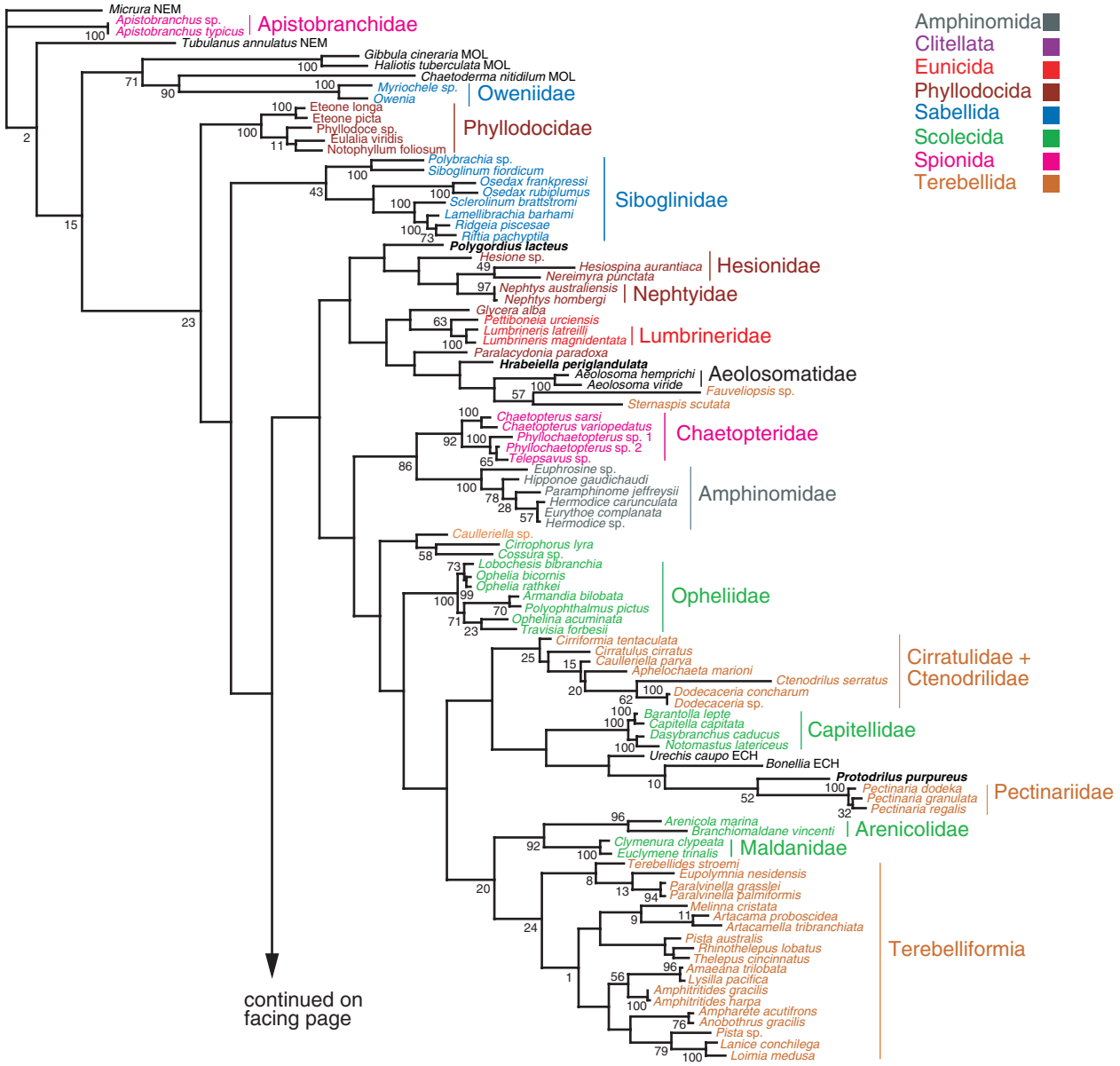

Fig. 2. Strict consensus tree from 20 trees obtained fom the analysis of the "restricted" data set. Numerals are jac values. More inclusive taxa (usually families) are provided after the species names, and traditional major annelid clades are indicated in color as specified in the upper right corner. Taxa in bold are polychaetes *incertae sedis*. Abbreviations following the names of the outgroup taxa refer to their phylum: ECH, Echiura; NEM, Nermertea; MOL, Mollusca; SIP, Sipuncula.

number of equally parsimonious solutions (144 trees) obtained for the complete data set than for the restricted data set (20 trees). Moreover, monophyly of Sabellidae was found in the analysis of the "restricted" data set but not in the analysis of the "complete" data set.

Discussion

This study was intended to be the most ambitious attempt yet to resolve annelid relationships. Still, overall resolution remains discouraging: rarely so many taxa have been sequenced for so many nucleotides with such sparing results. Considering that our analysis includes deep divergences that may go back to the Cambrian (see Rouse and Pleijel, 2001 and references within), or even further, the relative weakness of the phylogenetic signal for the most basal clade is not entirely surprising. More recent divergences appear to be better supported, much as has been observed in other studies (see Hall et al., 2004 and references within). Of the 41 annelid family ranked taxa represented by more than one taxon in our

Fig. 2. *Continued*

study, 28 (29 with the restricted data set) are found to be monophyletic and 27 are supported by a jac value over 50 in both analyses. There also is a much better resolution within some groups with a dense taxon sampling such as among the clitellates (18 representatives) and in the syllids (19).

The most recent classification of polychaetes is based on the morphological analyses of Rouse and Fauchald (1997), and slightly modified by Rouse and Pleijel (2001). However, the majority of the more inclusive clades proposed in those classifications have never been found to be monophyletic in molecular studies. Even

with an increase of the taxonomic sampling and combining the phylogenetic information of four different molecular markers, our results still do not support monophyly of the higher polychaetes groups as identified from morphological data.

Scolecida

The name Scolecida was first proposed by Rouse and Fauchald (1997), for a group that was only supported by two apomorphies, the presence of parapodia with similar rami and the possession of two or more pairs of pygidial cirri, though Rouse and Pleijel (2001) concluded that it would be "likely that further analysis will show that it is not a monophyletic grouping". Scolecida, as treated by most recent authors, include Arenicolidae, Capitellidae, Cossuridae, Maldanidae, Opheliidae, Orbiniidae, Paraonidae, Questidae and Scalibregmatidae. Our study, which includes 29 representatives covering all the family ranked taxa belonging to Scolecida (in green in our two figures), agrees with the previous molecular ones (e.g., Bleidorn et al., 2003b and Hall et al., 2004) showing that Scolecida is not a monophyletic group and should be abandoned or redelineated, though there are some well supported close relationships among some of these taxa. For instance, several recent studies suggest close relationships between questids and orbiniids (Erséus et al., 2000; Rota et al., 2001; Bleidorn et al., 2003a,b), possibly (with weaker support) with *Questa* nested within the orbiniids (see Bleidorn, 2005). Bleidorn et al. (2003b) also suggested transfer of *Travisia* from Opheliidae to Scalibregmatidae, a result that was corroborated by Hall et al. (2004) and Persson and Pleijel (2005). Morphologically, in terms of its body shape, distinct epidermis, and lack of a mid-ventral groove, *Travisia* resembles members of Scalibregmatidae. However, our analyses with new sequences for *Travisia* contradict a close relationship to Scalibregmatidae, and provide strong support for the monophyly of the traditional Opheliidae with *Travisia* included. Bleidorn et al. (2003b), Hall et al. (2004) and Persson and Pleijel (2005) used the same sequences of *Travisia*, whereas ours are based on newly collected specimens. For this reason we did not include the existing 18S rDNA sequence from GenBank. It cannot be excluded that a misidentification is involved and the matter warrants further investigation. With reference to other scolecid taxa, we found Arenicolidae to be the sister group to Maldanidae (89/92), a hypothesis with a long taxonomic history (Fauchald and Rouse, 1997). This result also has more recent morphological and molecular support (Bartolomaeus and Meyer, 1997; Rouse and Fauchald, 1997; Bleidorn et al., 2003b; Hall et al., 2004; Bleidorn, 2005; Persson and Pleijel, 2005). Concerning the particular position of the (Arenicolidae, Maldanidae) clade, our analyses suggest a sister group relationship to Terebelliformia (including Alvinellidae, Ampharetidae, Pectinariidae, Terebellidae and Trichobranchidae), a result found also in the previous molecular studies of Brown et al. (1999) and Hall et al. (2004). However, our results concerning this are far from conclusive owing to a very weak support (18/20) for this relationship and the unexpected position of pectinariids outside of the Terebelliformia clade.

Palpata

Palpata was formulated by Rouse and Fauchald (1997) for a group containing virtually all polychaetes except Scolecida and a few taxa *incertae sedis*. Given that the presence of palps and a limited peristomium were the only synapomorphies supporting this taxon, it is not surprising that later analyses (including ours) have not recovered the taxon. Palpata, following Rouse and Fauchald (1997), contains two major clades: Aciculata and Canalipalpata.

Aciculata

On the basis of parsimony analyses of morphological data, Rouse and Fauchald (1997) recognized Aciculata (the name referring to a particular chaetal type called aciculae, one of the apomorphies for the group). This group constituted one of the most strongly supported more inclusive taxa in their analyses with several identifiable synapomorphies (ventral sensory palps, prostomial antennae, dorsal cirri, ventral cirri, single pair of pygidial cirri, segmental organs). In agreement with all molecular studies done so far, our analyses, which involve a total of 67 representatives of the Aciculata clade, do not support the monophyly of the group. Aciculata was divided by Rouse and Pleijel (2001) into three major clades: Amphinomida, Eunicida and Phyllodocida.

Amphinomida

Amphinomida, according to Rouse and Pleijel (2001), contains approximately 200 nominal species, and comprises two major groups: Amphinomidae (known as fireworms) and Euphrosinidae. The monophyly of Amphinomida is supported in all of the morphological analyses of Rouse and Fauchald (1997) and by a number of morphological apomorphies (e.g., chaetal structure and composition, proboscis shape and presence of caruncle). Our molecular analyses, which included six representatives of Amphinomida (five amphinomids and one euphrosinid), agree with the morphological hypotheses and provide strong support for the monophyly of this taxon (99/100). Our results also support monophyly of Amphinomidae (88/78), in

that euphrosinids have a basal position in the group. Rouse and Fauchald, 1997) close relationship between Eunicida *sensu* Rouse and Pleijel (2001) and Amphinomida was not supported here; instead we obtained the highly unexpected sister group relationship between Amphinomida and Chaetopteridae (86/86). However, there is no morphological support whatsoever for this relationship, and we regard this result as suspicious.

Eunicida

Eunicida typically includes polychaetes with a ventral muscularized pharynx, ventral mandibles, dorsal maxillae and with the peristomium forming one or more rings (Rouse and Fauchald, 1997). This group, as formulated by Rouse and Pleijel (2001), includes Dorvilleidae (dinophilids included), Eunicidae, Hartmaniellidae, Histriobdellidae, Lumbrineridae, Oenonidae and Onuphidae. Our study included a total of 17 representatives, including three dinophilids, six dorvilleids, six eunicids and two lumbrinerids. Whereas most members of Eunicida are unequivocally supported by morphological characters (a ventral pharyngeal organ with a complex jaw apparatus), our results indicate that they are scattered among three separate clades, one with lumbrinerids, one with eunicids, onuphids and most dorvilleids, and one with dinophilids and the dorvilleid *Pettiboneia* (see Figs 1 and 2). Part of this non-monophyly of Eunicida accords well with prior studies (Struck et al., 2002b; Hall et al., 2004; Struck and Purschke, 2005), although on morphological grounds we find it difficult to accept that taxa such as lumbrinerids and eunicids are not closely related. As to Eunicidae and Onuphidae, Fauchald (1992) suggested that the special jaw asymmetry and aragonite mineralization constitute two apomorphies, and a sister group relationship between the two was advocated by Orensanz (1990). Several recent phylogenies (Struck et al., 2002b; Hall et al., 2004) have corroborated the monophyly of this group; however, all three of these studies also indicated that the recognition of Onuphidae as a family ranked taxon makes Eunicidae paraphyletic. In contrast, Rouse and Fauchald (1997) showed a sister group for Onuphidae with (Eunicidae (Lumbrineridae, Dorvilleidae)). Our results contradict the latter hypothesis and confirm the close relationship between Eunicidae and Onuphidae (99/100), further corroborating that the onuphids constitute a derived clade within the eunicids.

The interstitial and simple-bodied dinophilids have been viewed as members of Eunicida with a close relationship to the dorvilleids (see Rouse and Pleijel, 2001 and references within). Struck et al. (2002b) suggested that dinophilids are not a derived clade within Dorvilleidae and even not the sister group of any eunicidan taxon, and provided additional evidence for this in a more recent study (Struck and Purschke, 2005); the actual position of dinophilids, however, remained uncertain. The present result agrees that Dinophilidae are not a dorvilleid clade and are not sister to or member of Eunicida, instead it is sister to the aquatic clitellate *Capilloventer*, with both of these as sister to remaining Clitellata; albeit with very low jac support (see also further below under "Clitellata"). As to the dorvilleids, we found five of the six included ones to form a clade (12/65) that is sister to Eunicidae (incl. Onuphidae). The sixth dorvilleid, *Pettiboneia urciensis,* instead is sister to lumbrinerids. The polyphyly of Dorvilleidae and the placement of *P. urciensis* agree with Struck et al. (2002b). From a morphological point of view this is surprising, as *Pettiboneia* has many characters in common with the other dorvilleids, and possibly the sequences from this taxon should be re-examined.

Phyllodocida

Rouse and Fauchald (1997) presented strong support for the monophyly of Phyllodocida (anterior enlarged cirri, an axial muscular proboscis, ventral position of sensory palps, compound chaetae with a single ligament, lack of dorsolateral folds). However, as with results from other molecular studies (Brown et al., 1999; Struck et al., 2002a,b; Bleidorn et al., 2003a,b; Hall et al., 2004; Struck and Purschke, 2005), Phyllodocida, represented in our study by 43 terminal taxa, is not monophyletic. Based on the morphological cladistic analysis of Pleijel and Dahlgren (1998), Rouse and Pleijel (2001) recognized two clades within Phyllodocida: Aphroditiformia and Nereidiformia.

The monophyly of Aphroditiformia, scale-worms, is strongly supported by the present molecular data (100/100) with Polynoidae forming a grade owing to a derived position for Sigalionidae. The monophyly of scale-worms is in agreement with previous morphological studies (see Rouse and Pleijel, 2001 and references within), although two recent studies by Wiklund et al. (2005) and Struck et al. (2005), the former based on 18S rDNA, COI and morphology, and the latter on the same two genes, both show that Pisionidae (not included in the current analysis) are scale-worms with reduced scales and placed within Sigalionidae. Furthermore, either of these two studies agrees with our results that Polynoidae is paraphyletic.

In contrast to Aphroditiformia, our results contradict the monophyly of Nereidiformia as currently delineated, insofar as the members are scattered among five different clades (see Figs 1 and 2). Interestingly, our analyses suggest that pilargids form a clade with nereidids (70/72), calling into question the suggested close relationship between Pilargidae and Syllidae envisaged earlier (Fitzhugh and Wolf, 1990; Glasby, 1993). Pleijel and Dahlgren (1998) instead suggested that they are sister to a group, including Chrysopetalidae,

Hesionidae and Nereididae, although this latter group was not recovered here. Regarding other Phyllodocida, Böggemann (2002) in a revision of Glyceridae (bloodworms), indicated a sister group relationship between Glyceridae and Goniadidae, and Rouse and Pleijel (2001) treated them as the single unit Glyceriformia. However, this is not supported by our current analyses where *Goniada* with high support (97/98) comes out as sister to Acrocirridae and Flabeligeridae, a relationship that does not have precedent in the published literature to date. From a morphological point of view this is highly surprising, and the sequence data for *Goniada* may require further attention. In a recent study based on 18S rDNA and morphological data (Worsaae et al., 2005), the monophyly of Glyceriformia was not recovered either, although in that case they were closely related, such that Glyceridae was sister to Phyllodocidae, Goniadidae and Sphaerodoridae. Regarding Phyllodocida, all molecular analyses accomplished so far (including this study) suffer from sparse sampling in such speciose groups of polychaetes like Hesionidae (150 nominal species), Nereididae (500 nominal species) and Phyllodocidae (500 nominal species). This stands in contrast to the recently analyzed and revised (Nygren and Sundberg, 2003; Nygren, 2004) large group Syllidae (over 800 nominal species), where a much denser sampling provides high support values for virtually all clades.

Canalipalpata

Canalipalpata was proposed by Rouse and Fauchald (1997) for an inclusive group of polychaetes that encompasses about half of the described polychaete species. This clade is supported by a single apomorphy, the presence of grooved palps, and it is not surprising that the monophyly of Canalipalpata is not found by the present molecular data and has never been recovered in any molecular study done so far. Within Canalipalpata, three major clades were identified by Rouse and Fauchald (1997): Spionida, Sabellida and Terebellida.

Spionida

Among the three groups recognized in Canalipalpata, Spionida was the best supported clade in Rouse and Fauchald (1997), based on the presence of a pair of peristomial grooved palps, nuchal organs forming posterior projections, and anterior excretory nephridia and posterior segmental organs for gamete release. Here, Spionida is represented by two apistobranchids, six chaetopterids, one magelonid and eight spionids. Our analyses failed to recover a monophyletic Spionida. In both trees (Figs 1 and 2), the members are scattered in five different clades. Concerning the higher taxa belonging to Spionida, the main interesting result is the well supported monophyly of Chaetopteridae (92/92). This result was expected considering the particular morphology of the chaeptopterids, including a body with three highly characteristic body regions with differentiated parapodia. We conclude that the status of Spionida merits particular attention, especially relating to spionids, which is a highly speciose and diverse group of polychaetes with about 450 described species.

Sabellida

According to the classification of Rouse and Fauchald (1997), Sabellida contains Oweniidae, Sabellariidae, Sabellidae, Serpulidae and Siboglinidae. The fusion of the prostomium with the peristomium constituted the only apomorphy supporting this grouping. Our analyses do not support the monophyly of Sabellida and instead show all five family ranked taxa scattered across the trees, with none particularly close to one another. However, the monophyly of all families within Sabellida is recovered in our analyses, except for Sabellidae, which is monophyletic only in the "restricted" analysis (Fig. 2), though this is recovered as monophyletic in the jac tree of the "complete" analysis (result not shown). In a recent cladistic analysis using morphological and molecular (18S rDNA and 28S rDNA) data, Rousset et al. (2004) found a sister group relationship between Oweniidae and Siboglinidae, whereas Bleidorn et al. (2003b) and Hall et al. (2004) using 18S rDNA data found an unexpected close relationship between Oweniidae and Apistobranchidae (Canalipalpata, Spionida). Our analyses are not congruent with any of these results, showing that Owenidae with the aplacophoran mollusk *Chaetoderma* form a surprising well supported clade (93/90), and that Siboglinidae is close to *Chiton* (Mollusca) in the "complete" data set. The robust grouping between *Chaetoderma* and Oweniidae, two taxa showing long branches in our tree, may be the result of a long-branch attraction (LBA) phenomenon. The sister group to Siboglinidae has not been convincingly inferred in any analyses using only molecular data done so far (Bleidorn et al., 2003a,b; Hall et al., 2004; Struck and Purschke, 2005), and although we analyzed a very large data set, our results concerning this issue are still weak and elusive. It is more surprising that our results do not confirm the morphologically well established close relationships between Sabellidae and Serpulidae (see Rousset et al., 2004 and references within), and show consistently that Sabellariidae (sandmason or honeycomb worms) are sister to a group containing Aproditoidea plus Sigalionidae (75/85). Neither of these results can be considered as reliable. Until now, the analyses based on only molecular data (including this one) have failed to properly assess the interrelationships within Sabellida, probably suffering from a combination of weak phylogenetic signal and artifacts (as LBA). It

seems that a combined analyses using morphological and molecular data, as the one by Rousset et al. (2004), is promising and might be a way to overcome or evaluate some potential artifacts (see Bergsten, 2005).

Terebellida

Terebellida, as delineated by Dales (1962), includes Ampharetidae, Pectinariidae and Terebellidae, all polychaetes having multiple grooved palps. Thereafter, Terebellida was expanded by Rouse and Fauchald (1997) to include taxa that also have a single pair of palps (e.g., Acrocirridae, Cirratulidae, Flabeligeridae). Our taxonomic sampling included 40 terminal taxa belonging to Terebellida, with representatives from all the family ranked taxa. Although Rouse and Fauchald (1997) identified several synapomorphies for this overall grouping, our results indicate that it is polyphyletic, with the members scattered in six different clades (see Figs 1 and 2). This is also in agreement with recent studies (Hall et al., 2004; Rousset et al., 2004). Using morphological and molecular data, Rousset et al. (2004) showed that the synapomorphies previously identified for Terebellida are highly homoplastic. Rouse and Pleijel (2001) recognized two subgroups within Terebellida: Cirratuliformia and Terebelliformia. Cirratuliformia contains Acrocirridae, Cirratulidae, Ctenodrilidae, Fauveliopsidae, Flabeligeridae (including *Poeobius*) and *Sternaspis*. The group was delineated to include taxa with a single pair of grooved palps (with the exception of some Cirratulidae with seemingly multiple palps, and Ctenodrilidae and Fauveliopsidae without palps). Our analyses do not support the monophyly of Cirratuliformia. However, we find an interesting sister group relationship between *Sternaspis* and Fauveliopsidae [although poorly supported (57/52)], confirming the morphological results of Rouse and Pleijel (2003) and the putative homology between the ventral shields in *Sternaspis* and *Fauveliopsis*. Our results provide also strong support for a sister group relationship between Acrocirridae and Flabeligeridae (99/97), and corroborates the results of Rouse and Pleijel (2003) and Burnette et al. (2005) that *Poeobius* (Poeobiidae) is nested within Flabeligeridae. Owing to the morphological similarity between Acrocirridae and Cirratulidae (as anterior branchiae and anterior pair of segmental organs and paired palps), some authors (see Rouse and Pleijel, 2001) argued that the sister group for Acrocirridae could be within Cirratulidae. Rouse and Pleijel (2003) also demonstrated that Flabeligeridae may also be close to Acrocirridae and so that both could be nested in Cirratulidae. Our results do not show an acrocirrid and flabeligerid plus cirratulid relationship, but instead agree with Banse (1969) who showed that Acrocirridae in fact share more features with Flabeligeridae (such as the structure of the epidermal papillae and the compound hooks) than with Cirratulidae. Terebelliformia (also sometimes referred to as Terebellomorpha), as treated by recent authors (see, for example Rousset et al., 2003; Glasby et al., 2004), includes Alvinellidae, Ampharetidae, Pectinariidae, Terebellidae and Trichobranchidae. Terebelliformia, in contrast to Cirratuliformia, was recovered in our trees (admittedly with very low support of 20/24), but only at the exclusion of Pectinariidae. Monophyly of Terebelliformia has previously been established on the basis of morphological characters, including presence of neuropodial uncini, a looped gut, anterodorsal branchiae, non-eversible ventral pharyngeal organ (Rouse and Fauchald, 1997), DNA sequence data (Colgan et al., 2001) and a combination of morphological and molecular data (Rousset et al., 2004). It is noteworthy that using only 18S rDNA sequences, Hall et al. (2004) faced the same difficulty concerning the placement of pectinariid *Pectinaria dodeka*. In that study *Pectinaria dodeka* exhibits a long branch (see Hall et al., 2004, figs 1 and 2) and was placed within the terebelliforms in the maximum likelihood analysis, whereas it is close to the myzostomes (a clade that has also very long branch) in the parsimony analysis. In our study, we have only 18S sequences for the three pectinariids included, and our results indicate that the pectinariids are the sister group to the meiofaunal polychaete *Protodrilus purpureus* (Canalipalpata, *incertae sedis*), a taxon with a long branch. The problem with the position of Pectinariidae may be that parts of 18S sequences have evolved rapidly and thus are more subject to long-branch attraction. However, they are morphologically distinct from the other terebelliforms (Alvinellidae, Ampharetidae, Terebellidae and Trichobranchidae) so the possibility that the putative synapomorphies may be convergence may have to be considered. For instance, the uncini found in pectinariids are very similar to those seen in chaetopterids and sabellids as well as to those of terebelliforms.

With reference to relationships within Terebelliformia, few relationships show any support and more restricted analysis is probably needed. However, currently *Artacama* is placed in Terebellidae and *Artacamella* is in Trichobranchidae. However, both these groups show an extraordinary peristomial lower lip that has become a "proboscis" that is used for burrowing in sediment. For this reason, when Hartman (1955) first erected *Artacamella* she placed it with *Artacama* in the terebellid subfamily Artacaminae. Subsequently Hutchings (1977) and Holthe (1977) noted similarities between *Artacamella,* various trichobranchids. These were the long-handled thoracic hooks found in *Artacamella* and trichobranchids, and similarities of the prostomium and peristomium. Both authors recommended *Artacamella* be moved to Trichobranchidae. This separation of *Artacama* and *Artacamella* implies then that the remarkable protrusible proboscis is a convergent feature. The

results here, however, show that *Artacama* and *Artacamella* form a clade, albeit with low support and that further investigation is warranted.

Clitellata

With the exception of the position of dinophilids, the monophyly of Clitellata is corroborated in our trees (although there is no jac support for it). Based on morphology, this group is supported by several synapomorphies, such as a restriction of gonads to some anterior segments, hermaphroditism, sperm ultrastructure, direct development, relocation of the brain from the prostomium into a more posterior position and, of course, the clitellum (see Rouse and Fauchald, 1997 and references within), and the group has also been recovered in several previous molecular studies (e.g., Rota et al., 2001; Struck et al., 2002a,b; Bleidorn et al., 2003a; Erséus and Källersjö, 2004; Jördens et al., 2004; Struck and Purschke, 2005). As to dinophilids in our trees, they are the sister to *Capilloventer* (no jac support), and these two together constitute the sister to the remaining clitellates. Struck and Purschke (2005) recently demonstrated that dinophilids are not members of Eunicida, and a close relationship between dinophilids and clitellates was previously suggested by one of the analyses by Hall et al. (2004); in spite of the absence of support we believe this issue merits closer examination.

The actual position of Clitellata within Annelida remains highly uncertain. Two main hypotheses have been proposed about the position of clitellates, either that they are sister to a monophyletic Polychaeta or a derived taxon within the polychaetes (see Purschke, 2002 for a review). On the basis of hypotheses of either a limnetic (Clark, 1969; Timm, 1981; Brinkhurst, 1984, 1994; Brinkhurst and Nemec, 1987; Omodeo, 1998) or terrestrial origin of oligochaetes (Westheide, 1997; Purschke, 1999; Purschke et al., 2000; Purschke, 2002, 2003), various polychaete groups have been regarded as being closely related to Clitellata. For instance, Timm (1981), arguing for a limnetic origin of oligochaetes, proposed that Aeolosomatidae, minute worms living almost exclusively in freshwater habitats, are closely related to Clitellata.

Constraining Clitellata to be monophyletic requires an addition of five steps to the tree in Fig. 2, but this then leaves the group again without an obvious sister taxon outside of Capilloventridae. More strongly, our results reject a close relationship between Clitellata and Aeolosomatidae, confirming earlier molecular and morphological studies (see Struck and Purschke, 2005 and references within), a constrained sister group relationship between these two taxa requires 15 additional steps. Purschke (1999), proposing that the first clitellates were terrestrial, studied whether "clitellate characters" are present in the terrestrial or semiterrestrial polychaetes *Hrabeiella periglandulata* and Parergodrilidae (*Parergodrilus heideri* and *Stygocapitella subterranea*) as well. He found that these polychaetes have many features in common with the Clitellata but, adopting the earlier view (see for example, Rota, 1998) concluded that these similarities have arisen by convergence. Later, Purschke (2003) investigated *H. periglandulata* and the clitellate *Enchytraeus minutus* for potential homologies of the dorsal pharynx. He found that similarities between *H. periglandulata* and Clitellata are greater than between any of these and Parergodrilidae, and concluded that there is strong evidence for a close relationship between the former two taxa. However, our results as well as other molecular studies (see Jördens et al., 2004 and references within) neither support a position of Clitellata close to *H. periglandulata*, nor to Parergodrilidae; a constrained sister group relationship between Clitellata and *H. periglandulata* would require 20 extra steps, that between Clitellata and Parergodrilidae 12 extra steps (though the latter is reduced to six steps if Dinophilidae is allowed to remain sister to *Capilloventer*).

Monophyly and the root position of Annelida

The present analyses neither support the monophyly of Annelida nor that of Polychaeta. Indeed, our results indicate delineation problems with the annelids, showing several other protostomes as nested among the polychaetes. We are not the first to face this problem. In the majority of molecular studies dealing with the interrelationships of the major groups of annelids, some taxa of Arthropoda, Brachiopoda, Mollusca, Platyhelminthes, Sipuncula and Phoronida were also found as part of an annelid ingroup (Rota et al., 2001; Bleidorn et al., 2003b; Hall et al., 2004; Jördens et al., 2004; Struck and Purschke, 2005). At least some of these positions are likely due to a combination of tree reconstruction artifacts (such as LBA) and a lack of phylogenetic signal at the basis of the annelid tree, possibly caused by rapid radiation at the beginning of the history of annelids and mollusks (see, e.g., Balavoine and Adoutte, 1998 and Giribet, 2002).

However, for Echiura, in particular, there is growing evidence supporting a position within Annelida. The treatment of a separate phylum Echiura has been sustained mainly by the fact that echiurans are unsegmented. In their morphological analyses, Rouse and Fauchald (1995, 1997) scored segmentation simply as being absent, while Nielsen (1995, 1997) argued for a secondary loss of segmentation in this group. Purschke et al. (2000) also pointed out that a sister group relationship between echiurans and annelids should be viewed with caution, considering the problematic assessment of absent characters (i.e., here absence of segmentation in echiurans). Using elongation factor-1α

sequence data, McHugh (1997) found that echiurans are derived annelids, confirming Nielsen's hypothesis. However, Brown et al. (1999) found Echiura to be sister group to annelids in their combined analysis of three genes, though its position varied on the individual genes. Nevertheless recent studies of the organization of the nervous system (Hessling, 2002; Hessling and Westheide, 2002) also gave further evidence for regarding Echiura as an annelid group, although its sister taxon remains to be found. Using 18S rDNA data, Bleidorn et al. (2003a,b) and Hall et al. (2004) consistently found echiurans to be nested within polychaetes with a close affinity to Capitellidae (Scolecida). Our results partly confirm this; in the "complete" analysis (Fig. 1), they are the sister to capitellids, although the support for this is low (30) and in the "restricted" analysis (Fig. 2), they are also close to capitellids. Thus, although increasingly from a morphological point of view, a derived position of Echiura within Annelida needs further assessment with extended taxon and sequence sampling.

Even with the exclusion of putative outgroups (i.e., the restricted data set), our analysis failed to recover the monophyly of Annelida (see Fig. 2). Rouse and Pleijel (2001, 2003) pointed out that the rooting of the annelid tree is a major problem in animal systematics. So far, large-scale molecular studies including the present one have not been very encouraging; we lack data (or analytical methods) to deal with such deep divergences and therefore also knowledge about the closest relatives to annelids. As a result we are at present unable to specify a root position on the annelid tree.

Conclusions

Analyses of deep evolutionary relationships have never been easy, especially not when it concerns the evolution of diversified groups such as Annelida, and when using "limited" samples of taxa and data. Our results corroborate (or at least fail to refute) the monophyly of a vast majority of the annelid families represented by more than one terminal taxon in the study. It also provides new molecular support for some particular close relationships previously established only on the basis of morphological characters. However, although this is the largest data set compiled and analyzed for the annelids, our results regarding the delineation and resolution of the major clades of annelids are still ambiguous. Our analyses reveal that the more inclusive polychaete clades established on the basis of morphology by Rouse and Fauchald (1997), except Amphinomida and Aphroditiformia, are yet not supported by sequence data, underlining the need for further work. Concerning the sister group of Clitellata, there is as yet no clear candidate; earlier morphological hypotheses (suggesting, e.g., Aeolosomatidae, *Hrabeiella*, Parergodrilidae or *Questa*) are not supported.

Contrary to our expectation, a dense taxon sampling did not provide a notable improvement of the resolution in the deepest part of the annelid tree. We could neither support the monophyly of Annelida nor that of Polychaeta. This failure, i.e., the lack of phylogenetic signal in the deepest part of the tree, can be interpreted as support to the hypothesis of a period of fast diversification during the Cambrian suggesting an "explosive radiation" of annelids (for instance, Balavoine and Adoutte, 1998; Giribet, 2002; Conway Morris, 2003). A combination of the very short branches resulting from a period of fast evolution, combined with differences in evolutionary rates in other parts of the tree, may be the cause of our difficulties with the deepest clades of Annelida. As morphological data are less prone to LBA artifacts, we suggest a future approach that uses morphological and molecular data in combination for the analysis of higher-level annelid relationships, but this will also require further studies on particular issues at lower taxonomic levels (see, e.g., Burnette et al., 2005; Bleidorn, 2005; Worsaae et al., 2005). Nuclear ribosomal loci, in particular 18S, still appear to be useful, and taxon sampling for this gene should be extended for several polychaete groups, especially for those for which only limited information is available (such as Phyllodocidae or Spionidae). Needless to say, however, other markers are still badly needed to add information to the existing ribosomal data set.

Acknowledgments

We wish to thank the following persons and institutions for providing working facilities: Tor Bakke, NHM, Norway, Michel Bhaud, Laboratoire Arago, France, Philippe Bouchet (organizer of the expeditions SEAMOUNT 1, LIFOU 2000, and "the Fifth European Malacological Workshop" at Sicily), MNHN, Kristian Fauchald, USNM, Maria Cristina Gambi, Stazione Zoologica Anton Dohrn, Italy, Kristineberg Marine Research Station, Sweden, Tomoyuki Miura, Kagoshima University, Japan, Masaaki Morisawa, Misaki Marine Biological Station, Japan, Yoshihisa Shirahama, Seto Marine Biological Laboratory, Japan, and Jon-Arne Sneli, Trondhjem Biological Station, Norway, and the following persons for support and advice during the lab work: Liz Borda, Bodil Cronholm, Pia Eldenäs, Mari Källersjö, Laetitia Plaisance and Tripp Macdonald. Australian specimens were collected with permit G96/539 and G00/019 from the Great Barrier Reef Marine Park Authority. Financial support for VR was provided by the European Union HIGHLAT project at the Swedish Museum of Natural History (Molecular Systematics Laboratory), La Fondation des Treilles

(Paris), and an Annette Kade Fellowship for work at the American Museum of Natural History, for FP by Christensen Research Institute, Madang, Papua New Guinea (Palo Alto, California), Formas, grant no. 2004–0085, Japan Society for the Promotion of Science, Kristineberg Marine Research Station (European Community ARI-programme), the Royal Swedish Academy of Science, Trondheim Marine Systems Research Infrastructure (European Community ARI-programme), USNM (Carribean Coral Reef Ecosystems Program and Visiting Scientist Fellowship), for GWR by the Australian Research Council and South Australian Museum, and for CE by The Swedish Research Council, grant no. 621-2001-2788. This is CCRE contribution no. 773 from Carrie Bow Cay Field Laboratory.

References

Balavoine, G., Adoutte, A., 1998. One or three cambrian radiations? Science 280, 397–398.

Banse, K., 1969. Acrocirridae n. Fam. (Polychaeta Sedentaria). J. Fish. Res. Board Can. 26, 2595–2620.

Bartolomaeus, T., Meyer, K., 1997. Development and phylogenetic significance of hooked setae in Arenicolidae (Polychaeta, Annelida). Invertebr. Biol. 116, 227–242.

Bergsten, J., 2005. A review of long-branch attraction. Cladistics 21, 163–193.

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

Bleidorn, C., Vogt, L., Bartolomaeus, T., 2003a. A contribution to sedentary polychaete phylogeny using 18S rRNA sequence data. J. Zool. Syst. Evol. Res. 41, 186–195.

Bleidorn, C., Vogt, L., Bartolomaeus, T., 2003b. New insights into polychaete phylogeny (Annelida) inferred from 18S rDNA sequences. Mol. Phyl. Evol. 29, 279–288.

Böggemann, M., 2002. Revision of the Glyceridae Grube, 1850 (Annelida: Polychaeta). Abh. Senckenberg. Naturforsch. Ges. 555, 1–249.

Brinkhurst, R.O., 1984. The position of the Haplotaxidae in the evolution of oligochaete annelids. Hydrobiology 115, 25–36.

Brinkhurst, R.O., 1994. Evolutionary relationships within the Clitellata: an update. Megadrilogica 5, 109–112.

Brinkhurst, R.O., Nemec, A.F.L., 1987. A comparison of phenetic and phylogenetic methods applied to the systematics of Oligochaeta. Hydrobiology 155, 65–74.

Brown, S., Rouse, G.W., Hutchings, P., Colgan, D., 1999. Assessing the usefulness of histone H3, U2 snRNA and 28S rDNA in analyses of polychaete relationships. Aust. J. Zool. 47, 499–516.

Burnette, A.B., Struck, T.H., Halanych, K.M., 2005. Holopelagic *Poeobius meseres* ('Poeobiidae', Annelida) is derived from benthic flabelligerid worms. Biol. Bull. 208, 213–220.

Clark, R.B., 1969. Systematics and phylogeny: Annelida, Echiura, Sipuncula. In: Florkin, M., Scheer, B.T. (Eds.), Chemical Zoology. Academic Press, New York, pp. 1–68.

Colgan, D., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46, 419–437.

Colgan, D.J., Hutchings, P.A., Brown, S., 2001. Phylogenetic relationships within the Terebellomorpha. J. Mar. Biol. Assoc. U.K. 81, 765–773.

Conway Morris, S., 2003. The Cambrian 'explosion' of metazoans and molecular biology: would Darwin be satisfied? Int. J. Dev. Biol. 47, 505–515.

Dales, R.P., 1962. The polychaete stomodeum and the inter-relationships of the families of Polychaeta. Proc. Zool. Soc. Lond. 139, 389–428.

Erséus, C., Källersjö, M., 2004. 18S rDNA phylogeny of Clitellata (Annelida). Zool. Scr. 33, 187–196.

Erséus, C., Prestegaard, T., Källersjö, M., 2000. Phylogenetic analysis of Tubificidae (Annelida, Clitellata) based on 18S rDNA sequences. Mol. Phyl. Evol. 15, 381–389.

Fauchald, K., 1992. A review of the genus *Eunice* (Polychaeta: Eunicidae) based upon type material. Smith. Contr. Zool. 523, 1–422.

Fauchald, K., Rouse, G.W., 1997. Polychaete systematics: past and present. Zool. Scr. 26, 71–138.

Fitzhugh, K., Wolf, P.S., 1990. Gross morphology of the brain of pilargid polychaetes: taxonomic and systematic implications. Am. Mus. Novit. 2992, 1–16.

Giangrande, A., Gambi, M.C., 1998. Metamerism and the life-style within polychaetes: morpho-functional aspects and evolutionary implications. Ital. J. Zool. 65, 39–50.

Giribet, G., 2002. Current advances in the phylogenetic reconstruction of metazoan evolution. A new paradigm for the Cambrian explosion? Mol. Phyl. Evol. 24, 345–357.

Giribet, G., Carranza, S., Baguna, J., Riutort, M., Ribera, C., 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Mol. Biol. Evol. 13, 76–84.

Giribet, G., Carranza, S., Riutort, M., Baguña, J., Ribera, C., 1999. Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences. Phil. Trans. R. Soc. Ser. B, 354, 215–222.

Glasby, C.J., 1993. Family revision and cladistic analysis of the Nereidoidea (Polychaeta: Phyllodocida). Invertebr. Taxon. 7, 1551–1573.

Glasby, C., Hutchings, P., Hall, K.A., 2004. Assessment of monophyly and taxon affinities within the polychaete clade Terebelliformia (Terebellida). J. Mar. Biol. Assoc. U.K. 84, 961–971.

Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics, 15, 415–428.

Goloboff, P.A., Farris, J.S., Nixon, K., 2003. TNT: Tree Analysis using New Technology. Program and documentation, available at http://www.zmuc.dk/public/phylogeny/tnt

Halanych, K.M., Dahlgren, T., McHugh, D., 2002. Unsegmented annelids? Possible origins of four Lophotrochozoan worm taxa. Integr. Comp. Biol. 42, 678–684.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment, editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hall, K.A., Hutchings, P., Colgan, D., 2004. Further phylogenetic studies of the Polychaeta using 18S rDNA sequence data. J. Mar. Biol. Assoc. U.K. 84, 949–960.

Hartman, O., 1955. Endemism in the North Pacific Ocean, with emphasis on the distribution of marine annelids, and descriptions of new or little known species. In: Essays in the Natural Sciences in Honor of Captain Allan Hancock on the Occasion of his Birthday July 26, 1955. University of Southern California Press, Los Angeles, pp. 39–60.

Hessling, R., 2002. Metameric organisation of the nervous system in developmental stages of *Urechis caupo* (Echiura) and its phylogenetic implications. Zoomorphology 121, 221–234.

Hessling, R., Westheide, W., 2002. Are Echiura derived from a segmented ancestor? Immunohistochemical analysis of the nervous system in developmental stages of *Bonellia viridis*. J. Morphol. 252, 100–113.

Holthe, T., 1977. The systematic position of *Artacamella* Hartman, 1955 (Polychaeta, Terebellomorpha). Sarsia 63, 35–37.

Hutchings, P.A., 1977. Terebelliform polychaeta of the families Ampharetidae, Terebellidae and Trichobranchidae from Australia, chiefly from Moreton Bay, Queensland. Rec. Aust. Mus. 31, 1–38.
Jördens, J., Struck, T., Purschke, G., 2004. Phylogenetic inference regarding Parergodrilidae and *Hrabeiella periglandulata* ('Polychaeta', Annelida) based on 18S rDNA, 28S rDNA and COI sequences. J. Zool. Syst. Evol. Res. 42, 270–280.
Kojima, S., 1998. Paraphyletic status of Polychaeta suggested by phylogenetic analysis based on the amino acid sequences of elongation factor 1-alpha. Mol. Phyl. Evol. 9, 255–261.
Lê, H.L.V., Lecointre, G., Perasso, R., 1993. A 28S rRNA based phylogeny of the Gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. Mol. Phyl. Evol. 2, 31–51.
Martin, P., 2001. On the origin of the Hirudinea and the demise of Oligochaeta. Proc. R. Soc. Lond. Ser. B, 268, 1089–1098.
McHugh, D., 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. Proc. Natl Acad. Sci. U.S.A. 94, 8006–8009.
McHugh, D., 2000. Molecular phylogeny of the annelids. Can. J. Zool. 78, 1873–1884.
McHugh, D., 2005. Molecular systematics of polychaetes (Annelida). Hydrobiology 535/536, 309–318.
Nielsen, C., 1995. Animal Evolution. Oxford University Press, Oxford.
Nielsen, C., 1997. The Phylogenetic Position of the Arthropoda. Chapman & Hall, London.
Nygren, A., 2004. Revision of Autolytinae (Syllidae: Polychaeta). Zootaxa 680, 1–314.
Nygren, A., Sundberg, P., 2003. Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida). Mol. Phyl. Evol. 29, 235–249.
Omodeo, P., 1998. History of Clitellata. Ital. J. Zool. 65, 51–74.
Orensanz, J.M., 1990. The eunicemorph polychaete annelids from Antarctic and Subantarctic Seas. Antarctic Res. Ser., 52, 1–183.
Palumbi, S.R., Martin, A., Romani, W., McMillan, O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR, Version 2.0. University of Hawaii, Honolulu, Hawaii.
Persson, J., Pleijel, F., 2005. On the phylogenetic relationships of *Axiokebuita*, *Travisia* and Scalibregmatidae (Polychaeta). Zootaxa 998, 1–14.
Pleijel, F., Dahlgren, T., 1998. Position and delineation of Chrysopetalidae and Hesionidae (Annelida, Polychaeta, Phyllodocida). Cladistics 14, 129–150.
Purschke, G., 1999. Terrestrial polychaetes—models for the evolution of the Clitellata (Annelida)? Hydrobiology 406, 87–99.
Purschke, G., 2002. On the ground pattern of Annelida. Organisms Diversity Evol. 2, 181–196.
Purschke, G., 2003. Is *Hrabeiella periglandulata* (Annelida, 'Polychaeta') the sister group of Clitellata? Evidence from an ultrastructural analysis of the dorsal pharynx in *H. periglandulata* and *Enchytraeus minutus* (Annelida, Clitellata). Zoomorphology 122, 55–66.
Purschke, G., Hessling, R., Westheide, W., 2000. The phylogenetic position of the Clitellata and the Echiura—on the problematic assessment of absent characters. J. Zool. Syst. Evol. Res. 38, 165–173.
Rota, E., 1998. Morphology and adaptations of *Parergodrilus* Reisinger and *Hrabeiella* Pizl & Chalupsky, two enigmatic soil-dwelling annelids. Ital. J. Zool. 65, 75–84.
Rota, E., Martin, P., Erséus, C., 2001. Soil-dwelling polychaetes: enigmatic as ever? Some hints on their phylogenetic relationships as suggested by a maximum parsimony analysis of 18S rRNA gene sequences. Contr. Zool. 70, 127–138.
Rouse, G.W., Fauchald, K., 1995. The articulation of annelids. Zool. Scr. 24, 269–301.
Rouse, G.W., Fauchald, K., 1997. Cladistics and polychaetes. Zool. Scr. 26, 139–204.
Rouse, G.W., Pleijel, F., 2001. Polychaetes. Oxford University Press, Oxford.
Rouse, G.W., Pleijel, F., 2003. Problems in polychaete systematics. Hydrobiology 496, 175–189.
Rousset, V., Rouse, G.W., Féral, J.-P., Desbruyères, D., Pleijel, F., 2003. Molecular and morphological evidence of Alvinellidae relationships (Terebelliformia, Polychaeta, Annelida). Zool. Scr. 32, 185–197.
Rousset, V., Rouse, G.W., Siddall, M.E., Tillier, A., Pleijel, F., 2004. The phylogenetic position of Siboglinidae (Annelida) inferred from 18S rRNA, 28S rRNA and morphological data. Cladistics 20, 518–533.
Siddall, M.E., Apakupakul, K., Burreson, E.M., Coates, K.A., Erséus, C., Gelder, S.R., Källersjö, M., Trapido-Rosenthal, H., 2001. Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of Oligochaetes. Mol. Phyl. Evol. 21, 346–351.
Siddall, M.E., Borda, E., Rouse, G.W., 2004. Towards a tree of life for the Annelida. In: Cracraft, J., Donoghue, M.J. (Eds.), Assembling the Tree of Life. Oxford University Press, New York, pp. 237–251.
Sjölin, E., Erséus, C., Källersjö, M., 2005. Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. Mol. Phyl. Evol. 35, 431–441.
Struck, T.H., Purschke, G., 2005. The sister group relationship of Aeolosomatidae and Potamodrilidae (Annelida: "Polychaeta")—a molecular phylogenetic approach based on 18S rDNA and cytochrome oxidase I. Zool. Anz. 243, 281–293.
Struck, T., Hessling, R., Purschke, G., 2002a. The phylogenetic position of the Aelosomatidae and Parergordrilidae, two enigmatic oligochaete-like taxa of the 'polychaeta', based on molecular data from 18S rDNA sequences. J. Zool. Syst. Evol. Res. 40, 155–163.
Struck, T., Westheide, W., Purschke, G., 2002b. Progenesis in Eunicida ("Polychaeta", Annelida)—separate evolutionary events? Evidence from molecular data. Mol. Phyl. Evol. 25, 190–199.
Struck, T.H., Purschke, G., Halanych, K.M., 2005. A scaleless scale worm: Molecular evidence for the phylogenetic placement of *Pisione remota* (Pisionidae, Annelida). Mar. Biol. Res. 1, 243–253.
Swofford, D.L., 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, Massechusetts.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Timm, T., 1981. On the origin and evolution of aquatic Oligochaeta. Eesti NSV Tead. Akad. Toim. (Bioloogiline Seeria), 30, 174–181.
Westheide, W., 1997. The direction of evolution within the Polychaeta. J. Nat. Hist. 31, 1–15.
Westheide, W., McHugh, D., Purschke, G., Rouse, G.W., 1999. Systematization of the Annelida: different approaches. Hydrobiology 402, 291–307.
Wiklund, H., Nygren, A., Pleijel, F., Sundberg, P., 2005. Phylogeny of Aphroditiformia (Polychaeta) based on molecular and morphological data. Mol. Phyl. Evol. 37, 494–502.
Winnepenninckx, B., Backeljau, T., De Wachter, R., 1995. Phylogeny of protostome worms derived from 18S rRNA sequences. Mol. Biol. Evol. 12, 641–649.
Worsaae, K., Nygren, A., Rouse, G.W., Giribet, G., Persson, J., Sundberg, P., Pleijel, F., 2005. Phylogenetic position of Nerillidae and *Aberranta* (Polychaeta, Annelida), analysed by direct optimization of combined molecular and morphological data. Zool. Scr. 34, 313–328.