2. A contribution to sedentary polychaete phylogeny using 18S rDNA sequence data

Abstract - The phylogenetic position of the Annelida as well as their ingroup relationships are a matter of ongoing debate. A molecular phylogenetic study of sedentary polychaete relationships was conducted based on 70 sequences of 18S rRNA, including unpublished sequences of 18 polychaete species. The data set was analyzed with maximum parsimony and maximum likelihood methods. Clade robustness was estimated by parsimony-bootstrapping and -jackknifing, decay index, and clade support, as well as *posteriori probabilities*, which were calculated by a Bayesian inference. Irrespective of the applied method, some traditional sedentary polychaete taxa, as the Cirratulidae, Opheliidae, Orbiniidae, Siboglinidae, and Spionidae, were recovered by our phylogenetic reconstruction. A close relationship between Orbiniidae and *Questa* received a particularly strong support. Echiura appears to be a polychaete ingroup taxon which is closely related to *Dasybranchus* (Capitellidae). As in previous molecular analyses, no support was found for the monophyly of Annelida nor for that of Polychaeta. However, we suggest that an increase in taxon sampling may yield additional resolution in the reconstruction of polychaete ingroup phylogeny, although the difficulties in reconstructing the basal phylogenetic relationships within Annelida may be due to their rapid radiation.

2.1 Introduction

The phylogenetic position of the Annelida is still a matter of debate (Schmidt-Rhaesa et al. 1998; Westheide et al. 1999; Wägele & Miesof 2001). The quest for the sister group of the Annelida is intimately connected to the question for the interrelationships within the Annelida, which are largely unresolved (Rouse & Fauchald 1997, 1998; Westheide 1997; Bartolomaeus 1998), as well as to the question which taxa are to be included to the Annelida. According to traditional classifications the Annelida consist of Polychaeta and Clitellata. While the latter is clearly supported as a monophylum in both, molecular and morphological analyses, the status of the Polychaeta is still controversial (Rouse & Fauchald 1998, Westheide et al. 1999). Cladistic analyses of the classical morphological data recognize the Polychaeta as a monophyletic group (see Rouse & Pleijel 2001), while scenario based analyses (Westheide 1997) and some molecular analyses (McHugh 1997) support an inclusion of Clitellata in a polychaete clade. Any attempt to bring a solution to this debate has to start with phylogenetic analyses on lower taxonomic levels. This

is because the Annelida are highly diverse and represent an evolutionary old taxon that presumably radiated at or before the Precambrian-Cambrian border (Butterfield 1990; Conway-Morris & Peel 1995).

Diversity in annelids is expressed by tremendous morphological differences that become obvious when comparing different representatives of the polychaetes. In the past, therefore, taxonomists created up to 24 "orders" within this taxon (Fauchald 1977). As an "order" is a relatively high taxonomic rank, their large number likewise expressed the tremendous morphological differences between different polychaete taxa. A few of them could be substantiated as monophyletic groups by morphological data, but most of them, however, have never attained such support. Thus, more recent literature divides the polychaetes into more than 80 taxa, giving them the classical rang of families (Rouse & Fauchald 1997, Rouse 1999, Rouse & Pleijel 2001). However, there is still some doubt, whether all of them are monophyletic (Fauchald & Rouse 1997).

Recent detailed analyses of the ultrastructure and of the formation of certain organs could substantiate the hypothesis of monophyly for certain annelid taxa and provide evidence for a closer relationship among some of them (Meyer & Bartolomaeus 1996; Purschke & Tzetlin 1996; Purschke 1997; Bartolomaeus 1998; Hausen & Bartolomaeus 1998; Hausam & Bartolomaeus 2001; Hausen 2001). Several analyses, morphological as well as molecular, also provided first evidence that the Pogonophora and Vestimentifera are sister taxa to certain polychaetes, supporting the hypothesis that both are derived polychaete taxa (Kojima et al. 1993, Bartolomaeus 1995, Rouse & Fauchald 1995, 1997, Black et al 1997, McHugh 1997). Echiura seem to be another candidate for a possible inclusion into the Annelida (McHugh 1997, Ax 2000, Hessling & Westheide 2002).

Molecular analyses (Winnepenninckx et al. 1995, 1998; Koijima 1998; Brown et al. 1999; McHugh 2000) could not provide any evidence for a probable monophyly of the Polychaeta or even of the Annelida. The results of theses surveys were conflicting with regard to the position of the traditional polychaete families, irrespective of the molecules these studies were based on. Pogonophora and Vestimentifera turned out to be monophyletic (Halanych et al. 2001), supported by statistically significant bootstrap values. No such support was provided in most of the analyses for the remaining polychaete entities.

If one compares these analyses, it becomes obvious that despite of their comparatively low diversity a relative large amount of molecular data exists for the Pogonophora (Halanych et al. 2001). A similar observation can be made for the Clitellata (Martin 2001, Siddall et

al. 2001). Compared to the diversity in polychaetes, sequences of only a few polychaete taxa are available and in most cases only a single representative stands for a larger taxon (Kojima 1998, McHugh 2000). It therefore does not surprise that even those taxa that can be supported as monophyla on the basis of morphological data do not form a single clade in molecular analyses. A strong conviction is that the low number of polychaete species analyzed causes the low resolution and knowledge about whether an increased taxon sampling increases the resolution is essential, so that molecular data can be used to substantiate the monophyly of certain polychaeta taxa. In this study the usefulness of the 18S rRNA for the inference of polychaete phylogeny was investigated, focusing on sedentary taxa. Simulation studies have shown that an increase of the taxon sampling can improve the resolution of the phylogentic signal in a data set (Graybeal 1998). Special emphasis was laid in this study on possible monophyletic taxa within the polychaeta. In accordance with the assumption on the influence of taxon sampling on the resolution, it is expected to establish sister group relationships among sedentary polychaete groups.

2.2 Material and methods

Taxon sampling

Eighteen species of polychaetous annelids and one sipunculid species were collected from various sites (see table 1) and the complete 18S rRNA-sequence of each species was analyzed. Voucher specimen of the examined taxa are deposited in the collection of T. Bartolomaeus (Systematics and Evolution of Metazoa, Free University of Berlin, Germany). For alignment and phylogenetic analyses a total of 70 metazoan 18S rRNA sequences, including nearly all available polychaete 18S rRNA sequences, were chosen from GenBank (Bethesda, MD, USA) (see Appendix A). Additional to the annelid sequences, sequences of several other protostomian taxa were considered and serve as outgroups for the reconstruction of the phylogenetic relationships within Annelida. The sequence of *Capitella capitata* (U67323) was excluded from the analysis, as a reanalysis of this sequence (unpublished) suggests that U67323 is erroneous and probably from a misidentified specimen.

DNA extraction

Collected specimens were identified and then preserved in 100% ethanol for later extraction. Genomic DNA was extracted from specimens using Qiagen DNeasy™ Tissue Kit (Qiagen GmbH, 40724 Hilden).

PCR amplification, purification and sequencing

PCR amplification of the 18S rRNA gene was performed in three overlapping fragments of ~900bp each or in a whole with modificated primer pairs from Giribet et al. (1996) by using standard cycle sequencing protocols. Amplification reaction mixtures for 18S rDNA contained 25 µl Qiagen Taq PCR Master Mix (Qiagen GmbH, 40724 Hilden), 2 µl Template-DNA, 4 µl of each primer and 15 µl H_2O. Amplifications were carried out using an Eppendorf Mastercycler gradient (Eppendorf GmbH, 22331 Hamburg). The following PCR temperature file was used: 95˚C for 3 min; 35 cycles with 94˚C for 35 seconds, 45-50˚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 GmbH, 40724 Hilden). Sequencing of all amplified fragments in both directions was carried out by the *IIT* Biotech/Bioservice of the University of Bielefeld. Overlapping fragments of the 18S rRNA were combined by using BioEdit (Hall

Table 1: Collection sites and GenBank accession numbers of species sequenced newly for this study

Species	18S rRNA (bp)	Collection site	GenBank Accesssion-numbers
Aonides oxycephala	1735	Concarneau, France	AF448149
Apistobranchus typicus	1814	Qeqertarsuaq, Greenland	AF448150
Caulleriella parva	1807	Concarneau, France	AF448151
Clymenura clypeata	1805	Concarneau, France	AF448152
Dasybranchus caducus	1819	Concarneau, France	AF448153
Dodecaceria atra	1804	Concarneau, France	AF448154
Eteone longa	1814	Sylt, Germany	AF448155
Ophelia neglecta	1804	Concarneau, France	AF448156
Ophelia rathkei	1815	Sylt, Germany	AF448157
Ophelina acuminata	1681	Helgoland, Germany	
Orbinia bioreti	1828	Concarneau, France	AF448158
Orbinia latreilii	1847	Concarneau, France	AF448159
Owenia fusiformis	1809	Concarneau, France	AF448160
Polyophthalmus pictus	1811	Banyuls-sur-mer, France	AF448161
Proscoloplos cygnochaetus	1965	Roscoff, France	AF448162
Scalibregma inflatum	1833	Helgoland, Germany	AF448163
Scolelepis squamata	1848	Sylt, Germany	AF448164
Telepsavus spec.	1814	Concarneau, France	AF448165
Sipunculus nudus	1817	Arcachon, France	AF448166

1999). Disagreement among these fragments was corrected by reference to the original chromatograms. All sequences were submitted to Genbank (for accession numbers see table 1).

Sequence Alignment

All sequences were aligned by using CLUSTAL W (Thompson et al. 1994) under default settings 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 consideration of models of secondary structure of 18S rRNA only affected the position excluded within the alignment. The alignment is avaible by e-mailing the first author.

Data Analysis

All phylogenetic analyses were conducted with PAUP* version 4.0b8 (Swofford 2001). A chi-square test of homogeneity of base frequencies across taxa was performed. The program TreeView (Page 1996) was used for tree visualization. All trees were rooted *a posteriori* the analysis using the sequence of *Gordius aquaticus* (Nematomorpha), because no analysis reports them as an annelid taxon.

Maximum parsimony and clade support

An equally weighted maximum parsimony search was run with 1000 random addition replicates, heuristic search option with tree-bisection-reconnection (TBR) branch swapping, holding one tree per step, and keeping all most-parsimonious trees. Two separate analyses, using two different weighting schemes were conducted. In the first analysis, all transformations were weighted equally, in the second analysis, transversions were weighted three times as much as transitions, which is in accordance with the model of sequence evolution supported by the result of *modeltes*t (see below).

Bootstrap and Jackknife (Felsenstein 1985; Farris 1997) values were determined from 1000 replicates subject to full heuristic searches with simple taxon addition to provide measures of relative clade support. Additionally, Bremer support (Bremer 1994) was estimated with converse constraint heuristic searches based on 100 random sequence addition replicates (Baum et al., 1994). It was evaluated whether the most parsimonious (MP) trees that include the selected clades are significantly better supported than trees that lack them (Whitlock & Baum, 1999; Lee, 2000). This was achieved by comparing the pool of MP trees from a converse constraint search with the unconstrained MP trees using a Wilcoxon signed-rank test (Templeton, 1983) as implemented in PAUP*. For each clade, the *P* value (clade significance) reported is the highest obtained across the pair-wise comparisons. A clade is considered significantly supported if $P < 0.1$* (Lee, 2000).

Maximum Likelihood

For estimating the appropriate model of sequence evolution, different models were tested using the program *modeltest* version 3.06 (Posada & Crandall 1998, 2001). Both test criteria (hLRT and AIC) indicate that the Tamura Nei substitution model (Tamura & 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 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

For Bayesian analysis of the data set mrbayes 3.0B2 was used (Huelsenbeck and Ronquist 2001). A Bayesian analysis of phylogenies searches for the best set of trees that is consistent with a given model of sequence evolution and the data set under investigation (Rannala and Yang 1996; Mau et al. 1999). The consensus of this set of trees is used to estimate probabilities for node support, which is taken as an equivalent of bootstrap values (Hall 2001). These probabilities, the Bayesian a posteriori probabilities, are obtained from the likelihood and prior probabilities by applying Bayes' rule. The Bayesian a posteriori probability resembles the probability of the hypothesis, given the data. Whereas, the prior probability is the unconditional probability of the hypothesis without reference to the data, and the likelihood is the probability of the data, given the hypothesis (Lewis 2001).

mrmodeltest 1.1b was used for estimating the maximum likelihood (ML) parameters in mrbayes. This program is a simpli.ed version of modeltest 3.06 (Posada and Crandall 1998, 2001) and contains less models which are tested. The hLRT criterion indicates that the SYM + I + C (Zharkikh 1994) represents the optimal model in respect to the data. All priors were set according to this model. Each Markov chain, three heated and one cold, was started from a random tree and all four chains ran simultaneously for 2.5 · 106 generations, with trees being sampled every 250 generations for a total of 10,000 trees. After the likelihood of the trees of each chain converged, we discarded the first 1000 trees as burnin. The majority-rule consensus tree containing the Bayesian a posteriori probabilities of the phylogeny was determined from 9000 trees.

2.3 Results

Sequence data

The alignment of the 70 18S rDNA sequences resulted in 2,207 positions. After the exclusion of ambiguous sites, the remaining 1,519 positions were taken on into our data matrix. Overall, the data matrix consists of 865 variable positions (57%), of which 588 positions are parsimony informative (39%). Since the chi-square test of homogeneity of base frequencies across taxa resulted in no significant *P*-values (chi-square=89.1117, df=207, *P*=1.0), assuming that compositional bias has no effect on the recovery of phylogenetic signal seems justifiable.

Maximum parsimony and clade support

The equal weighted parsimony analysis resulted in 33 MP trees (length=4,590; consistency index [CI]=0.3316; consistency index excluding uninformative characters [CI']=0.2798; retention index [RI]=0.4170). The strict consensus tree from this analysis together with the bootstrap and jackknife frequencies is illustrated in Fig. 1. Decay indices and clade significance values for selected groups are shown in Fig. 2.

Some of the traditional polychaete-"families" represent well supported clades: **Opheliidae** (Bootstrap [BT]=99.8%; Jackknife [JK]=99.1%; decay index [DI]=9; clade significance [*P*]=0.5641), **Cirratulidae** (BT=100.0%; JK=99.8%; DI=15; *P*=0.2603), **Spionidae** (BT=54.7%; JK=56.5%; DI=6; *P*=0.9374), **Orbiniidae** (BT=68.0%; JK=67.8%; DI=4; *P*=0.7976). Whereas *Orbinia* seems to be paraphyletic, since *Orbinia latreillii* appears to be closer related to other orbiniids than to *Orbinia bioreti* (*O. latreillii* + *Scoloplos armiger*: BT=70.4, JK=74.4; *O. latreillii* + *S. armiger* + *Proscoloplos cygnochaetus*: BT= 65.0, JK=65.3). The strongest support among all clades receives a relationship between the **Orbiniidae** and ***Questa*** (BT=99.9%; JK=100.0%; DI=34; *P*=0.0538*). A clade consisting of the **Echiura** and ***Dasybranchus caducus*** (Capitellidae) is also supported (BT=91.5%; JK=91.2%; DI=10; *P*=0.4053). Furthermore, as the results of the analysis of Halanych *et al.* (2001) already have shown, the **Siboglinidae** (BT=100.0%; JK=99.9%; DI=16; *P*=0.2909) are well supported. They consist of the two well supported sistergroups **Vestimentifera** (BT=100.0%; JK=100.0%; DI=16; *P*=0.2010), and the **Frenulata** (BT=99.7%; JK=99.7%; DI=13; *P*=0.3505). In concordance with the traditional view of annelid systematics, the **Clitellata** (BT=88.1%; JK=85.9%; DI=6; *P*=0.7323) are also well supported.

The unequally weighted parsimony analysis yielded in four MP trees. All trees differ in detail from the results of the equally weighted parsimony analysis. However, most of the well supported clades described above are recovered in both analyses. Only the **Spionidae** and **Orbiniidae** receive no support above the 50%-level in BT and JK from the unequally weighted analysis. Nevertheless, further on, a monophylum consisting of

the **Orbiniidae** and ***Questa*** receives high support.

The results of the bootstrap and the jackknife analyses show a similar pattern. Both support a high resolution for the relationships within those well-supported groups, thereby exhibiting almost no conflicting evidence. In contrast to these findings are all other relationships generally weakly supported and many even do not reach the 50% support level.

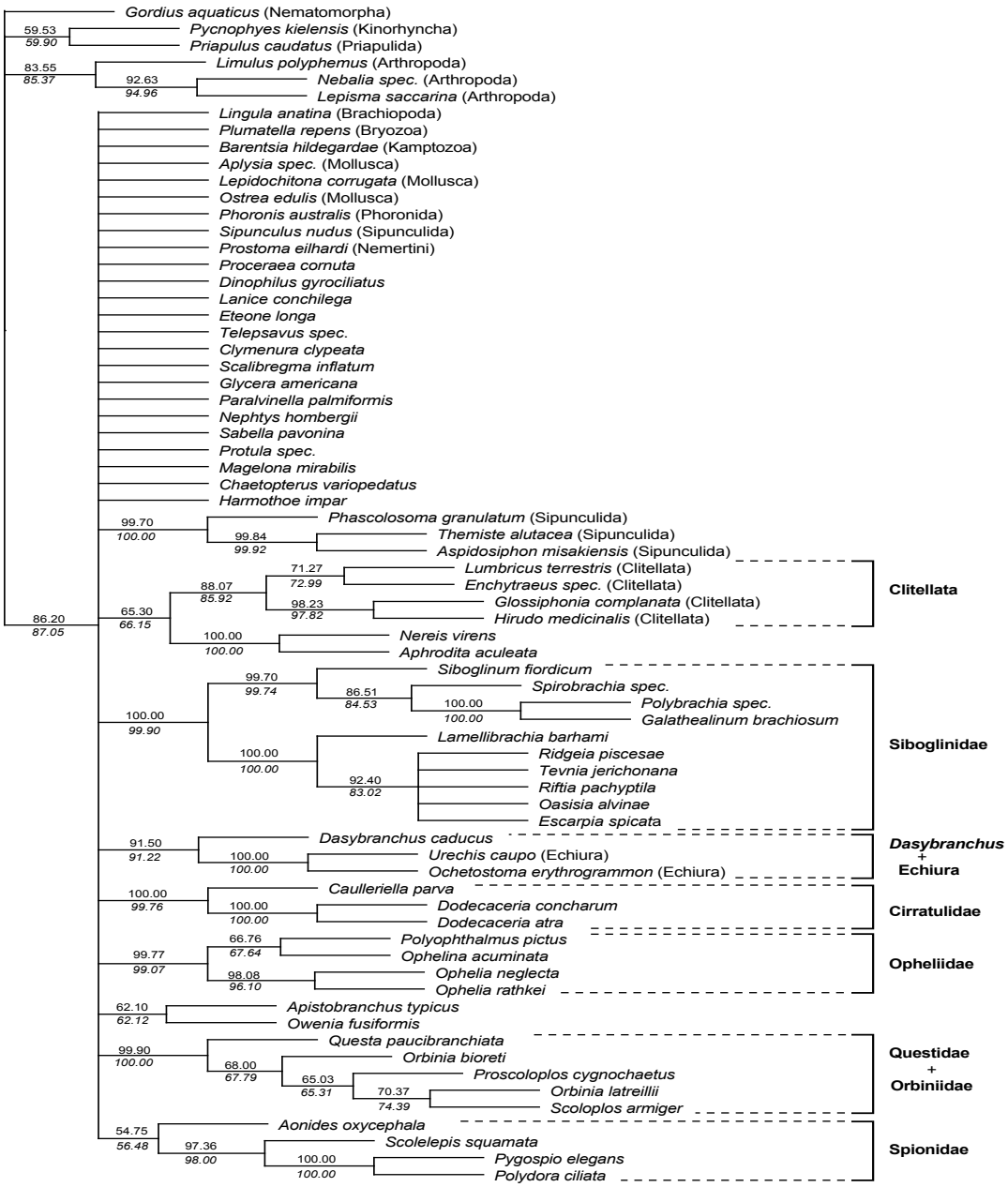

Figure 1. Strict consensus of 33 most-parsimonious trees of the equally weighted parsimony analysis (length= 4,590 steps; CI=0.33; RI=0.42). Bootstrap- and jackknife-frequencies are given above and below the branches.

Maximum Likelihood

The Tamura Nei substitution model (Tamura and Nei 1993) with equal base frequencies, invariant sites and gamma distribution (TrNef+I+Γ) represents the best fitting model for an explanation of the data of all the models that were considered in the modeltest. The most likely tree has a log-likelihood value of -22747.98730 and is illustrated in Fig. 3. The groups mentioned above are also supported by the likelihood analysis. Their ingroup topologies are congruent to those found in the consensus tree of the equally weighted parsimony analysis.

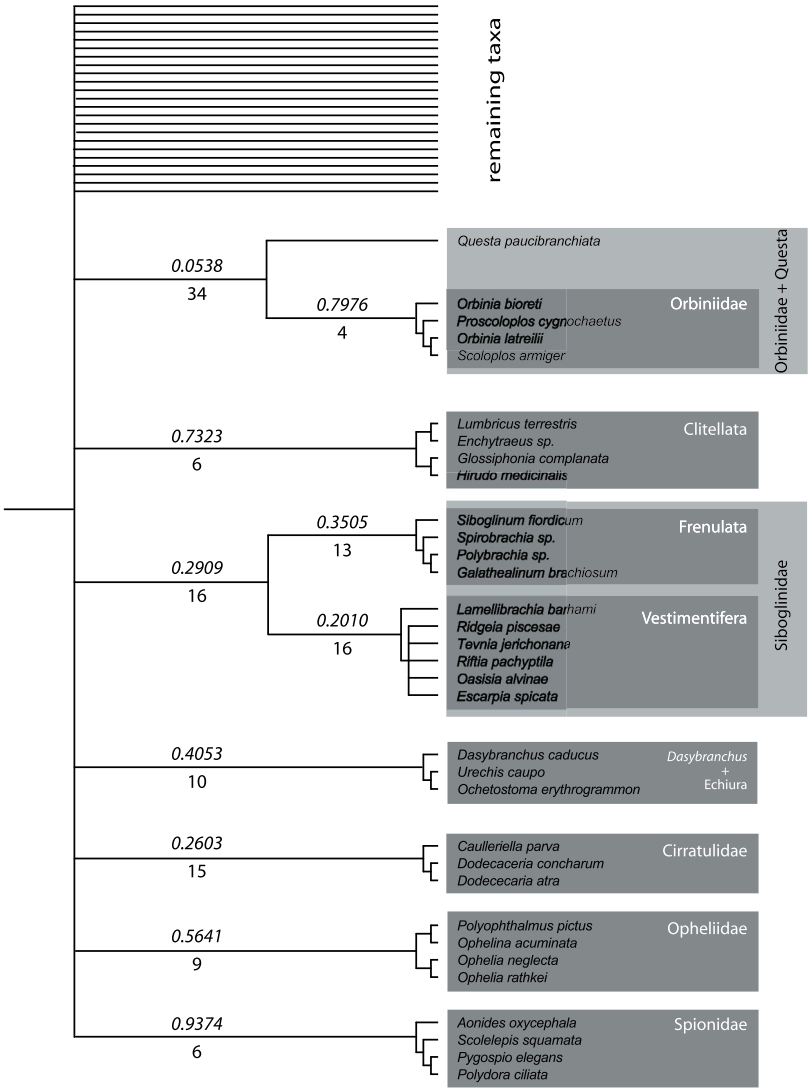

Figure 2. Phylogenetic relationship of selected groups (based on the strict consensus of the equally weighted parsimony analysis), with clade significance values and decay indices above and below the branches.

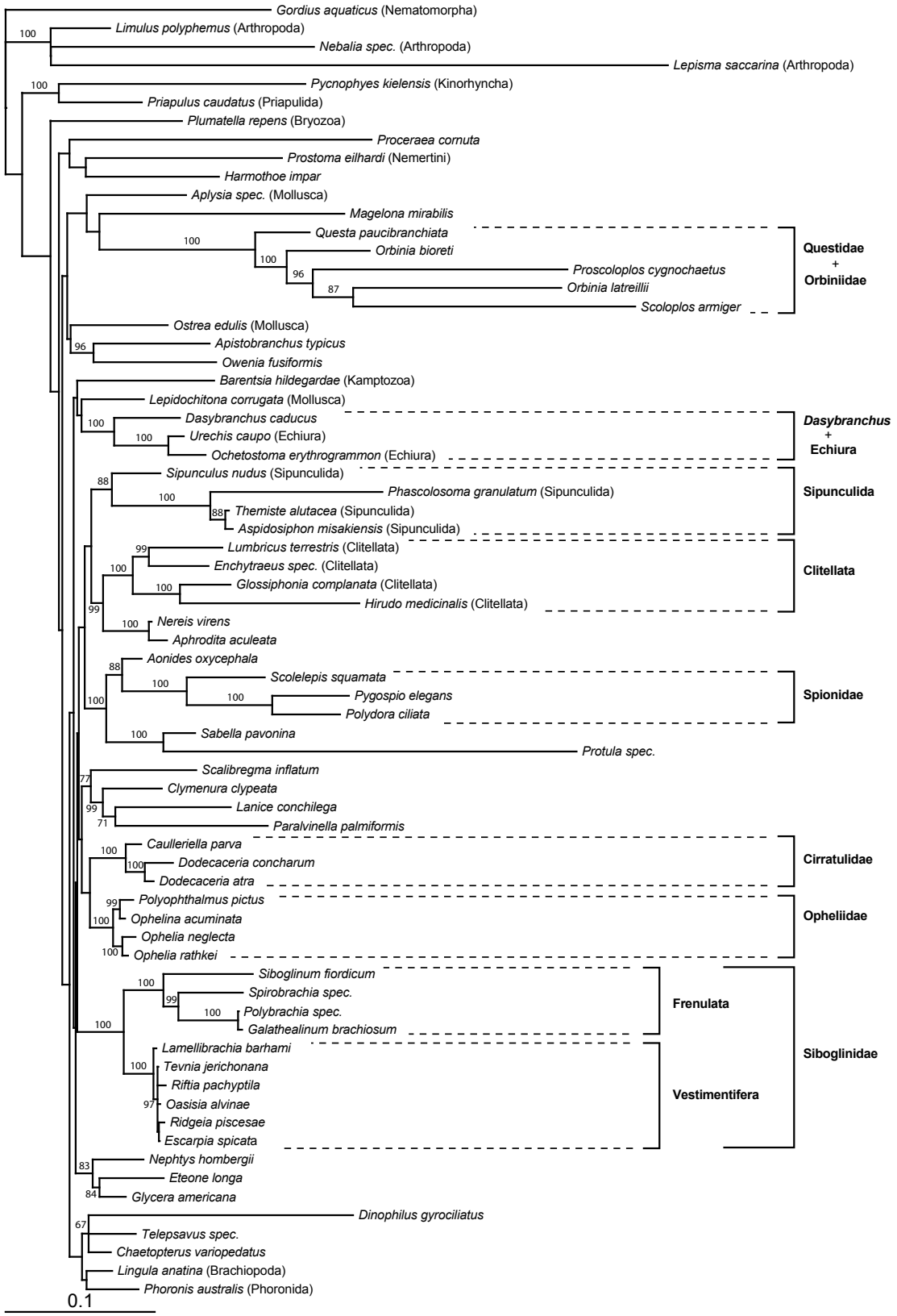

Figure 3. Maximum likelihood tree based on the TrNef+I+Γ model of sequence evolution (-log*L*=22747.9873). The posteriori probabilities of the Bayesian analysis are given above the branches.

Bayesian inference

The results of the Bayesian inference are presented as posteriori probabilities on the branches of the most likely tree (Fig. 3). Most obvious is that all clades with a high bootstrap support are also strongly supported in the Bayesian analysis. In concordance with Whittingham et al. (2002), it is found in the present study that some clades with a low bootstrap support are nevertheless well supported in the Bayesian analysis. For example, the clade formed by the Spionidae, which is supported by a low bootstrap value of 54,7%, is supported with a Bayesian probability of 88%. Another example are the Orbiniidae, who receive a support of 68% in the bootstrap analysis and a Bayesian probability of 100%.

2.4 Discussion

As already shown for other taxa (Blaxter et al. 1998), extended taxon sampling helps to increase the resolution of comparative 18S rRNA sequence analysis. Provided that enough sequences are available, this molecule seems to be suitable for the inference of some aspects of polychaete phylogeny. However, the present analysis also reflects the problems that arise when attempting to infer evolutionary events that took place during or before the Cambrium, which especially appears to apply to the 18S rRNA sequence data (Abouheif et al. 1998). The present data show that the 18S rRNA neither supports the hypothesis of the monophyly of Annelida or that of Polychaeta significantly, nor does it significantly support their paraphyly. This low resolution may be due to a rapid radiation of the Annelida that has intensely been discussed elsewhere (Brown et al. 1999, Rota et al. 2001), and is generally credited to an erosion of information during time. Provided that such an explosive radiation occurred in annelids, it may also have influenced morphological characters leading to the known problems in tree reconstruction (Rouse & Fauchald 1997). If these difficulties actually hint at a rapid radiation, at least some of the groups we analyzed must have radiated more recently.

Increasing the taxon sampling of 18S rRNA sequences of different polychaete groups indicates a promising methodological solution to the problem of reconstructing relationships which resulted from rapid radiation, yet there is no guarantee for its success.

The present analysis confirms that the pogonophoran and vestimetiferan species cluster in a single clade, representing the taxon Siboglinidae (see Mc Hugh 2000, Halanych et al. 2001). According to previous analyses, Siboglinidae represent a subordinate polychaete taxon (Bartolomaeus 1998, McHugh, 1997, Rouse & Fauchald 1997). Reduction of the gut lumen during development and persistence of its cells to house endosymbiontic

bacteria, as well as an extremely elongated first segment are strong arguments derived from morphological analyses which support the monophyly of Siboglinidae.
Clitellata also form a monophyletic group when 18S rRNA and other molecular data sets are analyzed (Rota et al. 2001, but see Martin 2001). A large number of morphological characters, like hermaphroditism, restriction of gonads to the anterior segments, sperm ultrastructure, modified and direct development, re-location of the brain from the prostomium into a more posterior position (Ferraguti 1984, Purschke et al. 1993, Rouse & Fauchald 1997) support the monophyly of this taxon. A specific glandular region posterior to the gonads, the clitellum, which produces a cocoon that encloses the eggs, also is apomorphic for clitellates.

Formation of clitellar material by a special glandular region and the restriction of gonads to a few segments are also characteristic for the Questidae and lead to the hypothesis of a questid-clitellate relationship (Giere & Riser1981). Subsequent studies, however, argued against such a position of the aberrant taxon *Questa* (Jamieson & Webb 1984, Rouse & Fauchald 1997, Giere & Erseus 1998). So far, their phylogenetic relationships remained uncertain. Our analysis, which is the first to entail several orbiniid sequences together with a sequence of *Questa*, provides strong evidence for the position of aberrant *Questa* as being closely related to the Orbiniidae, a result that was also recovered by the analysis of Rota et al. (2001).
The support of the monophyly of Orbiniidae is highly dependend on the choice of method. Although Bayesian probalities yield high support (100%), this taxon is not supported by bootstrap analysis of the unequally weighted parsimony analysis (<50%). Elevated parapodia in the posterior body region are regarded as a morphological autapomorphy for this taxon (Fauchald & Rouse 1997). However, this character is not present in all orbiniid taxa (Rouse & Pleijel 2001) and so the knowledge of ingroup relationships is essentiall for a correct phylogenetic interpretation of this character.

Anyway, while the Bayesian a posteriori probabilities seem to be what the scientist is looking for – as likelihood values are not easy to interpret, because they represent the probability of the data, given the hypothesis, instead of representing the probability of the hypothesis, given the data (Lewis 2001) – there is no generally accepted procedure of interpreting Bayesian a posteriori probabilities neither, as their nature is not well understood yet (Leaché and Reeder 2002). Some authors address this to the subjectivity of the prior probability (Lewis 2001). Nevertheless, there seems to be a general tendency of Bayesian analyses to yield higher support values than bootstrap analyses do (see also Buckley et al. 2002), rendering this method a less-conservative test.
The three 18SrRNA sequences of the cirratulids cluster together in a highly supported

clade. The same is true for opheliids and spionids. These results support the monophyly hypothesis gained from morphological data, and thus support traditional taxa within polychaetes.

A final and very interesting result concerns the position of the Echiura. Most textbooks regard them as taxon outside the Annelida. Based on alpha 1 elongation factor sequences, McHugh (1997), however, provided some evidence that the Echiura belong to the Annelida. The only morphological feature that might support inclusion of the Echiura into the Annelida are the chaetae (see Ax 2000). However, our analysis now provides evidence for a common ancestry of the Echiura and Capitellidae and this result is supported with a high bootstrap-value (91.5%) and a Bayesian probality of 100%. Additional morphological and molecular data to further test this hypothesis is awaited.

This study confirms the initial assumption that increased taxon sampling increases the resolution of annelid relationships based on the 18S rRNA sequences. As this group is an evolutionary ancient group, 18SrDNA sequences from additional species as well as further molecules will help to resolve polychaete phylogeny.

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Appendix A. List of sequences retrieved from GenBank

Species	18S rDNA (bp)	Source	GenBank Accesssion-numbers
Brachiopoda			
Lingula anatina	1813	GenBank	X81631
Phoronida			
Phoronis australis	1767	GenBank	AF119079
Bryozoa			
Plumatella repens	1813	GenBank	U12649
Kinorhyncha			
Pycnophyes kielensis	*1806*	*GenBank*	*U67997*
Priapulida			
Priapulus caudatus	*1750*	*GenBank*	*AF025927*
Nematomorpha			
Gordius aquaticus	*1799*	*GenBank*	*X80233*
Arthropoda			
Lepisma saccharina	1828	GenBank	X89484
Limulus polyphemus	1787	GenBank	U91490
Nebalia spec.	1805	GenBank	L81945
Mollusca			
Aplysia spec.	1826	GenBank	X94268
Lepidochitona corrugata	1821	GenBank	X91975
Ostrea edulis	1821	GenBank	L49052
Kamptozoa			
Barentsia hildegardae	1759	GenBank	AJ001734
Nemertini			
Prostoma eilhardi	1834	GenBank	U29494
Sipunculida			
Aspidosiphon misakiensis	1766	GenBank	AF119090
Phascolosoma granulatum	1841	GenBank	X79874

Themiste alutacea	1753	GenBank	AF119075
Echiurida			
Ochetostoma erythrogrammon	1814	GenBank	X79875
Urechis caupo	1772	GenBank	AF119076
Annelida			
Clitellata			
Enchytraeus sp.	1831	GenBank	Z83750
Glossiphonia spec.	1890	GenBank	Z83751
Hirudo medicinalis	1891	GenBank	Z83752
Lumbricus terrestris	1813	GenBank	AJ272183
Polychaeta			
Aphrodita aculeata	1810	GenBank	Z83749
Chaetopterus variopedatus	1692	GenBank	U67324
Dinophilus gyrociliatus	1784	GenBank	AF119074
Dodecaceria concharum	1701	GenBank	U50967
Glycera americana	1814	GenBank	U19519
Harmothoe impar	1736	GenBank	U50968
Lanice conchilega	1816	GenBank	X79873
Magelona mirabilis	1728	GenBank	U50969
Nephtys hombergii	1764	GenBank	U50970
Nereis virens	1814	GenBank	Z83754
Paralvinella palmiformis	1752	GenBank	AF168747
Polydora ciliata	1684	GenBank	U50971
Proceraea cornuta	1839	GenBank	AF212179
Protula sp.	1749	GenBank	U67142
Pygospio elegans	1758	GenBanK	U67143
Questa paucibranchiata	1788	GenBank	AF209464
Sabella pavonina	1726	GenBank	U67144
Scoloplos armiger	1769	GenBank	U50972
Siboglinidae			
Escarpia spicata	1764	GenBank	AF168741
Galathealinum brachiosum	1820	GenBank	AF168738
Lamellibrachia barhami	1759	GenBank	AF168742
Oasisia alvinae	1764	GenBank	AF168743
Polybrachia sp.	1820	GenBank	AF168739

Riftia pachyptila	1765	GenBank	AF168745
Ridgeia piscesae	1828	GenBank	X79877
Siboglinum fiordicum	1844	GenBank	X79876
Spirobrachia sp.	1754	GenBank	AF168740
Tevnia jerichonana	1763	GenBank	AF168746