

A molecular phylogeny of annelids

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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.

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Annelids are segmented worms that are found world-wide 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

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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 Winnepeninckx 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-1 α , 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-1 α (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 μ L of 10 μ M of primer pair mix, 1 μ L of template and 23 μ L 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 Masterecyclers. 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 μ L BigDye™ (Applied Biosystems, Perkin-Elmer Corporation, Foster City, CA), 1 μ L of 1 μ M primer and 3 μ L 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 μ L 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	<i>Capilloventer australis</i> Erséus, 1983	Victoria, Australia	AY365455	AY340384		AY340448
Enchytraeidae	<i>Fridericia tuberosa</i> Rota, 1995	Tuscany, Italy	AF209453	AY340394		AY340457
Haplotaenidae	<i>Haplotaen cf. gordioides</i> (Hartmann, 1821)	California, USA	AY365456	AY340398		AY340461
Lumbricidae	<i>Eisenia andrei</i> Bouché, 1972	Västergötland, Sweden	AY365460	AY340390	DQ779728	AY340454
Lumbricidae	<i>Lumbricus terrestris</i> Linnaeus, 1758	GB	AJ272183	AF185172	AF185262	
Lumbriculidae	<i>Eclidrilus frigidus</i> Eisen, 1881	GB	AY040692			
Lumbriculidae	<i>Rhynchelmis tetratheca</i> (Michaelsen, 1920)	Södermanland, Sweden	AY365464	AY340414		AY340477
Megascolecidae	<i>Pontodrilus litoralis</i> (Grube, 1855)	Florida, USA	AY365462	AY340410		AY340473
Phreodrilidae	<i>Antarctodrilus proboscidea</i> (Brinkhurst & Fulton, 1979)	Victoria, Australia	AY365465	AY340383	DQ779716	AY340447
Propappidae	<i>Propappus volki</i> Michaelsen, 1916	Blekinge, Sweden	AY365457	AY340412		AY340475
Tubificidae	<i>Heronidrilus gravidus</i> Erséus, 1990	Belize	AY340433	AY340399	DQ779736	AY340462
Tubificidae	<i>Heterochaeta costata</i> Claparède, 1863	Bohuslän, Sweden	AY340432	AY340397		AY340460
Tubificidae	<i>Tubificoides amplivasatus</i> (Erséus, 1975)	Bohuslän, Sweden	AY340445	AY340421		AY340483
Erpobdellidae	<i>Erpobdella octoculata</i> (Linnaeus, 1758)	Paimpont Forest, France	AF099949		DQ779729	
Glossiphoniidae	<i>Glossiphonia complanata</i> (Linnaeus, 1758)	Paimpont Forest, France	AF115982		DQ779733	
Glossiphoniidae	<i>Helobdella stagnalis</i> (Linnaeus, 1758)	Västergötland, Sweden	AF115986	AY340402	DQ779735	AY340465
Hirudinidae	<i>Hirudo medicinalis</i> Linnaeus, 1758	Paimpont Forest, France	AY786464		DQ779738	AF315058
Hirudinidae	<i>Piscicola geometra</i> (Linnaeus, 1758)	GB	AF115995			
Polychaeta						
Incertae sedis						
Aelosomatidae	<i>Aelosoma hemprichi</i> Ehrenberg, 1828	Cultures in Bologna, Italy/GB	AHE310500			DQ779600
Aelosomatidae	<i>Aelosoma viride</i> Stephenson, 1911	Cultures in Bologna, Italy	DQ779638			
<i>Hrabeiella</i>	<i>Hrabeiella periglandulata</i> Pizl & Chalupsky, 1984	GB	AJ310501			
Parergodrilidae	<i>Parergodrilus heideri</i> Reisinger, 1925	GB	AJ310504			
Parergodrilidae	<i>Stygocapitella subterranea</i> Knoellner, 1934	GB	AF412810			
Dinophilidae	<i>Dinophilus gyrocoelatus</i> O. Schmidt, 1857	GB	AF412805			AF380116

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

Table 1
Continued

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

Table 1
Continued

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

Terebelliformia	Flabelligeridae	<i>Flabelligera affinis</i> M. Sars, 1829	Iceland	DQ779650	DQ779688	DQ779614
	Flabelligeridae	<i>Pocobius meseres</i> Heath, 1930	Monterey Bay, USA	DQ779668	DQ779706	DQ779631
	Sternaspidae	<i>Sternaspis scutata</i> (Ranzani, 1817)	Banyuls, France	DQ779671	DQ779710	AY532353
	Alvinellidae	<i>Paralvinella grasslei</i> Desbruyères & Laubier, 1982	GB	AY577886		
	Alvinellidae	<i>Paralvinella palmitiformis</i> Desbruyères & Laubier, 1986	GB	AF168747		
	Ampharetidae	<i>Ampharete acutifrons</i> (Grube, 1860)	Iceland		DQ779673	DQ779601
	Ampharetidae	<i>Anobothrus gracilis</i> (Malmgren, 1866)	GB	AY611458	AF501670	
	Ampharetidae	<i>Melina cristata</i> (M. Sars, 1851)	Bohuslän, Sweden	AY611459	AY611445	DQ779624
	Pectinariidae	<i>Pectinaria dodeka</i> Hutchings & Peart, 2002	GB	AB106263		
	Pectinariidae	<i>Pectinaria granulata</i> (Linnaeus, 1767)	GB	AY577890		
	Pectinariidae	<i>Pectinaria regalis</i> Verrill, 1901	GB	AY040698		
	Terebellidae	<i>Anaeana trilobata</i> (M. Sars, 1863)	GB	AF508115	AF342695	
	Terebellidae	<i>Amphitritides gracilis</i> (Grube, 1860)	GB	AB106260	AF185158	AF185241
	Terebellidae	<i>Amphitritides harpa</i> Hutchings & Glasby, 1988	GB			
	Terebellidae	<i>Artacana proboscidea</i> Malmgren, 1866	Bohuslän, Sweden	AY344666	AY344667	
	Terebellidae	<i>Eupolymnia residens</i> (Delle Chiaje, 1828)	GB	AY611460		
	Terebellidae	<i>Lanice conchilega</i> Pallas, 1766	Brittany, France/GB	X79873	AY340403	
	Terebellidae	<i>Loimia medusa</i> (Savigny, 1818)	GB	AY040690		
	Terebellidae	<i>Lysilla pacifica</i> Hesse, 1917	GB	AB106259	AF342696	AF342703
	Terebellidae	<i>Pista australis</i> Hutchings & Glasby, 1988	GB	AB106261	AF185159	AF185242
	Terebellidae	<i>Pista</i> sp.	GB			
	Terebellidae	<i>Rhinothelepus lobatus</i> Hutchings, 1974	GB		AF342694	AF342701
	Terebellidae	<i>Thelepus cincinnatus</i> (Fabricius, 1780)	Trondheim, Norway/GB	AY611462	DQ779712	DQ779636
	Trichobranchidae	<i>Artacameilla tribranchiata</i> Hutchings & Peart, 2000	South Australia		DQ779677	DQ779605
Scolecida	Trichobranchidae	<i>Terebellides stroemi</i> M. Sars, 1835	GB	AY577893	X80658	AY577884
	Arenicolidae	<i>Arenicola marina</i> (Linnaeus, 1758)	Brittany, France	AI310502		AY340446
	Arenicolidae	<i>Branchiomaldane vincenti</i> (Langerhans, 1881)	GB	AF508117	DQ779718	AY569690
	Capitellidae	<i>Barantolla lepte</i> Hutchings, 1974	GB	AB106265		
	Capitellidae	<i>Capitella capitata</i> (Fabricius, 1780)	GB	AF508118		
	Capitellidae	<i>Dasybranchus caducus</i> (Grube, 1846)	GB	AF448153		
	Capitellidae	<i>Notomastus latericeus</i> M. Sars, 1851	Bohuslän, Sweden/GB	AY040697	AY340406	AY340469
	Cosuridae	<i>Cosaura</i> sp.	Iceland	DQ779646	DQ779684	DQ779610
	Maldanidae	<i>Clymenura clypeata</i> (de Saint Joseph, 1894)	Brittany, France	AY340423	AY340385	AY340449
	Maldanidae	<i>Euclymene trinalis</i> Hutchings, 1974	GB		AF185170	
	Opheliidae	<i>Armandia bilobata</i> Hartmann-Schröder, 1986	South Australia	DQ779641	DQ779676	DQ779604
	Opheliidae	<i>Lobochesis bibranchia</i> Hutchings & Murray, 1984	GB	AB106266		
	Opheliidae	<i>Ophelia bicornis</i> Savigny, 1818	GB	AF508122		
	Opheliidae	<i>Ophelia rathkei</i> McIntosh, 1908	GB	AF448157	X80651	

Table 1
Continued

Higher taxa	Species	Source	18S rDNA	28S rDNA	Histone H3	16S rDNA
ARTHROPODA	Opheliidae	<i>Ophelia acuminata</i> Örsted, 1843	Bohuslän, Sweden	AY340439	AY340408	DQ779749
	Opheliidae	<i>Polyphthalinus pictus</i> (Dujardin, 1839)	GB	AF448161	AF185171	AF185259
	Orbiniidae	<i>Orbinia bioreti</i> (Fauvel, 1919)	GB	AF448158		AY532334
	Orbiniidae	<i>Orbinia latreillii</i> Audouin & Milne-Edwards, 1833	GB	AY532355		AY532335
	Orbiniidae	<i>Prosclopos cygnochaetus</i> Day, 1954	GB	AF448162		AY532340
	Orbiniidae	<i>Protoarcia oerstedii</i> (Claparède, 1864)	GB	AF508123		AY532347
	Orbiniidae	<i>Protoarcia uncinata</i> Hartmann-Schröder, 1962	GB	AF508124		
	Orbiniidae	<i>Scoloplos armiger</i> (O.F. Müller, 1776)	Bohuslän, Sweden	AY340443	AY340417	AY340480
	Orbiniidae	<i>Scoloplos johnstonei</i> Day, 1934	GB	AF508126		AY532332
	Paraonidae	<i>Cirrophorus lyra</i> (Southern, 1914)	Bohuslän, Sweden	AY340424	AY340386	AY340450
	Questidae	<i>Questa paucibranchiata</i> Giere & Erséus, 1998	Bahamas/GB	AF209464	AY340413	AY340476
	Scalibregmatidae	<i>Hyboscolex</i> sp.	GB	AB106268	AY583707	AY583720
	Scalibregmatidae	<i>Lipobranchius jeffreysii</i> (McIntosh, 1869)	GB	AF508120		
	Scalibregmatidae	<i>Polyphysia crassa</i> (Örsted, 1843)	Bohuslän, Sweden	AY340440	AY340409	AY340472
	Scalibregmatidae	<i>Scalibregma inflatum</i> Rathke, 1843	Bohuslän, Sweden	AF448163	AY612624	AY532331
	Scalibregmatidae	<i>Travisia forbesii</i> Johnston, 1840	Normandie, France		DQ779713	DQ779637
	Symphyla	<i>Hansenella</i> sp.	GB	AY210823	AY210821	AF370864
	Xiphosura	<i>Limulus polyphemus</i> Linnaeus, 1758	GB	U91490	X90468	AF373606
	Branchiopoda	<i>Triops longicaudatus/australensis</i>	GB	AF144219	AY157606	AY115610
BRACHIOPODA		<i>Terebratulina retusa</i> (Linnaeus, 1758)	Bohuslän, Sweden/GB	U08324	AY340422	DQ779768
	Articulata	<i>Bonellia</i> sp./viridis	Great Barrier Reef, Australia/GB	AF123307	DQ779678	DQ779721
	Echiura	<i>Urechis caupo</i> Fisher & MacGinitie, 1928	GB	AF342805	AF342804	X58895
MOLLUSCA	Aplacophora	<i>Chaetoderma nitiidulum</i> Lovén, 1845	Bohuslän, Sweden/GB	AY377658	AY340387	AY340451
	Polyplacophora	<i>Chiton olivaceus</i> Spengler, 1797	GB	DQ779644	DQ779682	DQ779608
	Gastropoda	<i>Gibbula cineraria</i> (Linnaeus, 1758)	W. Norway	AY340430	AY340395	AY340458
		<i>Haliotis tuberculata</i> Linnaeus, 1758	GB	AF120511	AF327553	AY377775
		<i>Vampyroteuthis infernalis</i> Chun, 1903	GB	AY557459	AJ310260	AY557408
NEMERTEA		<i>Micrura fasciolata/alaskensis</i>	Bohuslän, Sweden/GB	AY340436	AY340404	AY340467
		<i>Tubulanus annulatus</i> (Montagu, 1804)	GB	AY210452	AY210473	AF103756
SIPUNCULA		<i>Antillesoma antillarum</i> (Grube & Örsted, 1858)	GB	AF519259		AF519311
		<i>Gloeosiphon aspergillus</i> (Quatrefages, 1865)	GB	AF519263		AF519316
		<i>Golfingia elongata</i> (Kieferstein, 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	TGAACCTCTCTTCAAAGTTCTTTTC	(Lê et al., 1993)
16S		
ArL	CGCCTGTTTATCAAAAACAT	(Palumbi et al., 1991)
BrH	CCGGTCTGACTCAGATCAGT	(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	GAGTCTCGTTCTTATCGGA	(Giribet et al., 1999)
18Sa2.0	ATGGTTGCAAAGCTGAAAC	(Giribet et al., 1999)
9R	GATCCTTCCGCAGGTTACCTAC	(Giribet et al., 1996)
Histone H3		
H3af	ATGGCTCGTACCAAGCAGACVGC	(Colgan et al., 1998)
H3ar	ATATCCTTRGGCATRATRGTCAC	(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 *Vampyroreuthis*. 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 *Scolecipis 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), Flabelligeridae (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),

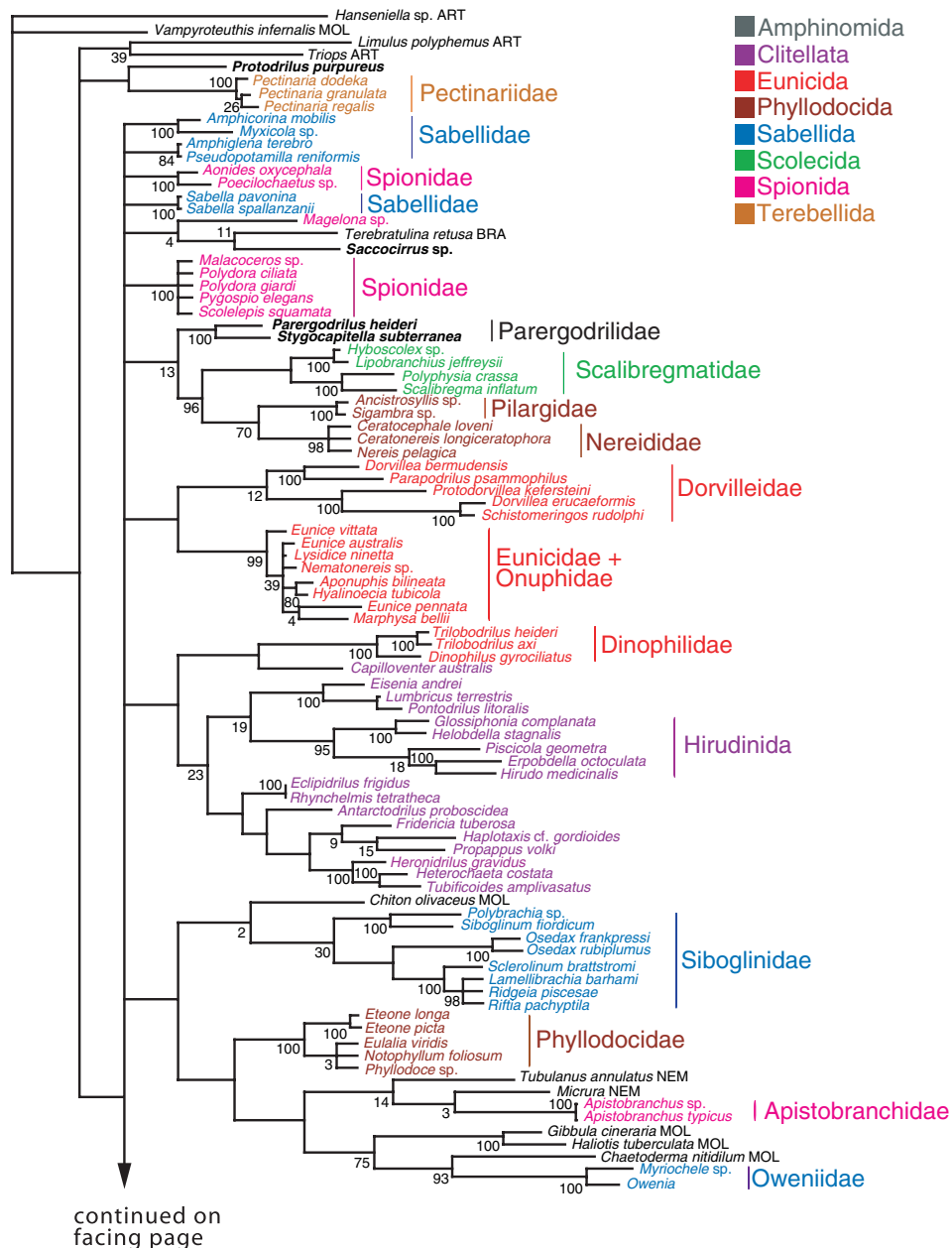


Fig. 1. Strict consensus tree from 144 trees obtained from the analysis of the “complete” data set. Numerals are jac values. More inclusive taxa (usually families) are provided after the species names, and traditional major annelid clades are indicated in color as specified in the upper right corner. 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 Acrociiridae and Flabelligeridae (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

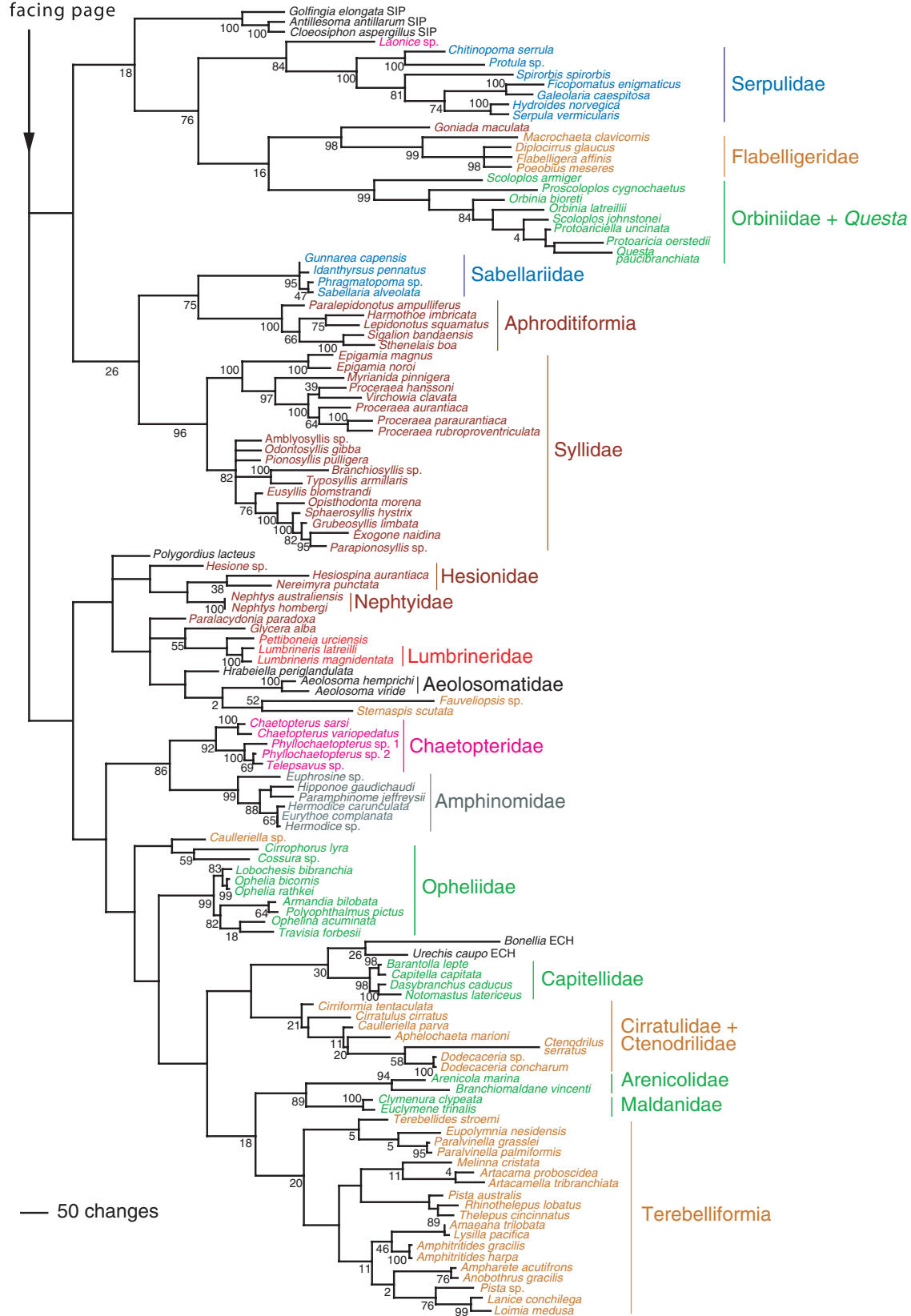
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Fig. 1. Continued

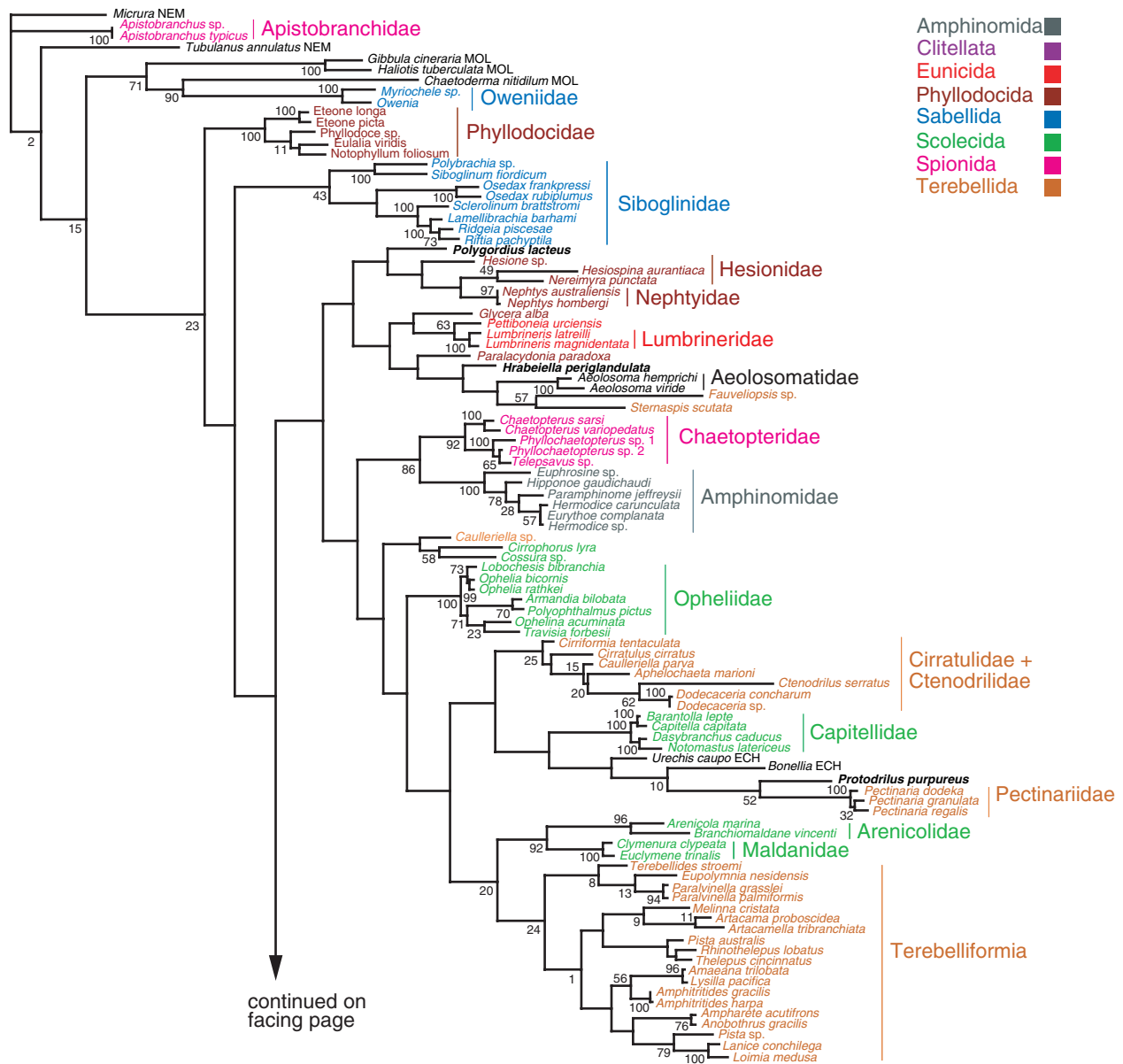


Fig. 2. Strict consensus tree from 20 trees obtained from the analysis of the “restricted” data set. Numerals are jac values. More inclusive taxa (usually families) are provided after the species names, and traditional major annelid clades are indicated in color as specified in the upper right corner. 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 Sabelliidae 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

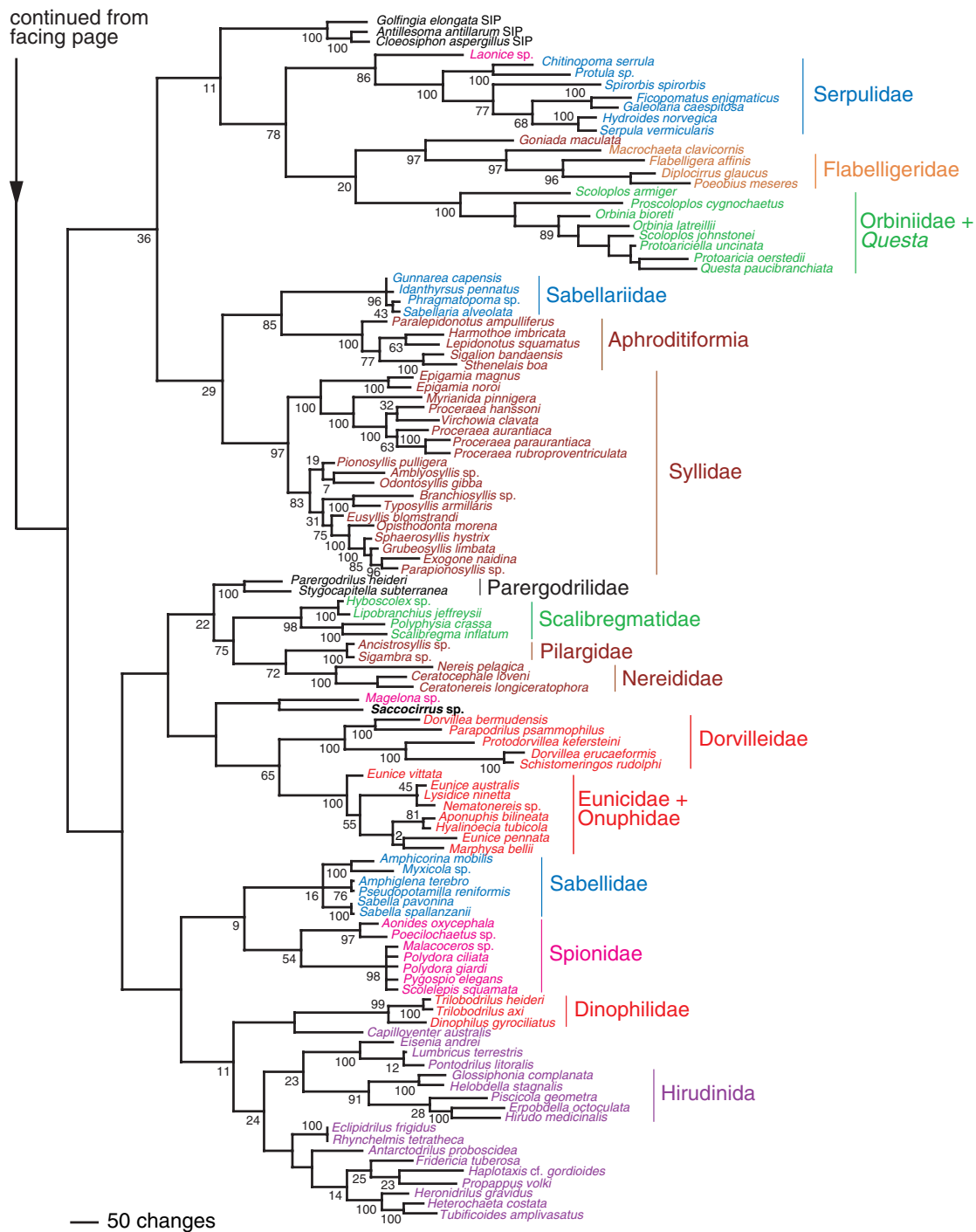
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Fig. 2. Continued

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 scolecoid 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, Oeononidae 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 jack 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 Flabelligeridae, 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 chaetopterids, 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 Oweniidae 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 Aporididae 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, Flabelligeridae). 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, Flabelligeridae (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 Flabelligeridae (99/97), and corroborates the results of Rouse and Pleijel (2003) and Burnette et al. (2005) that *Poeobius* (Poeobiidae) is nested within Flabelligeridae. 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 Flabelligeridae may also be close to Acrocirridae and so that both could be nested in Cirratulidae. Our results do not show an acrocirrid and flabelligerid plus cirratulid relationship, but instead agree with Banse (1969) who showed that Acrocirridae in fact share more features with Flabelligeridae (such as the structure of the epidermal papillae and the com-

pound 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-reversible 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 *Artacamella* is placed in Terebellidae and *Artacama* 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-1 α

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 (sug-

ing, 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 Phyllodoctidae or Spionidae). Needless to say, however, other markers are still badly needed to add information to the existing ribosomal data set.

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