

4. 18S-rDNA and polychaetes

Abstract - The monophyly of polychaetes as well as the phylogeny of their traditional “families” is controversial. Most recent molecular phylogenetic analyses of polychaetes are based on 18S-rDNA data. To date, analyses have been more or less focused on one or another polychaete group limiting our understanding of polychaete evolution. Herein the taxon sampling was increased so that many polychaete “families” are represented by multiple exemplars to better represent biodiversity. A detailed analysis of the data matrix of the 18S rDNA sequences was conducted prior to the phylogenetic reconstruction so that an appropriate evolutionary model would be employed.

Although polychaete phylogeny cannot be solved unambiguously, the paraphyly of polychaetes in regard to the Clitellata, Echiura and Siboglinidae and the monophyly of most of the traditional polychaete “families” is corroborated and the tree of Rouse and Fauchald (1997) is significantly rejected. In some cases, monophyly of a recognised “family” is not substantiated, closer examination of putative morphological apomorphies reveals they are usually weak characters. For instance, both “Dorvilleidae” and “Eunicida” were found to be paraphyletic and the opheliid genus *Travisia* should be transferred to the Scalibregmatidae. Thus, the 18S-rDNA seems to be a use-full molecular marker within the polychaetes to test the monophyly of a “family”.

4.1 Introduction

The phylogeny and evolution of polychaetes is still controversial. An issue is, whether polychaetes themselves are monophyletic within Annelida representing the sister group of the Clitellata, or whether they are paraphyletic with regard to the Clitellata (Meyer & Bartolomaeus, 1996; Purschke & Tzetlin, 1996; Purschke, 1997; Rouse & Fauchald, 1997, 1998; Westheide, 1997; Bartolomaeus, 1998; Hausen & Bartolomaeus, 1998; Westheide et al., 1999; Purschke, 2002, 2003). The cladistic analysis of Rouse and Fauchald (1997) based on morphological data favours a sister group relationship of Clitellata and “Polychaeta”, whereas other analyses support the paraphyly of polychaetes regarding to the Clitellata (e.g. Purschke, 2002, 2003). The latter is supported by molecular data throughout all molecular phylogenetic analyses (see McHugh, 2000, 2003). Furthermore, it remains unresolved if clitellate-like polychaetes like the Aeolosomatidae or *Hrabeiella periglandulata* are the sistergroup of the Clitellata or not (Purschke, 1999, 2003; Rota et al., 2001; Struck et al. 2002a).

Traditionally polychaete annelids have been classified into over 80 “families”, which are more or less supported by morphological data (Fauchald, 1977). In contrast only

a few taxa of a higher phylogenetic rank than “families”, like the Eunicida, are well supported by morphological data (Orensanz, 1990). Rouse and Fauchald (1997) suggest that the “Polychaeta” comprise two major clades, the Scolecida and Palpata. The most inclusive taxa within the Palpata are the Canalipalpata and the Aciculata, comprising the Phyllodocida and Eunicida. This view was challenged by Bartolomaeus (1998) and Hausen (2001), who question the monophyly of Scolecida, Palpata and Canalipalpata. Instead they present evidence for the monophyly of a taxon comprising all polychaete taxa with hooked setae (*sensu* Bartolomaeus, 1998). With respect to morphological data there is little possibility of solving the problem due to arguments over character scoring, homology and the lack of characters.

In recent studies molecular data and particularly sequences of the 18S rRNA are the most frequently used to analyse annelid phylogeny (see McHugh, 2000, 2003; Bleidorn et al., 2003a). However, the phylogeny within the Annelida and especially within the „Polychaeta“ has not been resolved by these approaches. Neither of the above mentioned suggestions can be supported nor be rejected by the molecular data at hand today (see McHugh, 2000, 2003). The scope of this study is to estimate relationship between the polychaete taxa with a special emphasis on the enigmatic clitellate-like taxa of the „Polychaeta“ based on molecular data using the sequences of the structural gene of the 18S rRNA. Although the taxon sampling of the polychaetes have been increased in the recent analyses (Rota et al., 2001; Struck et al., 2002a & b; Bleidorn et al., 2003a & b), the taxon sampling is still insufficient, reflecting only a maximum of one third of the polychaete „families“, most of them represented by only one member. Furthermore, these analyses are more or less strongly biased in the taxon sampling due to the special concern of each analysis (McHugh, 2003). Therefore, in this study the taxon sampling is increased and many polychaete „families“ are represented by more than one member, so that the biodiversity of polychaetes is much better recognized than in the previous analyses. Furthermore, a detailed analysis of the data matrix was conducted prior to the phylogenetic reconstruction to explore the specific evolutionary model of base substitution of the 18S rRNA within the Annelida in more detail.

4.2 Materials and methods

For this study comprising 159 operational taxonomic units the sequences of the 18S rDNA of 16 additional species of different polychaete taxa were determined (see Appendix A). The samples were preserved in 100% ethanol, in the detergent solution Persil MegaPerls (Bahl & Pfenniger, 1996) or in LTE-buffer (1 mM Tris-HCl pH 7.5, 0.1 mM EDTA) and stored at – 20°C (ethanol, detergent) or – 70°C (buffer). The extraction of DNA and the amplification of 18S rDNA via PCR were performed according to Struck et al. (2002b).

The sequences were determined with the automatic sequencer ABI Prism 377 (Perkin Elmer, Shelton, Connecticut, USA). The alignment was conducted using the program CLUSTAL W (Thompson et al., 1994) and subsequently corrected by hand in GeneDoc (Nicholas & Nicholas, 1997). Ambiguous positions were excluded from the subsequent analysis.

Preliminary Analyses

The decision between uniform rate and among-site rate variation is crucial for each phylogenetic reconstruction (Wakeley, 1994, 1996; Yang, 1996; Milinkovitch et al., 1996; Swofford et al., 1996; Van de Peer et al., 2000; Philippe & Germot, 2000). This is in particular true for 18S-rDNA, which exhibit an among-site rate variation of 18S rDNA from 0.00024 to 17 in the eukaryotic crown group, especially in the bilaterian clade (Van de Peer et al., 2000). Consequently, the alignment of the 18S rDNA shows great differences in the substitution rates among different positions.

Although the parsimony analysis is not based on an explicit evolutionary model these differences can be addressed by using specific weighting schemes (see Barker & Lanyon, 2000 and literature therein). Furthermore, the necessity and an increasing accuracy of the phylogenetic reconstruction using appropriate weighting schemes has been shown by different studies (Bull et al., 1993; Huelsenbeck & Hillis, 1993; Hillis et al., 1994; Miyamoto et al. 1994; Milinkovitch et al., 1996; Cunningham, 1997; Vogt, 2002). Unfortunately, to date there is no consensus about the approach to determine and justify weighting schemes *a priori* or *a posteriori* (Barker & Lanyon, 2000). Even though an untested apriorism of a truly empirical statement is introduced, an *a priori* justification is to favour about an *a posteriori* justification to avoid the circularity of the latter. Therefore, to gather some support for the untested apriorism in this study no arbitrarily weighting schemes were chosen but a weighting scheme determined and justified by the data set.

Beside using arbitrarily chosen weighting schemes one approach is the definition of different weighting schemes for stems and loops in the secondary structure of the 18S rDNA (Wheeler & Honeycutt, 1988; Dixon & Hillis, 1993; Soltis & Soltis, 1998; Soltis et al. 1999). However, this approach does not take into account the differences within stems and loops. Therefore, to examine the degree of variation within the alignment more precise a sliding window analysis was performed (Pesole et al., 1992; Sturmbauer & Meyer, 1992). The percentage of variation within windows of nine bases with three bases of overlap was determined. The genetic variation is expressed as a percentage of the 27 possible base substitutions in a window of 9 bases. Five classes of variation were defined to obtain more or less the same number of positions per class: 0 - 20%, 20 - 30%, 30 - 40%, 40 - 50%, 50 - 100%.

The rates of each type of substitution, the transition and transversion rates as well as

the degree of saturation were estimated for each of the classes of variation to determine appropriate weighting schemes for the parsimony analyses. The procedure is as follows. First, using the options “pairdiff”, “include”, and “exclude” of PAUP4.0b (Swofford, 2002), a pair wise comparison of the aligned sequences is performed for each class of variation. Second, the different substitution rates of each pair of sequence in each class of variation is estimated by dividing the amount of specific substitutions by the whole number of aligned positions of each pair and class. Third, the mean of the different substitution rates is determined for each rate in each class.

Although a saturation of transitions or transversions was not or only slightly detectable in any class of variation (Fig. 1), the clear differences shown between both the different classes of variation and the transition and transversion rates (Fig. 2 & Table 1) justify and require *a priori* the use of an appropriate weighting scheme in the parsimony analysis. The weighting scheme was defined for the transition and transversion rates of each region of variability. The weights were estimated based on the ratio of each rate, transitions and transversions, to the highest rate over all regions, which was set to a weight of four to differentiate more precisely between the rates (Table 1).

Parsimony

Phylogenetic reconstruction based on the parsimony criterion was performed by applying the weighting scheme using PAUP*4.0b. The reconstruction was done in several steps. In all steps the heuristic swapping algorithm tree bisection-reconnection (TBR) with random addition of the taxa and a rearrangement limit of 10^9 for each replicate was used. In the first step the random addition of taxa was performed ten times. In the next step the score of this solution was used as a “chuck score” limit; only scores with a better value were allowed to keep more than 100 trees. The number of replicates in the random taxa addition was increased to 100. This step was repeated till no better solution could be found in the 100 replicates. In all steps gaps in the alignment were treated as missing.

The reliability of the phylogenetic nodes was estimated by 100 bootstrap (BP) and 100 jack-knife with 40% data deletion (JK60) replicates (Felsenstein, 1985; Farris, 1997). The same settings as in the first step were used, except that the number of replicates in the random taxa addition was set to one to save computational time.

Maximum Likelihood

MrModeltest 1.1b (Nylander 2002) was used for estimating the appropriate model of sequence evolution. This program is a simplified version of Modeltest 3.06 (Posada & Crandall, 1998, 2001). The hLRT criterion indicates that the model SYM+I+ Γ (Zharkikh, 1994) represents the optimal model with respect to the data: base frequencies = equal; rate matrix = (1.1451 2.7281 1.1231 0.8893 3.8268); among-site rate variation with proportion

of invariable sites = 0.2483; gamma distribution shape parameter = 0.6761. A maximum likelihood analysis was performed with PAUP*, version 4.0b10 (Swofford, 2002) under the likelihood settings suggested by the result of the model test 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 3.0B4 (Huelsenbeck & Ronquist, 2001). All priors were set according to the SYM+I+ Γ model: lset nst=6 rates=invgamma; prset RevMatPr=dirichlet(1.0,1.0,1.0,1.0,1.0,1.0) StateFreqPr=dirichlet(1,1,1,1) ShapePr=uniform(0.05,50.0) PinVarPr=uniform(0.0,1.0). Each Markov chain, three heated (mcmc temp=0.3) and one cold, was started from a random tree and all four chains ran simultaneously for 10^7 generations, with trees being sampled every 500 generations for a total of 20,001 trees. After the likelihood of the trees of each chain converged, we discarded the first 14,001 trees as *burn in*. The majority-rule consensus tree containing the posterior probabilities of the phylogeny was determined from 6,000 trees. Concerning posterior probabilities it has been often noticed that Bayesian probabilities tend to be higher than comparable bootstrap values for the same node (e.g. Buckley *et al.* 2002) and there is no current consensus of how posterior probabilities should be interpreted relative bootstrap measures. Recent simulation studies on this topic came to the conclusion that both measures of support cannot be directly compared, but could be used as upper and lower bounds of node reliability (Alfaro *et al.* 2003; Douady *et al.* 2003, Erixon *et al.* 2003; Suzuki *et al.* 2002). This should be kept in mind while judging the results of our analyses.

Hypotheses testing

Even though a phylogenetic hypothesis is not recovered by the phylogenetic reconstruction this does not mean, that the hypothesis can be significantly rejected by the data set. Therefore, the test of Shimodaria & Hasegawa (1999) was used to compare different alternative hypotheses. The hypothesis of polychaete monophyly including Echiura and Siboglinidae to the exclusion of Clitellata [Constraint tree: (Outgroup ((Polychaeta (Clitellata)))] was compared without that specific constraint [Constraint tree: (Outgroup (Polychaeta Clitellata))]. Furthermore, constrained analyses conform to the best tree of Rouse and Fauchald (1997) [Constraint tree: (Outgroup (Echiura (Clitellata ((Scolecida Polygordiidae Protodrilida) ((Canalipalpata) ((Phyllodocida) (Eunicida))))))] as well as to the hypotheses of Bartolomaeus (1998) and Hausen (2001) [Constraint tree: (Outgroup (Echiura Clitellata “other polychaete families” (Arenicolidae Maldanidae Oweniidae Chaetopteridae Terebellida Sabellida Siboglinidae) (Spionidae Magelonidae Protodrilida)))] were also compared with the latter analysis [Constraint tree: (Outgroup

(Polychaeta Clitellata)]. The best trees were obtained by the parsimony analysis and the comparisons were performed by applying the test of Shimodaira and Hasegawa (1999) based on their likelihood scores.

4.3 Results

The alignment produced a data set of 2439 positions, from which 885 ambiguous positions were excluded. In the final alignment 731 sites were parsimony informative, 553 positions were constant and 270 sites were parsimony uninformative.

Preliminary Analyses

Based on the sliding window analysis different regions of variability can be defined. Each region contents 11.0% to 29.1% of the total positions (Table 1). These regions reveal different transition and transversion rates rising from 0.01181 to 0.10226 respectively from 0.00375 to 0.08827 with the increasing variability. Whereas, the transition rate of the whole data set is 0.05282 and the transversion rate 0.03859. The lowest rates thus are only a quarter or a tenth of the mean rate and the highest rates nearly or more than the twice. This means that about 60% of the data set shows clear differences from the mean rate.

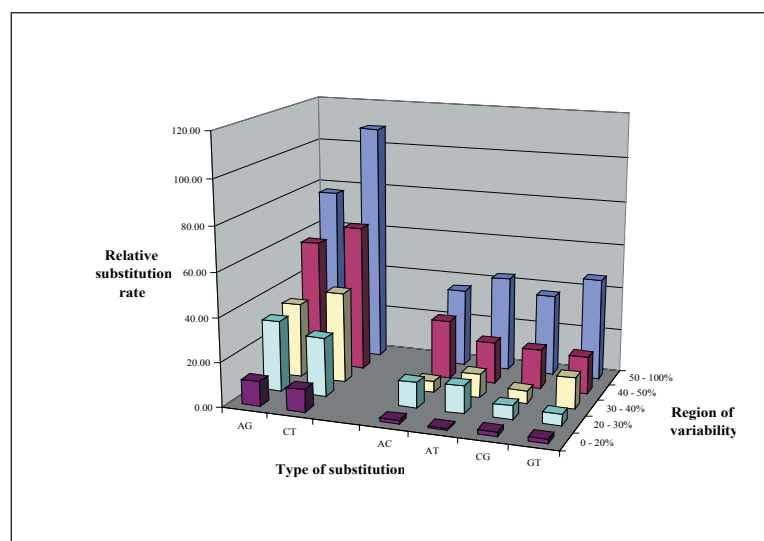


Figure 1. Saturation of transitions in the regions of variability. Transition rates of each species pair plotted against uncorrected pair wise distance p . Trend line implemented. **A.** Variation 50 - 100%. **B.** Variation 40 - 50% as an example for all other plots.

These differences can also be shown in the substitutions rates, which clearly reflect the great differences between the classes of variation (Fig. 2). The highest rate ($C \leftrightarrow T$ in the class 50 – 100%) is nearly 110fold higher than the lowest rate ($A \leftrightarrow T$ in the class 0 – 20%), and therefore more than hundred times more probable to occur. Transitions exhibit much higher rates than the transversion within each class of variation. On the other side, the differences between the rates within transitions and transversions are narrow with some exceptions. Thus grouping together these types of substitutions seems to be justified by the data set.

Phylogenetic Analyses

The topology of the strict consensus tree of the parsimony analysis (Fig. 3) as well as the ML-tree (Fig. 4) and the majority-rule consensus tree of the Bayesian analysis (Fig. 5) are congruent in many details. The parsimony analysis results in 1707 parsimonious trees with a tree length of 60663. The most likely tree of the Maximum Likelihood (ML) analysis has a ln-likelihood value of -35073.53. The chains of the Bayesian analysis reached the equilibrium at no later than $7 * 10^6$ generations. A majority-rule consensus tree was constructed from the remaining 6,000 trees and is shown with posterior probabilities above the branches in fig. 5.

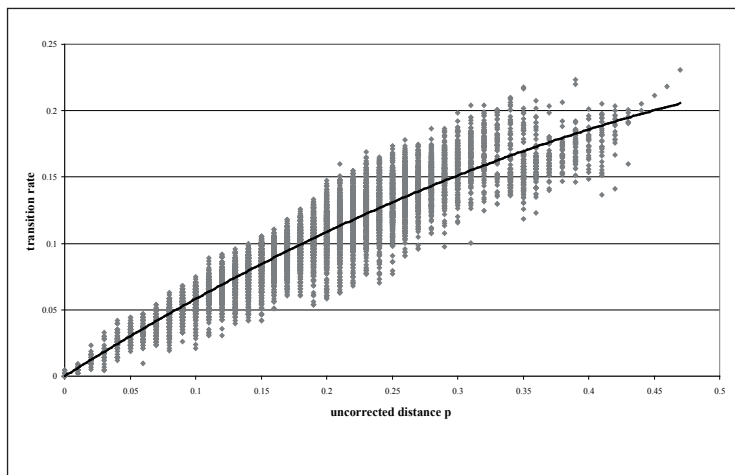


Figure 2. Substitution rates of the different substitution types in the different regions of variability relative to the lowest rate over all regions.

Table 1. Regions of variability and their rates of transition and transversion as well as the corresponding weighting scheme of transversions in relation to transitions

Region of variability	Number of positions	Rate of transitions	Rate of transversions	Weighting scheme
0 - 20%	444	0.01181	0.00375	109 to 35
20 - 30%	258	0.03252	0.01934	21 to 13
30 - 40%	171	0.04166	0.01979	21 to 10
40 - 50%	229	0.06846	0.04437	9 to 6
50 - 100%	452	0.10226	0.08927	5 to 4

Neither of these analyses recovers a monophyletic Annelida or Polychaeta. Lophotrochozoan taxa, like Kamptozoa, Brachiopoda, Phoronida or Nemertini cluster together with annelid taxa and the same holds true for molluscan taxa. The monophyly of the clitellate clade is supported with a posterior probability value (BP) of 100% as well as with bootstrap (BS) and jack-knife (JK60) values of 96% or 95% respectively and they appear as a highly derived taxon within polychaetes. A close relationship to

neither the Aeolosomatidae, nor the Parergodrilidae, nor *Hrabeiella periglandulata* can be revealed by these analyses. However, the SH-Test (Shimodaira & Hasegawa 1999) of the comparison of the monophyly of polychaetes (tree length = 61108) against their paraphyly (tree length = 61071) within monophyletic Annelida (including Echiura and Siboglinidae) reveals a non significant value of 0.759. Interestingly, all analyses sustain the close relationship of Echiura and Capitellidae (100%; 87%; 91%).

In none of the analyses groupings congruent to the systems of either Rouse and Fauchald (1997) or Bartolomaeus (1998) and Hausen (2001) are supported by any best tree. Only in the parsimony analysis (Fig. 3) a closer relationship of eunicidan taxa sensu Rouse and Fauchald (1997) can be shown. However, Dinophilidae and Amphinomidae are not incorporated in this clade. Furthermore, in all analyses monophyly of Terebellida, represented only by the Terebellidae and Alvinellidae, is supported (BP=100%; BS=60%; JK60%=83%). The Terebellidae are paraphyletic with regard to the Alvinellidae in all analyses. The comparison of both hypotheses against an analysis, which constrains the monophyly of Annelida including Echiura and Siboglinidae (tree length = 61071), by a test of Shimodaira and Hasegawa (1999) results in a value of 0.410 for the hypothesis of Bartolomaeus (1998) and Hausen (2001) (tree length = 61488) and a value of 0.001 for the hypothesis of Rouse and Fauchald (1997) (tree length = 62012). Therefore, the latter hypothesis is significantly rejected by the data set of the 18S-rDNA.

The relationships between polychaete families are poorly resolved, which can best be seen in the parsimony analysis as well as in the Bayesian analysis, both exhibit big polytomies at the basal nodes (Fig. 3 and 5). Only a sister group relationship between maldanids and arenicolids seems to be well substantiated (BP=100%; BS=60%; JK60=80%), as well as a clade consisting of *Pisione remota* and the investigated polynoids (100%; 99%; 100%). The monophyly of most of the polychaete “families” which are represented by more than one taxon are recovered by ML and parsimony analyses. However, not all taxa recovered taxa gain a significant support by BS and JK60 values (Fig. 3-5). In no analyses does the “Dorvilleidae” appear monophyletic. In the ML analysis and the Bayesian analysis are the Eunicidae paraphyletic with regard to the Onuphidae. The close relationship of these two taxa is substantiated in all analyses (BP=100%; BS=100%; JK60=100%). Interestingly, all analyses sustain the close relationship of the former opheliid *Travisia forbesii* with the Scalibregmatidae, especially with *Scalibregma inflatum* (100%; 90%; 97%). The monophyly of the remaining Opheliidae is also recovered in all analyses and highly corroborated by a posterior probability of 100%.

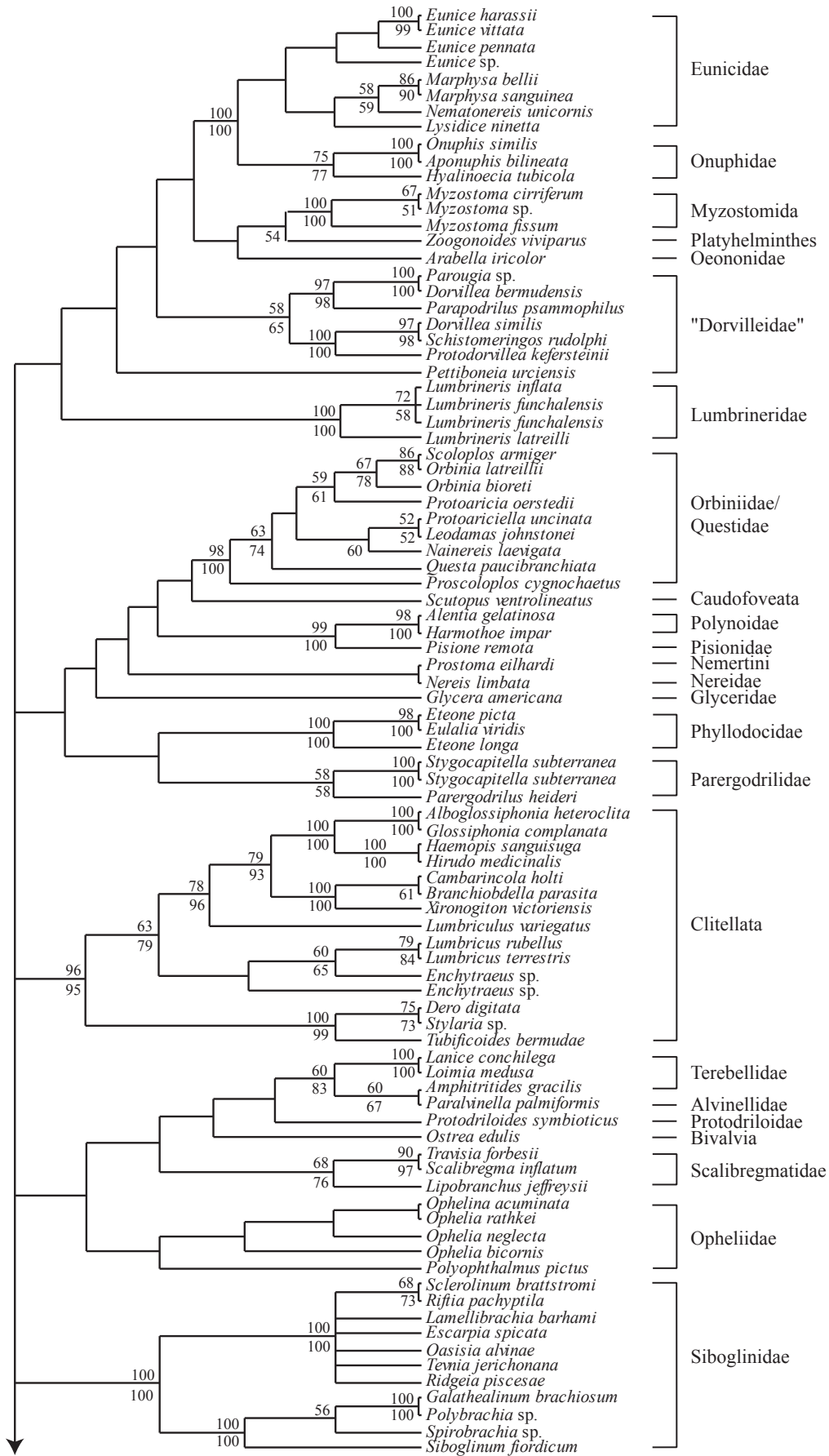


Figure 3B

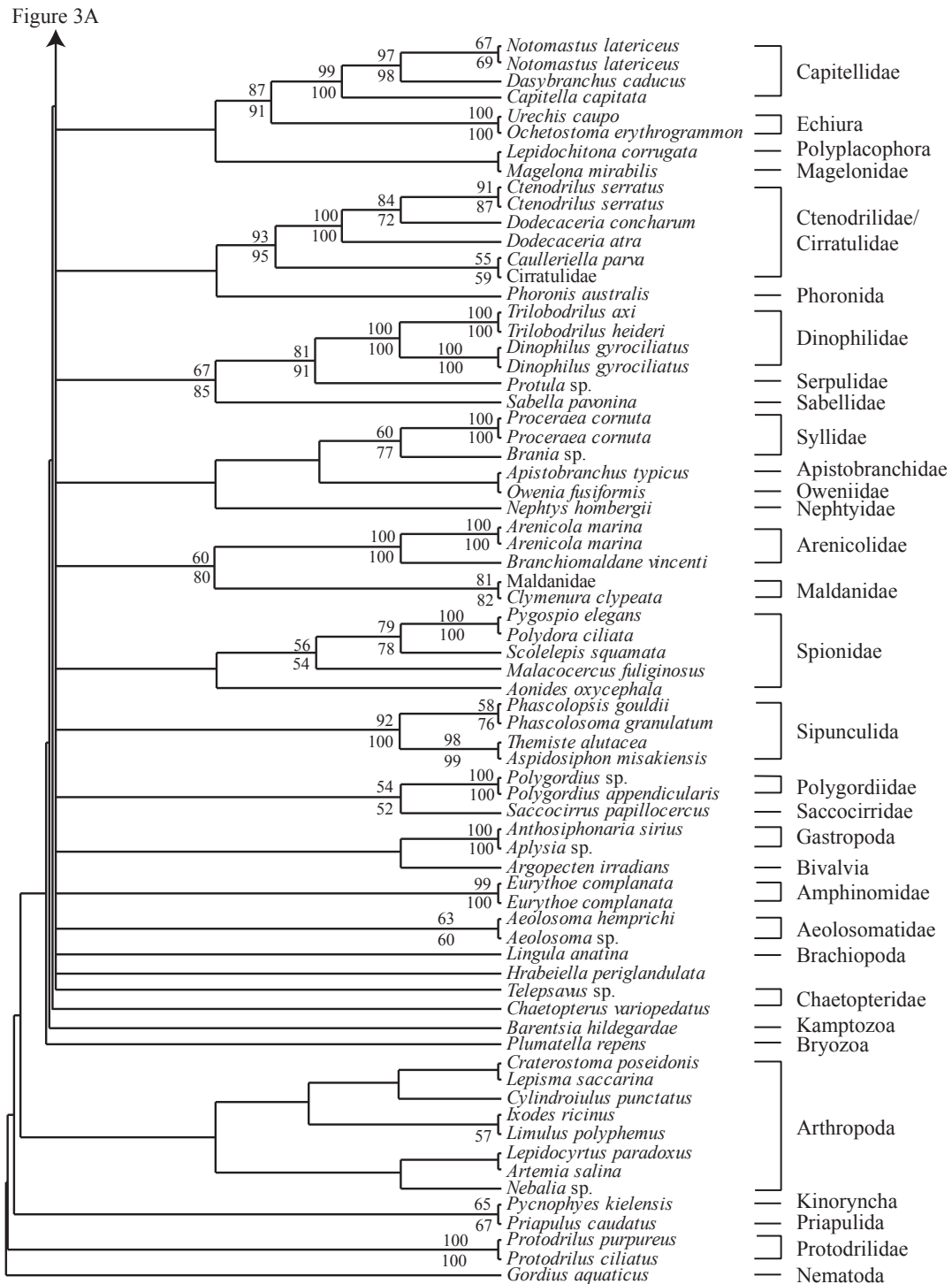


Figure 3. MP analysis with weighting scheme 2 (table 2). Strict consensus tree of 1707 parsimonious trees with a tree length of 60663. Gaps treated as missing. BS values above the branches; JK60 values below the branches. Only values above 50 are shown.

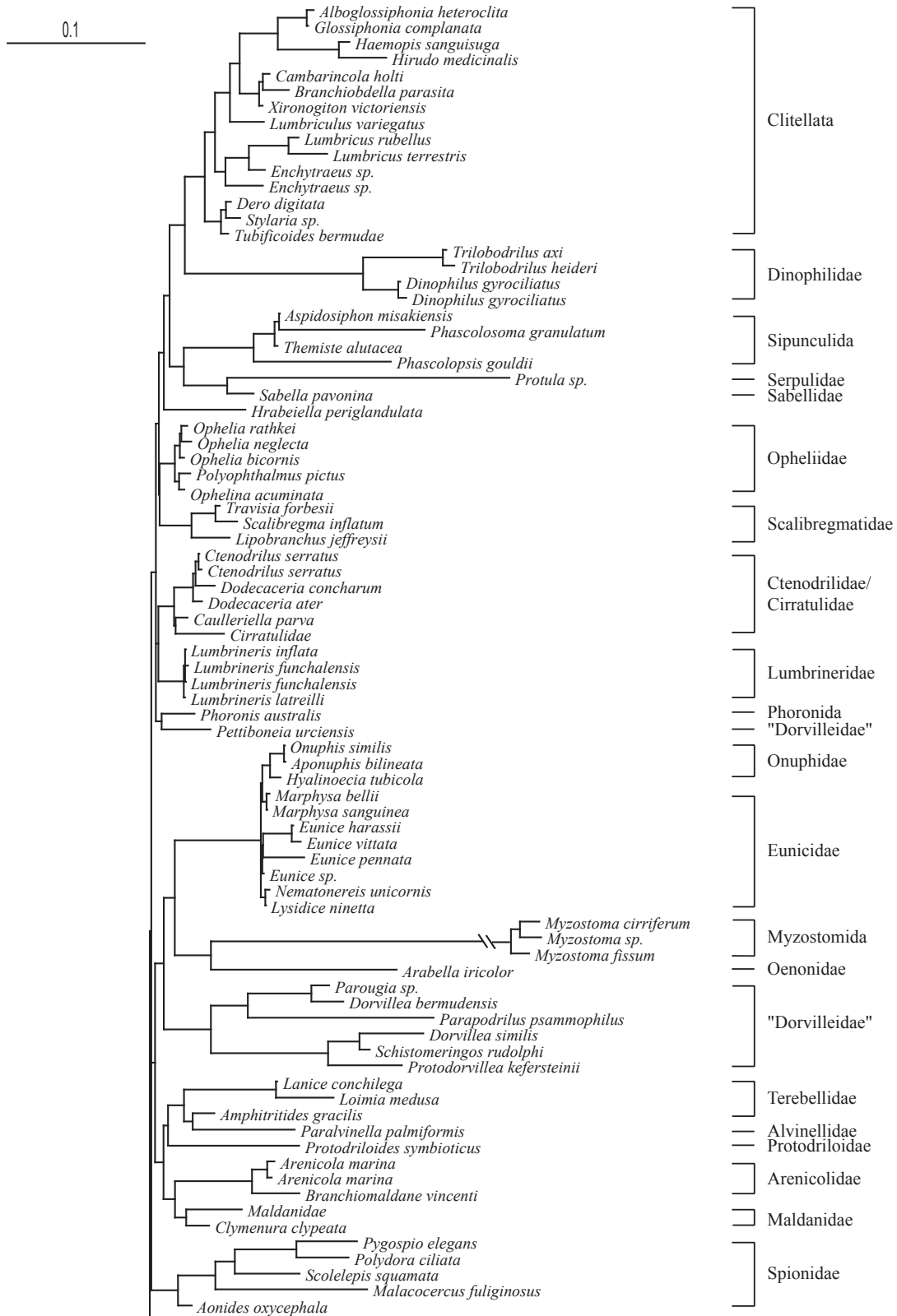


Figure 4B

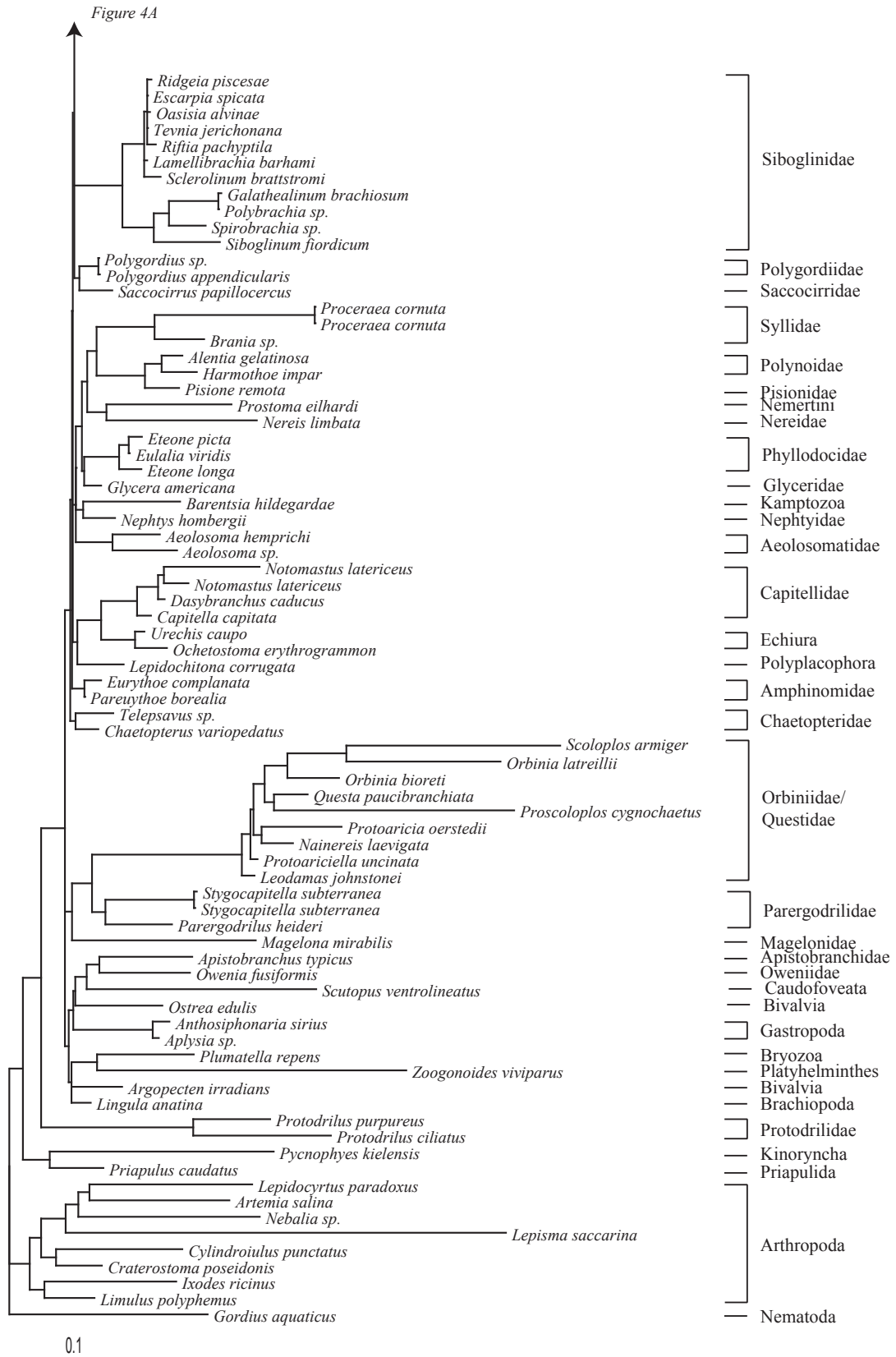


Figure 4. Maximum likelihood tree based on the SYM+I+Γ model of sequence evolution (-logL=35073.53)

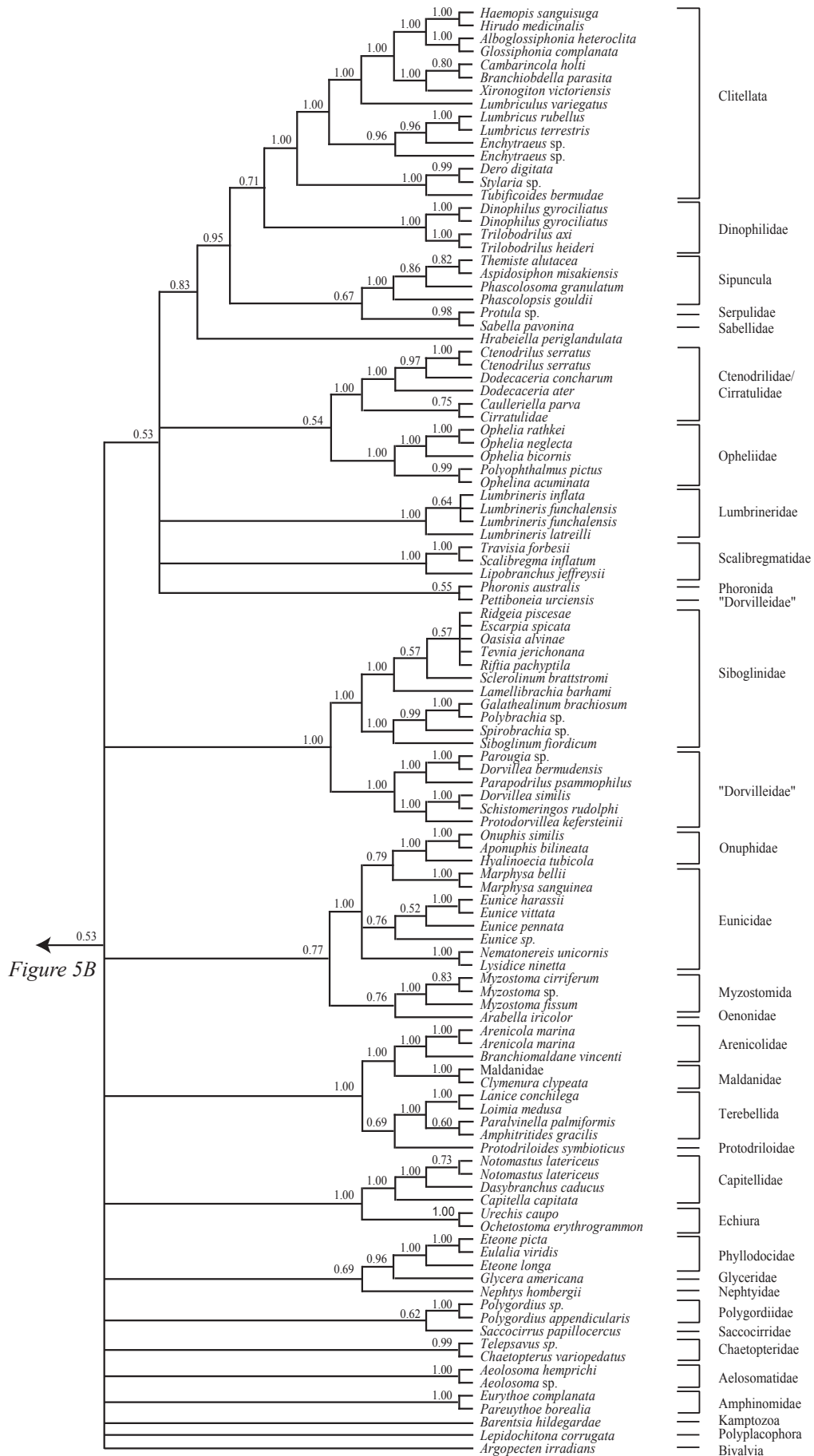


Figure 5B

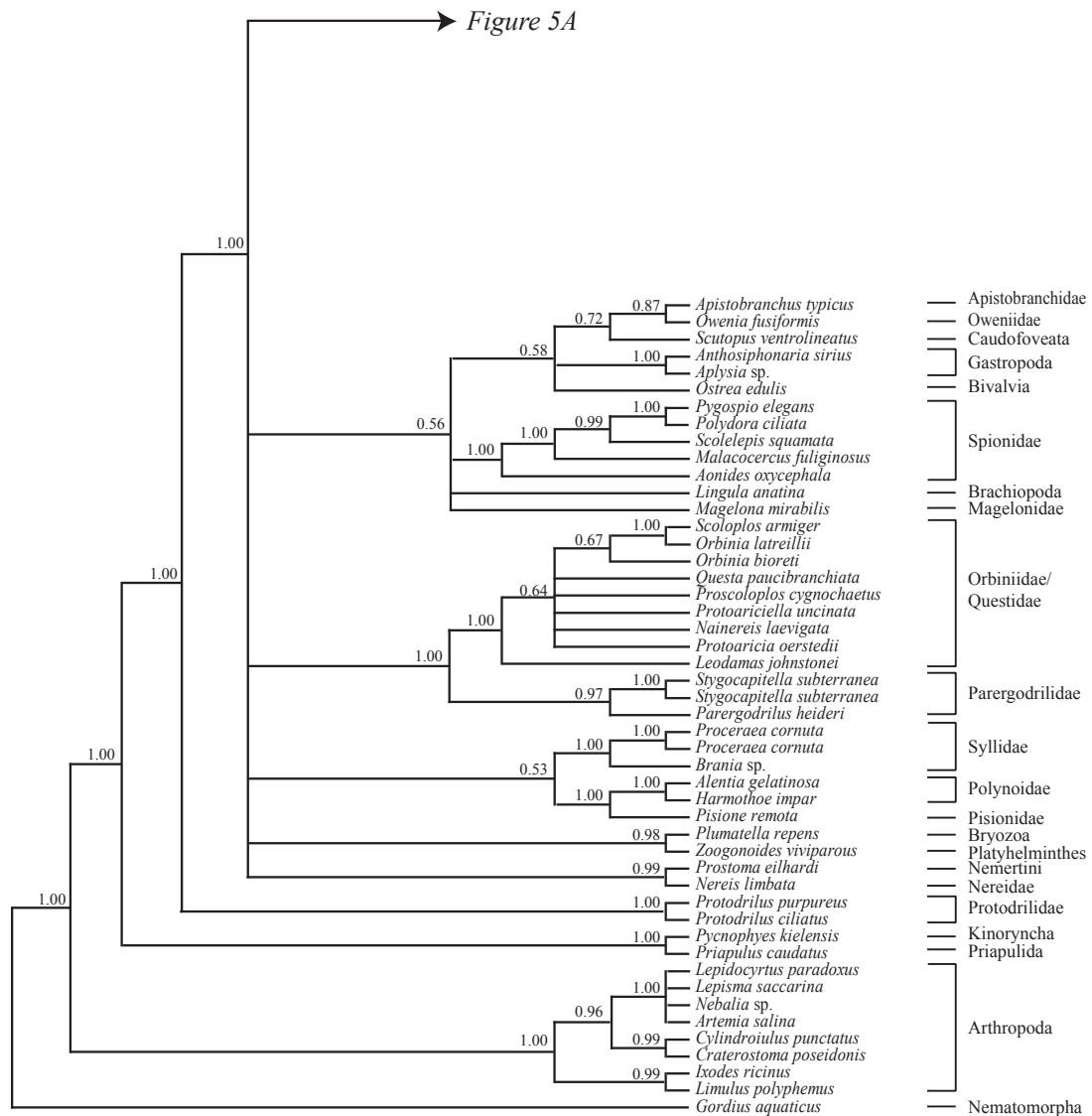


Figure 5. Majority-rule consensus tree of the 6.000 inferred trees of the Bayesian analysis. The posterior probabilities are given above the branches.

4.4 Discussion

Monophyly of polychaetes and the Position of Clitellata and clitellate-like Polychaetes

Though the analyses are based on appropriate evolutionary models the monophyly of Annelida is not recovered within the lophotrochozoan clade due to the well known effect that the 18S has a low phylogenetic signal at the basal nodes of this clade (e.g. Halaných, 1998; Giribet, 2002). This result corresponds to the results of former analyses (see McHugh, 2000, 2003). Furthermore, the basal nodes within this clade exhibit short branches and low supporting values independent of the chosen tree reconstruction method, evolutionary model and method of measure of support. Therefore, it is much more likely that these parts of the trees can rather be traced back on a low phylogenetic signal against a high homoplastic noise in the used 18S rRNA gene than on a true phylogeny

(see Phillippe et al., 1994; Regier & Shultz, 1998; McHugh, 2000, 2003; Giribet, 2002). A possible explanation of the low resolution in the hypothesis of an explosive radiation of the lophotrochozoan taxa in the Cambrian (McHugh, 2000, 2003; Rota et al., 2001; Giribet, 2002; Struck et al., 2002a).

Moreover, none of the analyses support the monophyly of the polychaetes. In all analyses the Clitellata are monophyletic and highly derived within clades comprising polychaetes (see also McHugh, 2000, 2003; Bleidorn et al., 2003a; Jördens et al., 2003). Therefore, considering the presently discussed alternatives of a sister group relationship of polychaetes and clitellates or the position of clitellates within the polychaete clade, the Clitellata are more likely highly derived polychaetes than their sistergroup. Based on morphological data and considerations about the evolutionary origin of the annelids rather in a marine environment than in a terrestrial-burrowing habitat, a highly derived position of the clitellates within the polychaetes was suggested previously (e.g. Westheide, 1997, Purschke, 2002, 2003). Unfortunately, the sister group of the Clitellata within the polychaetes cannot be determined.

Furthermore, in all analyses a close relationship neither to the Aeolosomatidae nor to the Parergodrilidae nor to *Hrabeiella* can be shown. All three taxa exhibit different combinations of clitellate-like characters, as for instance lack of parapodia, hermaphroditism and the possession of simple chaeta (Purschke, 1999; Hessling & Purschke, 2000; Purschke et al., 2000; Purschke & Hessling, 2002; Purschke, 2003), and non-clitellate characters. Especially, a closer relationship of Aeolosomatidae and “Oligochaeta” has been extensively discussed (Michelson, 1929; Stephenson, 1930; Bunke, 1967; Brinkhurst & Jamieson, 1971; Timm, 1981; Brinkhurst & Nemeč, 1987). Both recent ultra structural and immunohistochemical investigations failed to corroborate this hypothesis (Bunke, 1985, 1986, 1994; Hessling & Purschke, 2000; Purschke & Hessling, 2002) and considerations about a non-aquatic origin of the Clitellata (Westheide, 1997; Purschke, 1997, 1999). In their cladistic analyses Rouse and Fauchald (1997) found the Aeolosomatidae to cluster within the polychaetes although their exact placement remained uncertain. Early molecular analyses based on a small taxon sampling support a closer relationship to the Clitellata (Moon et al., 1996; Winnepenninckx et al., 1998), whereas more recent analyses could not give evidence for this position (Martin, 2000; Rota et al., 2001; Struck et al., 2002a). Thus, these analyses substantiate the latter hypothesis of an independent evolution of Clitellata and Aeolosomatidae.

Whereas, a convergent evolution of Parergodrilidae and Clitellata due to similar selection pressures has widely been accepted (Erséus & Rota, 1998; Rota, 1998; Purschke, 1999, 2001), fine-structural analyses demonstrate clear differences between the two taxa (Rota, 1998). However, their systematic position within the polychaetes remains unresolved (e.g. Rouse & Fauchald, 1997). Based on molecular analyses a closer relationship

of Parergodrilidae to Orbiniidae is suggested (Struck et al., 2002a; Bleidorn et al., 2003b; Jördens et al., 2003). This is also recommended by our ML-analysis and a high posterior probability of 100%. The position close to Orbiniidae and Questidae was also found in some of the cladistic analyses of Rouse and Fauchald (1997). Although such a position requires additional confirmation, this relationship is not precluded by further morphological data, e.g., in the pharyngeal structures (Purschke, 1987, 1988; Purschke & Tzetlin, 1996). However, the data do not unequivocally support a phylogenetic relationship of Parergodrilidae to any particular annelid taxon.

Furthermore, a close relationship of *Hrabeiella* and Parergodrilidae as suggested on morphological data (Pizl & Chalupsky, 1984; Rota & Lupetti, 1997; Rota, 1998; Purschke, 1999; Rouse & Pleijel, 2001) is rejected by the molecular data (see in addition Rota et al., 2001; Jördens et al., 2003). A close relationship of Clitellata and *Hrabeiella* also gains no support from our analyses. However, it is supported by ultra structural analyses of the pharynx (Purschke, 2003) and to some degree by a combined analysis of 18S- and 28S-rDNA (Jördens et al., 2003).

Phylogeny within in the polychaetes

From a morphological perspective, there is a great debate concerning the relationships of the polychaete families. The results of the cladistic analysis by Rouse and Fauchald (1997) are contradicted by the investigations of Bartolomaeus (1998) and Hausen (2001) and all this is intensively discussed elsewhere (see Rouse and Pleijel 2001). The problems which are connected to the attempt to unravel the phylogenetic relationships are reflected in the analysis using molecular data. Whereas most polychaete “families” represent well supported monophyla (see below), the relationships to one another remain obscure - a result which was also observed in former investigations (McHugh, 2000, 2003; Rota et al., 2001; Struck et al., 2002a & b; Bleidorn et al., 2003a & b). Nevertheless, based on the presented molecular data the hypothesis of annelid and polychaete ingroup relationships of Rouse and Fauchald (1997) can be significantly rejected.

Remarkable to us seems the placement of the Pisionidae with the Polynoidae. The affinities of the Pisionidae have been uncertain for a long time. First included in the Phyllodocidae (Grube 1857), Rouse and Fauchald (1997) found a clade consisting of the pisionids, Paralacydoniidae, Glyceridae and Goniadidae. Our results now support the ideas of Levinsen (1887), which have been revived by Pleijel and Dahlgren (1998), to treat this taxon as scale-worm relatives. Rouse and Pleijel (2001) mentioned that the presence of venom glands and piercing jaws supports this grouping.

Support of Traditional polychaete “Families” by 18S-rDNA

Although only a few interrelationships of the polychaete “families” are well established

by the 18S-rDNA the monophyly of most of the traditional polychaete “families” like Amphinomidae, Arenicolidae, Capitellidae, Lumbrineridae, Maldanidae, Onuphidae, Phyllodocidae, Polynoidae, Siboglinidae, Spionidae, and Syllidae are well substantiated and recovered in all analyses. In this way the “families” based on morphological data (see Fauchald, 1970; Rouse & Pleijel, 2001) receive additional and independent support from the results of the molecular data. However, in most cases the taxon sampling within the families is still low. Therefore, further analyses with a broader taxon sampling within these different taxa are necessary to corroborate this study.

Nevertheless, the monophyly of some polychaete “families” like “Dorvilleidae”, Eunicidae, Opheliidae, and Scalibregmatidae gains no support from the presented analyses. Thus, these results sustain former molecular analyses (Struck et al., 2002b; Bleidorn et al., 2003a & b) and seem to contradict in part the morphological data. However, in most cases a closer examination of the supposed morphological autapomorphies reveals their weakness as exemplified here with three examples.

A) The Eunicidae are well-known group of polychaetes and their close relationship to Onuphidae is well established by both morphological characters, like the special type of a labibognath jaw apparatus (“Paulinites theme”) and the mineralization of the jaws with aragonite (Kielan-Jaworoska, 1966; Colbath, 1986; Paxton, 1986; Orensanz, 1990), and molecular data (this analysis and Struck et al., 2002a & b). The monophyly of the Onuphidae is throughout all analyses recovered and defined by strong autapomorphies like the unique frontal lips, the distinct ceratophores at the antennae and palps and the peristomium with only one ring (Paxton, 1998; Rouse & Pleijel, 2001). However, the definition of the Eunicidae reveals that they possess only morphological characters which they have in common with either the Onuphidae or the other eunicidan taxa (Rouse & Pleijel, 2001). Therefore, the paraphyly of Eunicidae estimated in this analysis and in former analyses is not surprisingly (McHugh, 2003).

B) The same holds true for the paraphyly of the “Dorvilleidae”, another eunicidan family which no supported by any morphological character (for detailed discussion see Struck et al. 2002b). Since monophyly of the “Dorvilleidae” and Eunicida is neither supported by morphological nor by molecular data, these groups should be considered as paraphyletic and marked by quotation marks until the contrary has been proven.

C) Both the Opheliidae and the Scalibregmatidae are worldwide distributed with many described species and are grouped together in traditional classifications (Hartmann-Schröder, 1996). However, there is no convincing apomorphy known for the two taxa (Fauchald & Rouse, 1997) and Rouse and Pleijel (2001) annotated that the Opheliidae might be paraphyletic with regard to Scalibregmatidae. Typical scalibregmatid characters as the rugose epidermis and segmental annuli can be found in the Opheliidae as well. Of special interest is the opheliid taxon *Travisia*, which closely resembles scalibregmatid

species (Bellan et al., 1990; Hartmann-Schröder, 1996), and may be related to them (Blake, 2000a & b). The well developed ventral grooves, which are found in all other opheliid taxa, are generally absent or only poorly developed if present in *Travisia* (Dauvin & Bellan, 1994). Furthermore, Storch (1988) pointed out that *Travisia* possesses a stratified epidermis, which is unusual for invertebrates. Summarizing these findings, uncertainties regarding the monophyly of opheliids and scalibregmatids are due to the placement of *Travisia* as a basal opheliid. Our analysis strongly supports a common ancestry of *Travisia* and scalibregmatids and thus sustains the analysis of Bleidorn et al. (2003a). After transferring *Travisia* to the Scalibregmatidae it has to be proofed whether the presence of lateral grooves and a strong ventral groove might support the monophyly of the remaining new combined Opheliidae, which is supported in all of our analyses independent of the choice of method. A rugose epidermis can be assessed as an apomorphy for a taxon consisting of the Scalibregmatidae and *Travisia*.

Conclusions

Although the phylogeny of the polychaetes cannot be solved unambiguously, the paraphyly of the polychaetes in regard to the Clitellata and the monophyly of most of the traditional polychaete “families” is corroborated. Furthermore, when the monophyly of a “family” is not substantiated a closer examination of the supposed morphological autapomorphies reveals their weakness. For instance, both “Dorvilleidae” and “Eunicida” shall be considered as paraphyletic and the opheliid genus *Travisia* be transferred to the Scalibregmatidae. Thus, the 18S-rDNA seems to be a use-full molecular marker within the polychaetes to test the monophyly of a “family”.

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Appendix A: List of the taxa used in the analysis in Alphabetical Order; 18S rDNA Sequences determined by Authors shown in bold

Higher Taxon	Species	GeneBank Accession Nos.
Annelida		
	<i>Hrabeiella periglandulata</i> Pizl & Chalupsky 1984	<i>AJ310501</i>
Aeolosomatidae	<i>Aeolosoma hemprichi</i> Ehrenberg 1828	AJ310500
	<i>Aeolosoma</i> sp.	Z8374
Alvinellidae	<i>Paralvinella palmiformis</i> Desbruyères & Laubier 1986	AF168747
Amphinomidae	<i>Eurythoë complanata</i> (Pallas 1766) (<i>GenBank</i>)	AY040685
	<i>Eurythoë complanata</i> (Pallas 1766) (<i>Osnabrück, Germany</i>)	<i>AY364851</i>
Apistobranchidae	<i>Apistobranchus typicus</i> (Webster & Benedict 1887)	AF448150
Arenicolidae	<i>Arenicola marina</i> (Linneaus 1758) (<i>GenBank</i>)	AJ310502
	<i>Arenicola marina</i> (Linneaus 1758) (<i>Arcachon, France</i>)	<i>AF508116</i>
	<i>Branchiomaldane vincenti</i> Langerhans 1881	<i>AF508117</i>
Capitellidae ^a	<i>Capitella capitata</i> (Fabricius 1780)	AF508118
	<i>Dasybranchus caducus</i> (Grube 1846)	<i>AF448153</i>
	<i>Notomastus latericeus</i> Sars 1851 (<i>GenBank</i>)	<i>AY040697</i>
	<i>Notomastus latericeus</i> Sars 1851 (<i>Sylt, Germany</i>)	<i>AF508121</i>
Chaetopteridae	<i>Chaetopterus variopedatus</i> (Renier 1804)	U67324
	<i>Telepsavus</i> sp.	<i>AF448165</i>
Clitellata	<i>Alboglossiphonia heteroclita</i> (Linneaus 1761)	AF103730
	<i>Branchiobdella parasita</i> Henle 1835	<i>AF310690</i>
	<i>Cambarincola holti</i> Hoffman 1963	<i>AF115975</i>
	<i>Dero digitata</i> (Müller 1773)	<i>AF021879</i>
	<i>Enchytraeus</i> sp.	Z83750
	<i>Enchytraeus</i> sp.	U95948
	<i>Glossiphonia complanata</i> (Linneaus 1758)	Z83751
	<i>Haemopsis sanguisuga</i> (Linneaus 1758)	X91401
	<i>Hirudo medicinalis</i> Linneaus 1758	Z83752
	<i>Lumbriculus variegatus</i> (O.F. Müller 1774)	<i>AF209457</i>
	<i>Lumbricus rubellus</i> Hoffmeister 1843	Z83753
	<i>Lumbricus terrestris</i> Linneaus 1758	<i>AJ272183</i>
	<i>Stylaria</i> sp.	U95946
	<i>Tubificoides bermudae</i> Rasmark & Erséus 1986	<i>AF209467</i>
	<i>Xironogiton victoriensis</i> Gelder & Hall 1990	<i>AF115977</i>
Cirratulidae	<i>Caulleriella parva</i> Gillandt 1979	AF448151
	Cirratulidae	<i>AY040682</i>
	<i>Dodecaceria atra</i> (Quartefages 1866)	<i>AF448154</i>
	<i>Dodecaceria concharum</i> Oersted 1843	U50967
Ctenodrilidae ^a	Ctenodrilus serratus (Schmidt 1857) (Helgoland, Germany)	AY364850

	<i>Ctenodrilus serratus</i> (Schmidt 1857) (Helgoland, Germany)	AF508119
Dinophilidae	<i>Dinophilus gyrociliatus</i> O. Schmidt 1857 (Laboratory culture)	AF412805
	<i>Dinophilus gyrociliatus</i> O. Schmidt 1857 (GenBank)	AF119074
	<i>Trilobodrilus axi</i> Westheide 1967	AF412806
	<i>Trilobodrilus heideri</i> Remane 1925	AF412807
„Dorvilleidae“	<i>Dorvillea bermudensis</i> Åkesson & Rice 1992	AF412802
	<i>Dorvillea similis</i> Crossland 1924	AF412803
	<i>Parapodrilus psammophilus</i> Westheide 1965	AF412800
	<i>Parougia</i> sp.	AF412798
	<i>Pettiboneia urciensis</i> Campoy & San Martin 1980	AF412801
	<i>Protodorvillea kefersteinii</i> (McIntosh 1869)	AF412799
	<i>Schistomeringos rudolphi</i> (Chiaje 1828)	AF412804
Echiura	<i>Ochetosoma erythrogrammon</i> Rüppell & Leuckart 1830	X79875
	<i>Urechis caupo</i> Fisher & MacGinitie 1928	AF119076
Eunicidae ^a	<i>Eunice harassii</i> Audouin & Milne-Edwards 1833 (Roscoff, France)	???????
	<i>Eunice pennata</i> (O.F. Müller 1776)	AY040684
	<i>Eunice</i> sp.	AF412791
	<i>Eunice vittata</i> (Chiaje 1828)	AF412790
	<i>Lysidice ninetta</i> Audouin & Milne-Edwards 1834	AF412793
	<i>Marphysa bellii</i> (Audouin & Milne-Edwards 1834)	AF412789
	<i>Marphysa sanguinea</i> (Montagu 1815) (Concarneau, France)	???????
	<i>Nematonereis unicornis</i> (Grube 1840)	AF412792
Glyceridae	<i>Glycera americana</i> Leidy 1855	U19519
Lumbrineridae	<i>Lumbrineris funchalensis</i> (Kinberg 1865) (Roscoff, France)	AF412796
	<i>Lumbrineris funchalensis</i> (Kinberg 1865) (Roscoff, France)	AF412797
	<i>Lumbrineris inflata</i> (Moore 1911) (AquaZoo Düsseldorf, Germany)	???????
	<i>Lumbrineris latreilli</i> Audouin & Milne-Edwards 1834 (Innsbruck, Austria)	???????
Magelonidae	<i>Magelona mirabilis</i> (Johnston, 1845)	U50969
Maldanidae	<i>Clymenura clypeata</i> (Saint Joseph 1894)	AF448152
	Maldanidae	AY040694
Nephtyidae	<i>Nephtys hombergii</i> Savigny 1818	U50970
Nereidae ^a	<i>Nereis limbata</i> Ehlers 1868	U36270
Oeononidae	<i>Arabella iricolor</i> (Montagu 1804) (Banyuls-sur-Mer, France)	???????
Onuphidae	<i>Aponuphis bilineata</i> (Baird, 1870)	AF412795
	<i>Hyalinoecia tubicola</i> O.F. Müller 1776	AF412794
	<i>Onuphis similis</i> (Fauchald 1968) (Anse Forbans, Mahé, Seychelles)	???????
Opheliidae ^a	<i>Ophelia bicornis</i> Savigny 1818	AF508122

	<i>Ophelia neglecta</i> Aimé Schneider 1892	AF448156
	<i>Ophelia rathkei</i> McIntosh 1908	AF448157
	<i>Ophelina acuminata</i> Oersted 1843	AY083310 + AY083311
	<i>Polyophthalmus pictus</i> (Dujardin 1839)	AF448161
Orbiniidae	<i>Leodamas johnstonei</i> (Day 1934)	AF508126
	<i>Naineris laevigata</i> (Grube 1855)	AY040696
	<i>Orbinia bioreti</i> (Fauvel 1919)	AF448158
	<i>Orbinia latreillii</i> (Audouin & Milne Edwards 1833)	AF448159
	<i>Proscoplos cygnochaetus</i> Day 1954	AF448162
	<i>Protoaricia oerstedii</i> (Claparède 1864)	AF508123
	<i>Protoariciella uncinata</i> Hartmann-Schröder 1962	AF508124
	<i>Scoloplos armiger</i> (O.F. Müller 1776)	U50972
Oweniidae	<i>Owenia fusiformis</i> Delle Chiaje 1844	AF448126
Parergodrilidae	<i>Parergodrilus heideri</i> Reisinger 1925	AJ310504
	<i>Stygocapitella subterranea</i> Knöllner 1934 (<i>Sylt, Germany</i>)	AF412810
	<i>Stygocapitella subterranea</i> Knöllner 1934 (<i>GenBank</i>)	AJ310505
Phyllodocidae	<i>Eteone longa</i> (Fabricius 1780)	AF448155
	<i>Eteone picta</i> Quatrefages 1865 (<i>Roscoff, France</i>)	???????
	<i>Eulalia viridis</i> (Linnaeus 1767) (<i>Corcarneau, France</i>)	???????
Pisionidae	<i>Pisione remota</i> (Southern 1914) (<i>Cefalù, Sicilia, Italy</i>)	???????
Polygordiidae	<i>Polygordius appendicularis</i> (Fraipont 1887) (<i>Helgoland, Germany</i>)	???????
	<i>Polygordius</i> sp.	AF412809
Polynoidea	<i>Alentia gelatinosa</i> (Sars 1835) (<i>Cocardeau, France</i>)	???????
	<i>Harmthoë impar</i> Johnston 1839	U50968
Protodrilidae	<i>Protodrilus ciliatus</i> Jägersten 1952 (<i>Helgoland, Germany</i>)	???????
	<i>Protodrilus purpureus</i> (Schneider 1868)	AJ310506
Protodriloidae	<i>Protodriloides symbioticus</i> (Giard 1904)	AF508125
Questidae	<i>Questa paucibranchiata</i> Giere & Erséus 1998	AF209464
Sabellidae	<i>Sabella pavonina</i> Savigny 1820	U67144
Saccocirridae	<i>Saccocirrus papillocercus</i> Bobretzky 1871	AF412808
Scalibregmatidae	<i>Lipobranthus jeffreysii</i> (McIntosh 1869)	AF508120
	<i>Scalibregma inflatum</i> Rathke 1843	AF448163
	<i>Travisia forbesii</i> Johnston 1840	AF508127
Serpulidae	<i>Protula</i> sp.	U67142
Siboglinidae	<i>Escarpia spicata</i> (Jones 1985)	AF168741
	<i>Galathealinum brachiosum</i> (???????)	AF168738
	<i>Lamellibrachia barhami</i> Webb 1969	AF168742
	<i>Oasisia alvinae</i> Jones 1985	AF168743
	<i>Polybrachia</i> sp.	AF168739
	<i>Ridgeia piscesae</i> Jones 1985	X79877

	<i>Riftia pachyptila</i> Jones 1981	AF168745
	<i>Sclerolinum brattstromi</i> Webb 1964	AF315061
	<i>Siboglinum fiordicum</i> Webb 1963	X79876
	<i>Spirobrachia</i> sp.	AF168740
	<i>Tevnia jerichonana</i> Jones 1985	AF168746
Spionidae	<i>Aonides oxycephala</i> (Sars 1872)	AF448164
	<i>Malacocercus fuliginosus</i> (Claparède, 1868) (St. Efflau, France)	???????
	<i>Polydora ciliata</i> (Johnston 1838)	U50971
	<i>Pygospio elegans</i> Calparède 1863	U67143
	<i>Scolecopsis squamata</i> (O.F. Müller 1789)	AF448164
Syllidae	<i>Brania</i> sp. (unkown locality)	???????
	<i>Proceraea cornuta</i> (Agassiz 1862)	AF212179.2
	<i>Proceraea cornuta</i> (Agassiz 1862) (GenBank)	AF212179
Terebellidae	<i>Amphitritides gracilis</i> (Grube 1860)	AF508115
	<i>Lanice conchilega</i> (Pallas 1766)	X79873
	<i>Loimia medusa</i> (Savigny 1818)	AY040694
Arthropoda	<i>Artemia salina</i> (Linnaeus 1751)	X01723
	<i>Craterostigma tasmanianus</i> Pocock 1902	AF000774
	<i>Cylindroiulus punctatus</i> (Leach, 1815)	AF005448
	<i>Ixodes ricinus</i> (Linnaeus 1758)	Z74479
	<i>Lepidocyrtus paradoxus</i> Uzel, 1891	U61301
	<i>Lepisma saccharina</i> Linnaeus 1758	X89484
	<i>Limulus polyphemus</i> (Linnaeus 1758)	U91490
	<i>Nebalia</i> sp.	L81945
Brachiopoda	<i>Lingula anatina</i> Lamarck 1801	X81631
Byozoa	<i>Plumatella repens</i> (Linnaeus 1758)	U12649
Kamptozoa	<i>Barentsia hildegardae</i> Wasson 1997	AJ001734
Kinoryncha	<i>Pycnophyes kielensis</i> Zelinka 1928	U67997
Mollusca		
Bivalvia	<i>Argopecten irradians</i> (Lamarck, 1819)	L11265
	<i>Ostrea edulis</i> Linnaeus 1758	L49052
Caudofoveata	<i>Scutopus ventrolineatus</i> Salvini-Plawen, 1968	X91977
Gastropoda	<i>Anthosiphonaria sirius</i> (Pilsbry, 1894)	X98828
	<i>Aplysia</i> sp.	X94268
Polyplacophora	<i>Lepidochitona corrugata</i> (Reeve 1848)	X91975
Myzostomida	<i>Myzostoma cirriferum</i> F. Leuckart 1827	AF260585
	<i>Myzostoma fissum</i> von Graff 1884	AF260584
	<i>Myzostoma</i> sp.	AF123305
Nematomorpha	<i>Gordius aquaticus</i> Linnaeus 1758	X80233
Nemertini	<i>Prostoma eilhardi</i> (Montgomery, 1894)	U29494
Phoronida	<i>Phoronis australis</i> Haswell 1883	AF119079
Priapulida	<i>Priapulius caudatus</i> Lamarck 1816	AF025927
Sipunculida	<i>Aspidosiphon misakiensis</i> Ikeda 1904	AF119090

	<i>Phascolopsis gouldii</i> (Fisher 1950)	AF342796
	<i>Phascolosoma granulatum</i> Leuckart 1828	X79874
	<i>Themiste alutacea</i> Grube & Oersted 1858	AF119075
Platyhelminthes	<i>Zoogonoides viviparous</i> (Olsson 1868)	???????

^a the following sequences have been excluded from the analyses due to well funded evidence that they are not correct (Bleidorn *et al.* 2003a & b; Martin 2001; personal communication Arné Nygren; own observations): *Aphrodita aculeata* (Z83749), *Armandia maculata* (AY040681), *Capitella capitata* (U67323), Ctenodrilidae sp. n. (AJ310503), *Marphysa sanguinea* (AY040682), *Nereis virens* (Z83754), *Pectinaria regalis* (AY040698).