3. New insights into polychaete phylogeny (Annelida) inferred from 18S rDNA sequences

Abstract - Annelid systematics and the ingroup relationships of polychaete annelids are matter of ongoing debates in recent analyses. For the investigation of sedentary polychaete relationships a molecular phylogenetic analysis was conducted based on 94 sequences of 18S rDNA, including unpublished sequences of 13 polychaete species. The data set was analyzed with maximum parsimony and maximum likelihood methods, as wells as Bayesian inference. As in previous molecular analyses the monophyly of many traditional polychaete families is confirmed. No evidence has been found for a possible monophyly of Canalipalpata or Scolecida. In all analyses a placement of the Echiura as a derived polychaete ingroup with a close relationship to the Capitellidae is confirmed. The orbiniids appear paraphyletic with regard to *Questa*. *Travisia* is transferred from Opheliidae to Scalibregmatidae. The remaining opheliids include a yet undescribed ctenodrilid species from Elba, wheras the other investigated ctenodrilid *Ctenodrilus serratus* groups with the Cirratulidae and shows a close affinity to the cirratulid genus *Dodecaceria*. A common ancestry of *Branchiomaldane* and *Arenicola*, which has been predicted on morphological data, is confirmed by the analysis and a sistergroup relationship between Arenicolidae and Maldanidae is also recovered.
These results support our assumption that on the basis of a broader taxon sampling the phylogenetic position of controversially discussed taxa can be inferred by using 18S rDNA sequence data.

3.1 Introduction

In the traditional classification polychaete annelids have been classified into over 80 families (Fauchald, 1977). The phylogenetic relationships of these polychaete taxa are matter of ongoing debates in recent papers on annelid morphology (Meyer and Bartolomaeus, 1996; Purschke, 1997; Rouse and Fauchald, 1997, 1998; Westheide, 1997; Bartolomaeus, 1998; Hausen and Bartolomaeus, 1998; Westheide *et al.*, 1999; Hausen, 2001). Rouse and Fauchald (1997) suggest that the Polychaeta comprise two major clades, the Scolecida and the Palpata. The highest ranked taxa within the Palpata are the Canalipalpata (comprising the remaining so-called “Sedentaria”) and the Aciculata (formerly termed Errantia). This view was challenged by Bartolomaeus (1998) and Hausen (2001), who question the monophyly of these taxa.

Apart from the discussion about the phylogenetic relationships of polychaete taxa, there is still no agreement as to whether the Echiura represent a derived polychaete taxon. Traditionally Echiura are regarded as closely related to the Annelida (Rouse & Fauchald 1995, 1997; Rouse 1999), but recent studies of the organisation of the nervous system (Hessling & Westheide 2002) and comparative analyses of molecular data (McHugh 1997, 1999; Brown et al. 1999) give evidence for including the Echiura into the Annelida, although the annelid sister taxon of the Echiura remains to be found.

Whereas the above-mentioned hypotheses on polychaete systematics have all been obtained by analysing morphological data, in recent years many attempts have been made to unravel polychaete relationships using molecular data (Kojima, 1998; Brown *et al*.; 1999; McHugh, 1997, 2000; Halanych *et al*., 2001; Rota *et al*., 2001; Struck *et al*., 2002; Bleidorn *et al*., 2003). While none of these analyses can be regarded as a major breakthrough in polychaete systematics, some of them have shown that traditional polychaete families are often well supported (Struck *et al*., 2002; Bleidorn *et al*., 2003). As molecular data have proved to be an important tool for solving problems on the relationships at lower taxonomic levels, we used 18S rDNA sequence data to study the phylogenetic relationships among polychaetes. Increased taxon sampling, particularly of "scolecid" taxa, gives the possibility to test the hypotheses on polychaete systematics developed through the cladistic analysis by Rouse and Fauchald (1997). Increasing the taxon sampling seems to be a promising method to find the sister taxon of the Echiura within the Annelida.

3.2 Material and methods

Taxon sampling

Thirteen newly determined 18S rDNA sequences of several sedentary polychaete taxa (specimen are deposited in the collection of T. Bartolomaeus) were aligned together with 81 metazoan 18S rDNA sequences from GenBank, including nearly all available sequences of polychaete taxa from GenBank (see Appendix A). Collection sites for the newly sequenced annelids were as follows: Arcachon, France (*Arenicola marina, Ophelia bicornis*); Buenos Aires, Argentina (*Protoariciella uncinata*, collected by Dr. Rodolfo Elias); Cape Town, South Africa (*Scoloplos (Leodamas) johnstonei*, collected by Bilke Hausam); Collioure, France (*Protoaricia oerstedii*); Concarneau, France (*Branchiomaldane vincenti, Lipobranchus jefreysii*); Helgoland, Germany (*Ctenodrilus serratus*); Kristinenberg, Sweden (*Travisia forbesii*, collected by Alexander Gruhl & Andreas Unger); Roscoff, France (*Amphitritides gracilis*, *Notomastus latericeus*); Sylt, Germany (*Capitella capitata*, *Protodriloides symbioticus*). The following polychaete

18S rDNA sequences from GenBank have been excluded from the analysis because there exists evidence for their inaccuracy: *Pectinaria regalis* (AY040698), *Marphysa sanguinea* (AY040695), *Armandia maculata* (AY040681), *Nereis virens* (Z83754), *Aphrodite aculeata* (Z83749), *Capitella capitata* (U67323) and *Magelona mirabilis* (U50969) (own data, pers. comm. Torsten Struck, Osnabrueck)

DNA extraction, PCR amplification, purification and sequencing

Genomic DNA was extracted from specimen using Qiagen DNeasy™ Tissue Kit according to the manufacturer's instructions. PCR amplification of the 18S rDNA gene was performed in two overlapping fragments of ~900bp and ~1400bp each with modificated primer pairs from Giribet *et al*. (1996) by using standard cycle-sequencing protocols. Amplifications were carried out using an Eppendorf Mastercycler gradient. The following PCR temperature file was used: 95˚C for 3 min; 35 cycles with 94˚C for 35 seconds, 45-55˚C for 45 seconds to 1 min, and 72˚C for 1 min; final extension at 72˚C for 10 min. After detection by gel electrophoresis the products were purified with the Qiaquick PCR Purification Kit (Qiagen). Sequencing of all amplified fragments in both directions was carried out by the *IIT* Biotech/Bioservice of the University of Bielefeld. All sequences were submitted to GenBank (for accession numbers see Appendix A).

Sequence Alignment

Sequences were aligned with CLUSTAL W (Thompson *et al*., 1994) using the default parameters for gap opening and gap penalty and subsequently manually edited by eye using BioEdit (Hall, 1999). Gap positions and regions that could not be aligned unambiguously were excluded from the analysis. The alignment is available by emailing the first author.

Data Analysis

A chi-square test of homogeneity of base frequencies across taxa was performed. All trees were rooted *a posteriori* the analysis using the sequence of *Gordius aquaticus* (Nematomorpha).

Maximum Likelihood

For estimating the appropriate model of sequence evolution, a hierarchical likelihood ratio test (hLRT) was carried out as implemented in the program modeltest version 3.06 (Posada and Crandall, 1998, 2001).The test criteria indicate that the Tamura Nei substitution model (Tamura and Nei, 1993) with equal base frequencies, invariant sites and gamma distribution (TrNef+I+Γ) represents the optimal model in respect to the data. A maximum likelihood analysis was performed with PAUP*, version 4.0b8 (Swofford,

2001) under the likelihood settings suggested by the result of the modeltest using the heuristic search option with TBR branch swapping and simple sequence addition.

Bayesian inference

A Bayesian analysis of the data set was conducted by using MrBayes 3.0B2 (Huelsenbeck and Ronquist, 2001). We used MrModeltest 1.1b for estimating the ML parameters in MrBayes. This program is a simplified version of Modeltest 3.06 (Posada & Crandall 1998, 2001) and contains less models which are tested. The *hLRT* criterium indicates that the SYM+I+Γ (Zharkikh 1994) represents the optimal model in respect to the data. All priors were set according to this model: lset nst=6 rates=invgamma; prset revmatpr=unif orm(0.0,50.0) statefreqpr=fixed(equal) shapepr=uniform(0.0,100.0) pinvarpr=uniform(0. 0,1.0). Each Markov chain, three heated and one cold, was started from a random tree and all four chains ran simultaneously for $5 * 10^6$ generations, with trees being sampled every 400 generations for a total of 12,501 trees. After the likelihood of the trees of each chain converged, we discarded the first 2,500 trees as *burn in*. The majority-rule consensus tree containing the posterior probabilities of the phylogeny was determined from 10,001 trees.

Maximum parsimony

The data set was analyzed by parsimony, using PAUP*, version 4.0b8 (Swofford, 2001). Maximum parsimony searches were run with 1,000 random addition replicates, heuristic search option with tree-bisection-reconnection (TBR) branch swapping, holding one tree per step, and keeping all most-parsimonious trees. Transition-transversion ratios were weighted in accordance with the model of sequence evolution supported by the result of *modeltest.* Bootstrap values (Felsenstein, 1985) were determined from 1,000 replicates subject to full heuristic searches with random taxon addition to provide measures of relative clade support.

3.3 Results

Sequence data

After the exclusion of ambiguous sites, the alignment contains 1,574 positions, of which 709 are parsimony informative. The chi-square test of homogeneity of base frequencies across taxa resulted in no significant *P*-values (chi-square=144.546402, df=279, *P*=1.0). Assuming that compositional bias has no effect on the recovery of phylogenetic signal

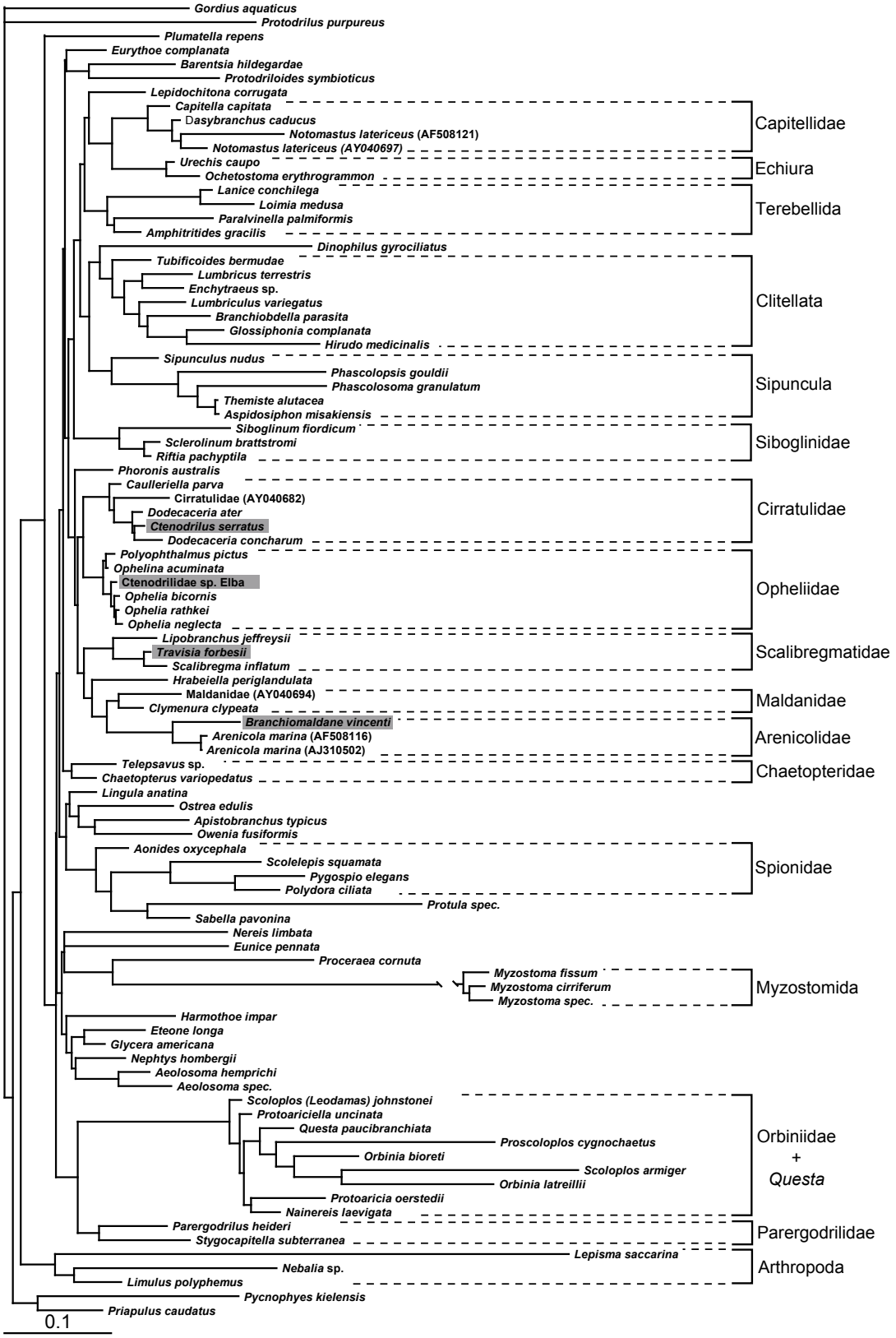

Figure1. Maximum likelihood tree based on the TrNef+I+Γ model of sequence evolution (-log*L*=244,060.72728). Taxa which are discussed in detail in the discussion are shaded with grey.

Figure 2. Majority-rule consensus tree of the 10.001 inferred trees of the Bayesian analysis. The posterior probabilities are given above the branches.

seems justifiable.

Maximum Likelihood and Bayesian inference

The most likely tree has a log-likelihood value of 24060.73 and is illustrated in Fig. 1. Fig.2 shows the results of the Bayesian inference, which are presented as posterior probabilities on the branches of the majority-rule consensus tree of the 10,001 inferred trees.

No evidence has been found for the monophyly of Annelida and Polychaeta – instead some non annelid protostome taxa cluster together with polychaete groups (e.g. *Barentsia hildegardae* (Entoprocta)), but without significant support. Many of the traditional polychaete-"families" are recovered by ML and are supported through Bayesian probabilities, e.g. Siboglinidae (Bayesian probability =100%), Capitellidae (100%), Parergodrilidae (99%), and Maldanidae (100%). The inclusion of *Branchiomaldane vincenti* in the Arenicolidae is also well supported (100%). A monophyletic Terebellida including Terebellidae (represented by *Amphtitrides gracilis, Lanice conchilega* and *Loimia medusa*) and Alvinellidae (*Paralvinella palmiformis*) yields high support (100%), whereas the Terebellidae appear paraphyletic in regard to the Alvinellidae. The Spionidae appear paraphyletic in the ML-analysis in regard to the Sabellida, but a monophyletic Spionidae is weakly supported through the Bayesian analysis (58%) The opheliid *Travisia forbesii* joins the scalibregmatids *Lipobranchus jefreysii* and *Scalibregma inflatum* (100%), and a close relationship between *Scalibregma* and *Travisia* is also strongly supported (100%). The remaining opheliids cluster together in a strongly supported clade (100%). Monophyly of the Ophelininae as represented by *Polyophthalmus pictus* and *Ophelina acuminata* is also supported (92%). The Opheliidae are joined by the undescribed ctenodrilid species from Elba, which clusters together with the three considered *Ophelia* species (98%). In contrast to this, the other here regarded Ctenodrilid *Ctenodrilus serratus* joins the Cirratulidae (99%) and appears as ingroup of *Dodecaceria* (90%). A clade consisting of the orbiniids and *Questa* is well supported (100%), whereas the Orbiniidae appear paraphyletic in regard to *Questa* (85%). A sistergroup relationship between the Orbiniidae + *Questa* cluster and the Parergodrilidae receives strong support (100%). Further relationships between polychaete families are recovered by ML as follows: A sistergroup relationship between Cirratulidae and Scalibregmatidae (97%); the echiurids cluster together with the Capitellidae (100%).

Maximum parsimony

The equally weighted parsimony analysis results in 25 MP trees (length=6,598; consistency index [CI]=0.2766; consistency index excluding uninformative characters [CI']=0.2356; retention index [RI]=0.4594). The strict consensus tree from this analysis

together with the bootstrap frequencies is illustrated in Appendix B.

Most of the strongly supported taxa of the Bayesian analysis are also supported through the MP Bootstrap-analyses, though often with a lower degree: Capitellidae (Bootstrap= 98%), Chaetopteridae (66%), Maldanidae (95%), Arenicolidae (including *Branchiomaldane vincenti*) 100%), Parergodrilidae (80%), Siboglinidae (99%). As in ML, *Ctenodrilus serratus* joins the cirratulids (100%) and appears as ingroup of *Dodecaceria* (99%). This analysis also recovers the inclusion of the undescribed ctenodrilid from Elba in the Opheliidae (99%) and supports a cluster of this taxon together with *Ophelia* (87%). *Travisia forbesii* as an ingroup of Scalibregmatidae yields high support (100%) and clusters together with *Scalibregma inflatum* (100%). The result of a capitellid - echiurid relationship is also confirmed in this analysis (94%). A clade consisting of *Questa* and Orbiniidae yields strong support (100%), whereas ingroup relationships of this taxon are only weakly supported. As in ML, the Orbiniidae appear paraphyletic in regard to *Questa*. In contrast to Bayesian inference no support (above 50%) is given for the Spionidae, Terebellida, Arenicolidae + Maldanidae, Orbiniidae + *Questa* + Parergodrilidae and Sipunculida.

3.4 Discussion

Annelids

Like many analyses of molecular data sets in the past (Kojima, 1998; Brown *et al*., 1999; McHugh, 2000; Rota *et al*., 2001; Struck *et al*., 2002; Bleidorn *et al*., 2003), this analysis gives no support for a monophyletic Polychaeta or a monophyletic Annelida. The low resolution may be due to a rapid radiation of the Annelida that has intensely been discussed intensely elsewhere (Brown *et al*., 1999; McHugh, 2000; Rota *et al*., 2001) and is generally credited to an erosion of information during time (Abouheif *et al*., 1998).

Position of the Echiura

Most remarkable is the confirmation of our former result (Bleidorn *et al*., 2003) that Echiura are a polychaete ingroup taxon with a close affinity to the Capitellidae. This relationship is well supported through bootstrap values and Bayesian probabilities. The phylogenetic position of these unsegmented marine worms is controversial. In the analyses of Rouse and Fauchald (1995, 1997) and Rouse (1999) they are treated as sister taxon of the Articulata (Annelida + Arthropoda). Molecular analyses instead place them as derived polychaetes (McHugh, 1997, 1999; Brown *et al*., 1999; Bleidorn *et al*., 2003). This view is congruent with the findings of Hessling and Westheide (2002) that

Echiura show serially repeated units in their nervous system which correspond to typical metameric ganglia of the Annelida. The placement as derived polychaetes favors the hypothesis of a secondary loss of segmentation in Echiura.

"Scolecida" and "Orbiniidae"

Our analyses include a broad sampling of scolecid taxa including Capitellidae (4 sequences), Arenicolidae (3), Maldanidae (2), Scalibregmatidae (3), Opheliidae (6), *Questa* (1), and Orbiniidae (8). None of our analyses yield support for a common ancestry of these taxa. Instead, we find the Capitellidae clustering together with Echiura. The inferred phylogenetic position of the Opheliidae depends on the choice of method. Whereas ML recovers a cirratulid-opheliid relationship, Bayesian inference gives no hint for a possible sistergroup of opheliids and, instead, supports a scalibregmatid-cirratulid clade, which is is also recovered by MP. In accordance with previous analyses (Kojima, 1998; Brown *et al*., 1999; McHugh, 2000; Rota *et al*., 2001; Struck *et al*., 2002; Bleidorn *et al*., 2003), most hypotheses on relationships among polychaete families are only weakly supported. Orbinidae + *Questa* are closely related to the Parergodrilidae, a result supporting the analysis of Struck *et al*. (2002). Interestingly, this relationship was also found in some of the cladistic analyses by Rouse and Fauchald (1997). Irrespective of the method used, our analysis suggests a probable paraphyly of the Orbiniidae with regard to *Questa*. The ingroup relationships of the Orbiniidae inferred from the 18S rDNA sequence data are in contrast to the traditional assumption (see Hartman, 1957) and a recent cladistic analysis by Blake (2000a).

According to Rouse and Fauchald (1997), the results of our ML analysis confirm the maldanid-arenicolid relationship, a result which lacks support in the MP Bootstrap- and Jackknife analyses. The placement of *Branchiomaldane*, an arenicolid taxon with maldanid-like anatomy (Rouse and Pleijel, 2001), with *Arenicola* is confirmed by all analyses with strong support. No evidence has been found for a possible inclusion of this taxon in the Maldanidae.

Our analysis strongly supports a common ancestry of *Travisia* and scalibregmatids, whereas *Travisia* can be treated as ingroup taxon of Scalibregmatidae. This result supports the view of Blake (2000b), who in the same way hypothesizes a close relationship between these taxa. Monophyly of the remaining Opheliidae is also strongly supported in all of our analyses independent of the choice of method.

Monophyly and the position of Ctenodrilidae

The first described ctenodrilid, *Ctenodrilus serratus*, was originally included in the

rhabdocoel Turbellaria by Schmidt (1857). Fauvel (1927) and Day (1967) considered the ctenodrilids to be a part of the Cirratulidae, while Hartmann-Schröder (1971) retains them as a separate drilomorph family and later (1996) as part of the order Ctenodrilida, together with Parergodrilidae. An examination of the nervous system of *Ctenodrilus serratus* by Gelder and Palmer (1976) reinforced the affinities with the Cirratulidae. In the cladistic analysis of Rouse and Fauchald (1997) they form a clade together with Fauveliopsidae, Poebiidae and Sternaspidae. In the maximum parsimony analysis of an 18S rDNA dataset by Rota *et al.* (2001) the new ctenodrilid species forms a clade with *Arenicola* (Arenicolidae) and *Dodecaceria* (Cirratulidae), but lacks sufficient bootstrap support.

Surprisingly, the newly-discovered ctenodrilid species from Elba (Rota *et al*., 2001) branches off between the newly combined opheliids and a common ancestry of these taxa is strongly supported irrespective of the method used. This suggests that in fact this new species will proove to be a juvenile or progenetic opheliid. Further biological data on this species are neccessary to confirm one of the two hypotheses. *Ctenodrilus serratus*, the other ctenodrilid species included in the analysis, clusters together with the cirratulids and shows a close affinity to the two included sequences of *Dodecaceria*. The systematic placement of the ctenodrilids as part of the Cirratulidae has a long tradition (Mesnil and Caullery, 1897; Fauvel, 1927) and is herewith confirmed, contradicting Hartmann-Schröder (1996) and Rouse and Fauchald (1997) who treated them as a separate taxon outside the Cirratulidae. This result is validated through high support of Bayesian probabilities, Bootstrap- and Jackknife-frequencies. Nevertheless, it still has to be tested wether all remaining ctenodrilid taxa should be included in the Cirratulidae.

These results support our assumption that on the basis of a broader taxon sampling the phylogenetic position of controversial discussed taxa can be inferred by using 18S rDNA sequence data.

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3.5 References

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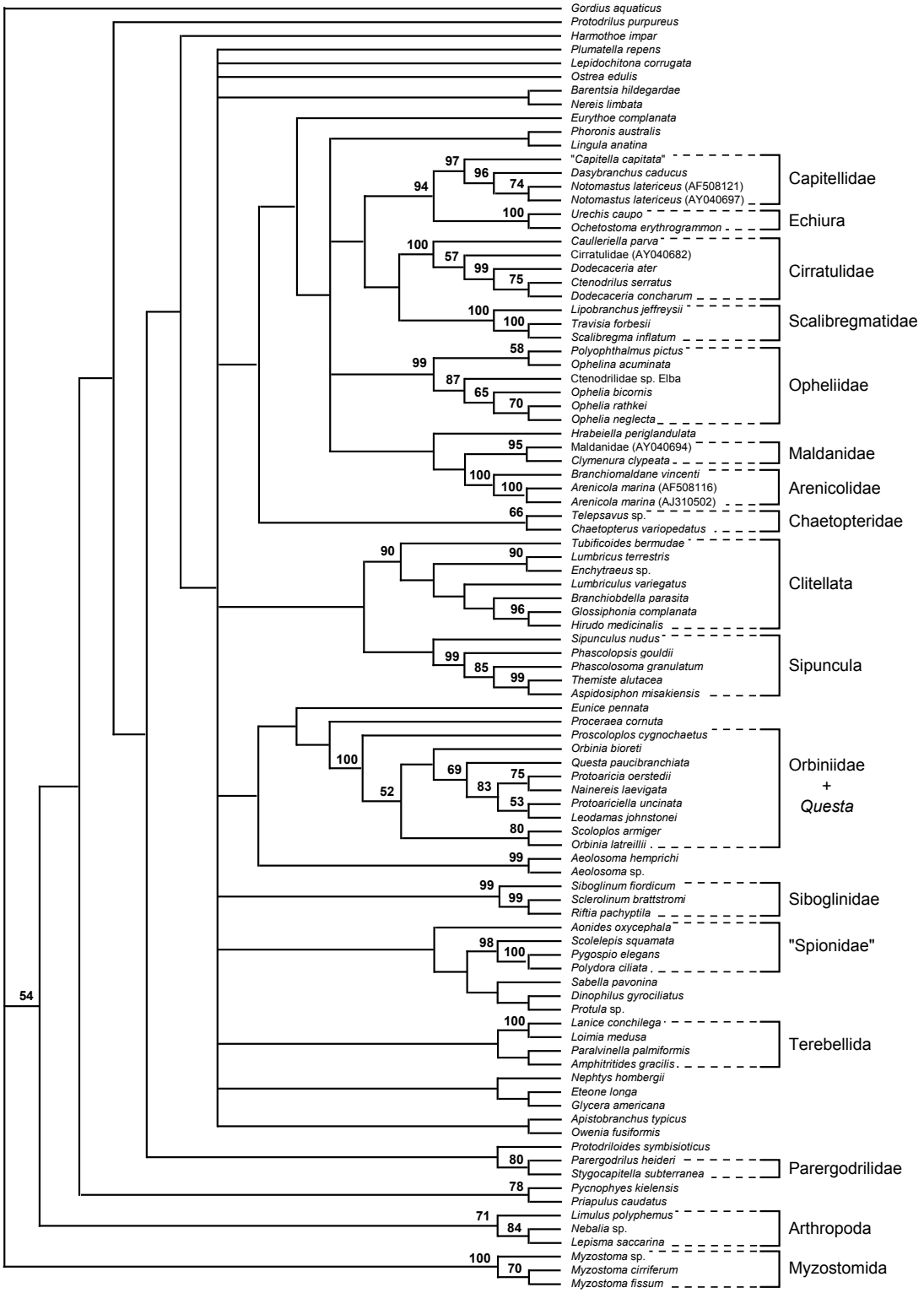
Appendix A. List of taxa used in the analysis with 18S rDNA sequence accession numbers.

Higher Taxon	Species	GenBank Accession No.
Annelida		
Aelosomatidae	*Aelosoma hemprichi* Ehrenberg, 1828	AJ310500
	Aelosoma sp.	Z83748
Alvinellidae	*Paralvinella palmiformis Desbruyères & Laubier, 1986*	AF168747
Amphinomida	*Eurythoe complanata* (Pallas, 1766)	AY040685
Aphroditoidea	*Harmothoe impar* Johnston, 1839	U50968
Apistobranchidae	*Apistobranchus typicus* (Webster & Benedict, 1887)	AF448150
Arenicolidae	*Arenicola marina* (Linné, 1758) *(GenBank)*	AJ310502
	Arenicola marina (Linné, 1758) *(Arcachon, France)*	AF508116
	Branchiomaldane vincenti Langerhans, 1881	AF508117
Capitellidae	*Capitella capitata* (Fabricius, 1780)	AF508118
	Dasybranchus caducus (Grube, 1846)	AF448153
	Notomastus latericeus Sars, 1851 *(GenBank)*	AY040697
	Notomastus latericeus Sars, 1851 *(Sylt, Germany)*	AF508121
Chaetopteridae	*Chaetopterus variopedatus* (Renier, 1804)	U67324
	Telepsavus sp.	AF448165
Clitellata	*Branchiobdella parasita* Henle, 1835	AF310690
	Enchytraeus sp.	Z83750
	Glossiphonia complanata (Linné, 1758)	Z83751
	Hirudo medicinalis Linné, 1758	Z83752
	Lumbriculus variegatus (O.F. Müller, 1774)	AF209457
	Lumbricus terrestris Linné, 1758	AJ272183
	Tubificoides bermudae Rasmark & Erséus, 1986	AF209467
Cirratulidae	*Caulleriella parva* Gillandt, 1979	AF448151
	Cirratulidae sp. (GenBank)	AY040682
	Dodecaceria ater (Quatrefages, 1866)	AF448154
	Dodecaceria concharum Oersted, 1843	U50967
Ctenodrilidae	*Ctenodrilidae* sp. (Elba)	AJ310503
	Ctenodrilus serratus (Schmidt, 1857)	AF508119
Dinophilidae	*Dinophilus gyrociliatus* O. Schmidt, 1857	AF119074
Eunicidae	*Eunice pennata* (O.F. Müller, 1776)	AY040684
Glyceridae	*Glycera americana* Leidy, 1855	U19519
Hrabeiella	*Hrabeiella periglandulata* Pizl & Chalupsky, 1984	AJ310501
Maldanidae	*Clymenura clypeata* (Saint Joseph, 1894)	AF448152
	Maldanidae sp. *(GenBank)*	AY040694

Myzostomida	*Myzostoma cirriferum* F. Leuckart, 1827	AF260585
	Myzostoma fissum von Graff, 1884	AF260584
	Myzostoma sp.	AF123305
Nephtyidae	*Nephtys hombergii* Savigny, 1818	U50970
Nereidae	*Nereis limbata* Ehlers, 1868	U36270
Opheliidae	*Ophelia bicornis* Savigny, 1818	AF508122
	Ophelia neglecta Aimé Schneider, 1892	AF448156
	Ophelia rathkei McIntosh, 1908	AF448157
	Ophelina acuminata Oersted, 1843	AY083310 +
AY083311		
	Polyophthalmus pictus (Dujardin, 1839)	AF448161
	Travisia forbesii Johnston, 1840	AF508127
Orbiniidae	*Naineris laevigata* (Grube, 1855)	AY040696
	Orbinia bioreti (Fauvel, 1919)	AF448158
	Orbinia latreilii (Audouin & Milne Edwards, 1833)	AF448159
	Proscoloplos cygnochaetus Day, 1954	AF448162
	Protoaricia oerstedii (Claparède, 1864)	AF508123
	Protoariciella uncinata Hartmann-Schröder, 1962	AF508124
	Scoloplos armiger (O.F. Müller, 1776)	U50972
	Scoloplos (Leodamas) johnstonei Day, 1934	AF508126
Oweniidae	*Owenia fusiformis* Delle Chiaje, 1844	AF448160
Paergodrilidae	*Parergodrilus heideri* Reisinger, 1925	AJ310504
	Stypocapitella subterranea Knöllner, 1934	AJ310505
Phyllodocidae	*Eteone longa* (Fabricius, 1780)	AF448155
Protodrilida	*Protodrilus purpureus* (Schneider, 1868)	AJ310506
	Protodriloides symbisioticus (Giard, 1904)	AF508125
Questa	*Questa paucibranchata* Giere & Erséus, 1998	AF209464
Sabellidae	*Sabella pavonina* Savigny, 1820	U67144
Scalibregmatidae	*Lipobranchus jeffreysii* (McIntosh, 1869)	AF508120
	Scalibregma inflatum Rathke, 1843	AF448163
Serpulidae	*Protula sp.*	U67142
Siboglinidae	*Scerolinum brattstromi* Webb, 1964	AF315061
	Siboglinum fiordicum Webb, 1963	AF315060
	Riftia pachyptila Jones, 1981	AF168745
Spionidae	*Aonides oxycephala* (M. Sars, 1872)	AF448149
	Polydora ciliata (Johnston, 1838)	U50971
	Pygospio elegans Claparéde, 1863	U67143
	Scolelepis squamata (O.F. Müller, 1789)	AF448164
Syllidae	*Proceraea cornuta* (Agassiz, 1862)	AF212179
Terebellidae	*Amphitritides gracilis* (Grube, 1860)	AF508115
	Lanice conchilega (Pallas, 1766)	X79873

	Loimia medusa (Savigny, 1818)	AY040694
Echiura	*Ochetostoma erythrogrammon* Rüppell & Leuckart, 1830	X79875
	Urechis caupo Fisher & MacGinitie, 1928	AF119076
Sipunculida	*Aspidosiphon misakiensis* Ikeda, 1904	AF119090
	Phascolopsis gouldii (Fisher, 1950)	AF
	Phascolosoma granulatum Leuckart, 1828	X79874
	Sipunculus nudus Linné, 1767	AF448166
	Themiste alutacea GrÅbe & Oersted 1858	AF119075
Brachiopoda	*Lingula anatina* Lamarck, 1801	X81631
Ectoprocta	*Plumatella repens* (Linné, 1758)	U12649
Phoronida	*Phoronis australis* Haswell, 1883	AF119079
Mollusca	*Lepidochitona corrugata* (Reeve, 1848)	X91975
	Ostrea edulis Linné, 1758	L49052
Entoprocta	*Barentsia hildegardae* Wasson, 1997	AJ001734
Arthropoda	*Lepisma saccharina* Linné, 1758	X89484
	Limulus polyphemus (Linné, 1758)	U91490
	Nebalia sp.	L81945
Kinorhyncha	*Pycnophyes kielensis* Zelinka, 1928	U67997
Nematomorpha	*Gordius aquaticus* Linné, 1758	X80233
Priapulida	*Priapulus caudatus* Lamarck, 1816	AF025927



Appendix B. Strict consensus tree of the Maximum Parsimony analysis. Bootstrap - frequencies are given above the branches.