

Molecular systematics of polychaetes (Annelida)

Damhnait McHugh

Biology Department, Colgate University, Hamilton, NY 13346, USA

E-mail: dmchugh@mail.colgate.edu

Key words: Annelida, Polychaeta, phylogeny, 18S rRNA, root

Abstract

Some progress has been made in the field of molecular systematics of polychaetes over the past couple of years. In particular, phylogenetic analyses of sequence data from the 18S rRNA gene have included increasing numbers of taxa, and explicit hypothesis testing of sister-group relationships is being incorporated into the most recent studies. An increasing number of analyses of relationships within polychaete groups are being undertaken, with specific inferences being drawn regarding the evolution of characters such as reproductive mode. Despite this progress, the unanswered questions regarding annelid relationships outlined by McHugh (2000, p. 1881) remain: “what are the relationships among the polychaete annelids, what group is sister to the Clitellata, what extant group is most basal on the annelid tree, and what group is sister to Annelida?” Continued expansion of taxon sampling and further combined investigation of conserved nuclear coding genes, in conjunction with rRNA genes, may help to resolve some of these issues. Furthermore, only by expanding molecular systematic studies of polychaetes to analyses of nuclear coding genes for comprehensive taxon samples will it become clear whether the lack of basal-node resolution observed in analyses of 18S rRNA reflects a rapid radiation of the group, or is a feature associated with the 18S rRNA gene itself. Genomic-level data (e.g., mitochondrial gene order) may also be informative, and the cautious use of gene copies in phylogenetic analyses may point to a root of the annelid tree.

Introduction

Molecular phylogenetic analyses indicate that “Polychaeta” includes not only the polychaetes as we generally recognize them; derived positions of Clitellata, the siboglinids, and likely also the echiurids within a paraphyletic polychaete grade are supported to varying degrees (see McHugh, 2000; Martin, 2001; Rota et al., 2001; Siddall et al., 2001; Mallatt & Winchell, 2002; Struck et al., 2002a,b; Bleidorn et al., 2003). The most comprehensive phylogenetic analyses of the polychaetes based on morphological characters support a monophyletic “Polychaeta” that includes Siboglinidae (Rouse & Fauchald, 1997; Rouse, 1999; Rouse & Pleijel, 2001). However, the secondary absence of morphological characters may account for the exclusion from the polychaete clade of the clitellates (e.g., nuchal organs secondarily absent:

see Westheide et al., 1999; Purschke et al., 2000; Purschke, 2002) and the echiurids (segmentation secondarily absent: see Purschke et al., 2000; Hessling, 2002; Hessling & Westheide, 2002; Purschke, 2002) in those analyses.

Accepting paraphyly of “Polychaeta” on the basis of molecular phylogenetic analyses, the task then becomes deciphering the relationships among polychaete groups and identifying the basal-most extant annelid groups. Here, the focus is on the results of molecular analyses of polychaete relationships that have appeared since a recent synopsis of the molecular phylogeny of annelids (McHugh, 2000). Since that time, several broad studies of polychaete relationships based on 18S rRNA gene sequences have appeared, and some within-group analyses for a few polychaete families have been published. Despite this progress, lack of resolution of basal nodes is a recurring issue in molecular

studies of polychaete systematics, and rooting of the annelid tree remains an outstanding problem. Some avenues for future research that might help address these difficulties are suggested in the hopes that a more complete understanding of annelid evolution will soon be possible.

Polychaete phylogeny

A few wide-ranging analyses of polychaete relationships based on molecular sequence data have been published in the past couple of years (e.g., Rota et al., 2001; Struck et al., 2002a,b; Bleidorn et al., 2003). They are similar to most previous analyses of annelid phylogeny in that they use sequences of the 18S ribosomal RNA (18S rRNA) gene (e.g., Winnepeninckx et al., 1995, 1998; Kim et al., 1996; Moon et al., 1996; Eernisse, 1997). They differ, however, in that the taxon sampling has been expanded and the approach of testing hypotheses of specific sister-group relationships has been established (e.g., Struck et al., 2002b).

Rota et al. (2001) undertook a parsimony analysis of 18S rRNA sequences from 46 taxa (including 27 polychaetes, 11 clitellates, and an echiurid) to examine the relationships of the soil-dwelling, non-clitellate annelids, *Parergodrilus heideri* and *Hrabeiella periglandulata*. The parsimony analyses presented by Rota et al. (2001) were based on two different alignments of the 18S rRNA sequences (done using DCSE (De Rijk & De Wachter, 1993) or ClustalW (Thompson et al., 1994)). As with other analyses of 18S rRNA sequences for annelids, few relationships beyond sister groupings of terminal taxa are strongly supported in this study (Rota et al., 2001). Monophyly of Annelida (including polychaetes, Clitellata, and echiurids) is not supported, with three molluscan taxa and a sipunculan falling within the group. Furthermore, some expected groupings based on Rouse & Fauchald (1997) or Rouse (1999) are not recovered. While this study was relatively comprehensive in its sampling of taxa, only 20 of the approximately 80 family-designated groups of polychaetes were represented, and Rota et al. (2001) see this uneven representation as a possible explanation for the poor resolution of basal nodes.

The analyses did support a sister relationship between *Parergodrilus heideri* and *Stygocapitella*

subterranea (Bootstrap proportion (BP) > 78), thereby affirming monophyly of Parerogodrilidae; however, the position of Parerogodrilidae remains unresolved (Rota et al., 2001). *Hrabeiella periglandulata* apparently represents an independent case of the evolution of terrestriality in a polychaete; it is sister to the meiofaunal freshwater group, Aeolosomatidae, in both analyses although there is only weak support for this relationship (Rota et al., 2001).

In a similar study, Struck et al. (2002a) examined the phylogenetic position of Parerogodrilidae (*Stygocapitella subterranea*) and Aeolosomatidae (*Aeolosoma* sp.), using 18S rRNA sequences from 49 annelids (including 40 polychaetes and 9 clitellates), and designating molluscs and arthropods as outgroups. Unlike Rota et al. (2001), Struck et al. (2002a) explored various weighting schemes based on detailed analyses of the alignment prior to tree construction using maximum parsimony, distance, and maximum likelihood methods, but none resolved the position of these clitellate-like groups. However, neither *Stygocapitella subterranea* nor *Aeolosoma* sp. appeared within or as sister to the monophyletic clitellate clade on any of the trees. It would have been interesting to see some specific hypothesis testing in the studies by both Rota et al. (2001) and Struck et al. (2002a), e.g., Shimodaira–Hasegawa tests of significance for Bunke's 1967 hypothesis of Parerogodrilidae and Aeolosomatidae as sister taxa.

In both Rota et al. (2001) and Struck et al. (2002a), a sister relationship between Questidae and Orbiniidae was strongly supported (BP = 100), which argues against an early hypothesis of a questid–clitellate relationship. Monophyly of Clitellata, Hirudinea, Dinophilidae and Spionidae was also well supported, and a monophyletic Eunicida (*sensu* Rouse & Fauchald, 1997) was weakly supported in one maximum parsimony analysis (Struck et al., 2002a). On the other hand, some well-supported unexpected groupings indicated by the analyses suggest the need to confirm the identification of sequences deposited in public databases. For example, the strongly supported grouping of *Aphrodita aculeata* with *Neanthes virens*, to the exclusion of other species of *Neanthes* and other representatives of Aphroditidae warrants caution (Martin, 2001; Rota et al., 2001; Struck et al., 2002a; Bleidorn et al., 2003).

Struck et al. (2002b) focused on Eunicida and addressed a specific question regarding the evolution of small body size and simple body plans in some dorvilleids and in Dinophilidae: Did progenesis evolve more than once independently in these groups? Some authors consider Dinophilidae as a separate family (e.g., Orensanz, 1990), while others include them within Dorvilleidae (e.g., Rouse & Pleijel, 2001). Struck et al. (2002b) used maximum parsimony, maximum likelihood and minimum evolution tree-building methods to analyze 18S rRNA sequences from 43 taxa (including 31 polychaeta and 9 clitellates). The jawless species, *Parapodrilus psammophilus* fell within a dorvilleid clade (BP > 84%), indicating that it arose by progenesis from a dorvilleid ancestor. Kishino–Hasegawa tests rejected the hypothesis of a common origin for *P. psammophilus* and the small, simple-bodied Dinophilidae ($p < 0.05$); however, Templeton tests did not ($p > 0.05$). Another dorvilleid species, *Pettiboneia uriciensis*, was more closely related to Lumbrineridae than Dorvilleidae in all analyses, but non-parametric tests did not support rejection of dorvilleid monophyly on this basis (Struck et al., 2002b). Paraphyly of Eunicidae, with the inclusion of onuphid species in the eunicid clade, is perhaps not an unexpected result of the analysis (Struck et al., 2002b), given the sharing of jaw asymmetry and aragonite mineralization by the two groups (see Rouse & Pleijel, 2001). Interestingly, no clear morphological synapomorphy has been identified for either the Eunicidae or the Dorvilleidae (Struck et al., 2002b). Monophyly of Dinophilidae was strongly supported (BP = 100), but its position relative to the eunicidan groups in the analysis was unresolved.

Bleidorn et al. (2003) conducted a phylogenetic analysis of relationships among sedentary polychaeta using 18S rRNA gene sequences from 70 taxa (including 47 polychaeta and four clitellates) (Fig. 1). As with other 18S rRNA analyses, monophyly of several well-established polychaete groups for which two or more taxa were included was supported (Cirratulidae, Opheliidae, Orbiniidae, Spionidae, Siboglinidae), irrespective of the tree-building method used in the analysis (maximum parsimony or maximum likelihood). As with Rota et al. (2001) and Struck et al. (2002a), a close relationship between questids and orbiniids is well supported in the Bleidorn et al. (2003) study.

Furthermore, a sister relationship between a capitellid and the two echiurids (BP = 91) indicates additional support for a derived position of the echiurids within a polychaete grade (McHugh, 1997, 2000). While the study by Bleidorn et al. (2003) includes the most annelid taxa in a molecular analysis to date, the taxon sampling is very uneven. For example, 10 siboglinids are included, but only one terebellid. The authors acknowledge the need to increase taxon sampling and suggest that it may help to resolve polychaete relationships. Alternatively, as has been done by several others (see McHugh, 2000; Martin, 2001; Rota et al., 2001; Struck et al., 2002a,b), Bleidorn et al. (2003) suggest that a rapid radiation may explain the lack of resolution of basal annelid nodes in phylogenetic analyses of 18S rRNA sequences. This explanation will be supported if multiple independent gene sequences for extensive taxonomic samples also yield poor resolution in phylogenetic analyses.

Phylogenetic relationships within polychaete groups

In addition to broad scale analyses of annelid relationships, several recent studies have focused on relationships within and among particular polychaete groups. In several cases, combined analyses of both molecular and morphological data are used to examine these relationships. For example, Rousset et al. (2003) used sequences of 28S rRNA (D1 domain) and 52 morphological characters to examine the phylogenetic position of Alvinellidae, a group of polychaetes known only from hydrothermal vents, and originally classified as a subfamily of Ampharetidae (Desbruyères & Laubier, 1980). With a maximum parsimony analysis of just 13 taxa in their molecular data set, Rousset et al. (2003) found a trichobranchid as sister to one of the two alvinellids in the analysis; a combined analysis of 16 taxa (3 lacking molecular data) showed weak support for a sister group relationship between Alvinellidae and the trichobranchid, as well as monophyly of Terebellidae and Ampharetidae. However, the very limited taxon sampling leaves the issue of alvinellid relationships unresolved.

Focussing on the same taxonomic groups, Colgan et al. (2001) analyzed the phylogenetic relationships of terebellomorph polychaetes using a combined data set based on three nuclear genes

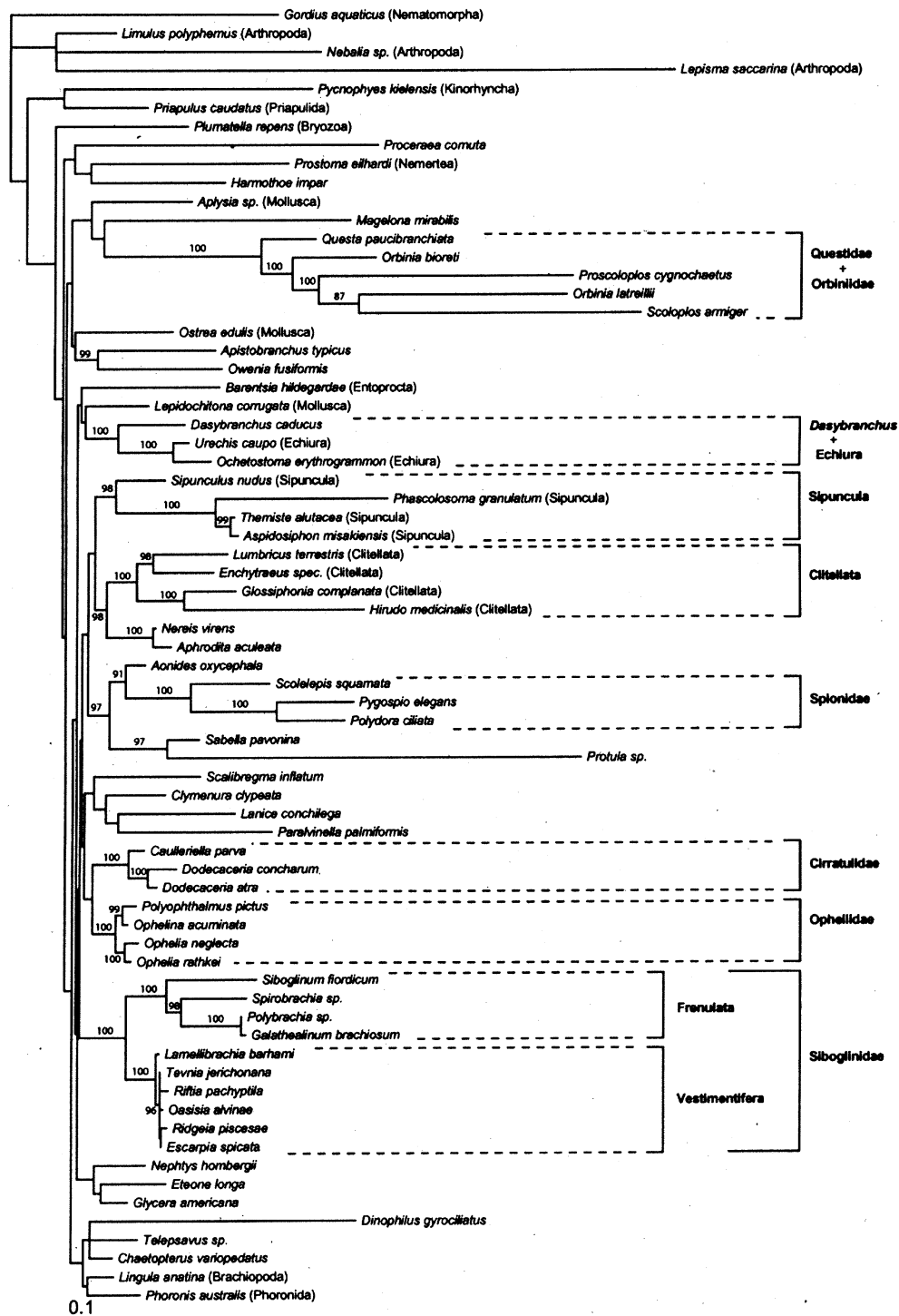


Figure 1. Results of a maximum likelihood analysis of 18S rRNA sequences from 70 taxa (from Bleidorn et al., 2003). Numbers above the nodes represent posterior probabilities from a Bayesian analysis of selected groups. Monophyly of the Clitellata, Spionidae, Cirratulidae, Opheliidae, Orbiniidae, and Siboglinidae is supported; a sister-group relationship between Questidae and Orbiniidae is also supported. Polychaetes do not form a monophyletic group and none of the orders of Fauchald (1977) or the clades proposed by Rouse & Fauchald (1997) are supported.

(Histone H3, U2 snRNA, and two regions of 28S rRNA) and the mitochondrial gene, cytochrome oxidase I (COI). Using a single clitellate outgroup to root the tree, Colgan et al. (2001) restricted their study of the data to maximum parsimony analysis of equally weighted characters. The topology resulting from analysis of all characters available for 25 taxa supported monophyly of Cirratulidae, but showed several other well-established groups to be polyphyletic (Terebellidae, Alvinellidae, Ampharetidae, Trichobranchidae). Despite the analysis of sequence data from multiple genes, no nodes on the tree below terminal-taxon sister relationships are supported by bootstrap proportions greater than 50%. In addition to the limited taxon sampling, these results likely also reflect the fact that some of the genes chosen lack the phylogenetic signal needed to resolve the relationships among annelid groups (e.g., Histone H3 (see McHugh, 2000) and COI (Nylander et al., 1999)).

In a recent study of another group of polychaeta, Nereidiformia, Dahlgren et al. (2000) undertook a parsimony analysis of COI sequences for nine taxa representing Hesionidae, Pilargidae, Nereididae, Chrysopetalidae, and Pisionidae. All but one node on the resulting topology was weakly supported (BP < 50%). Combining their molecular data with previously analyzed morphological data for 13 taxa, Dahlgren et al. (2000) hypothesized a sister relationship between the two chrysopetelids and the nereid, albeit with weak support (BP = 52%); the Hesionidae was polyphyletic. Again, this study is so limited in the scope of taxonomic sampling that few conclusions can be drawn from the results.

The body of molecular phylogenetic studies of polychaete relationships that move beyond tree-building to infer the evolution of features such as reproductive mode, feeding habit, larval development mode, heterochrony, etc. is building slowly. One example of such a study focuses on the relationships of species of *Ophryotrocha*, a group of small worms that displays a full range of reproductive modes from sequential hermaphroditism to gonochorism (Dahlgren et al., 2001). Using 16S rRNA gene sequence data, Dahlgren et al. (2001) carried out parsimony, distance, and maximum likelihood analyses to reconstruct relationships among 22 taxa, including 18 assigned to *Ophryotrocha*. While the basal rela-

tionships among the ingroup taxa were not resolved, the resulting trees allowed the inference that the reproductive mode of *Ophryotrocha* changed once and that simultaneous hermaphroditism is the reproductive mode of the immediate ancestor to the sequential hermaphroditic *Ophryotrocha* clade (Dahlgren et al., 2001).

Schulze et al. (2000) were also interested in the evolution of reproductive modes in a polychaete group. In this case, the authors used COI sequences to hypothesize relationships among populations of *Streblospio*, a polychaete group known to exhibit poecilogony, i.e., the presence of more than one developmental mode within a species. Maximum parsimony and distance analyses of 88 sequences from individuals of *S. benedicti* and *S. gynobranchiata* along the east and west coasts of North America supports paraphyly of *S. benedicti* with respect to *S. gynobranchiata*, and also corroborates poecilogony in this group (Schulze et al., 2000). Using molecular clock estimates of divergence times, Schulze et al. (2000) proposed that divergence times among clades of *Streblospio* are recent and thus the evolutionary changes in larval developmental modes have been rapid.

In a recent study of syllid polychaetes, Nygren & Sundberg (2003) analyzed 16S rRNA and 18S rRNA gene sequences for 47 taxa to reconstruct relationships and infer patterns of change in epitokous reproduction in the group. Irrespective of the tree-building method they used (maximum parsimony, maximum likelihood, or Bayesian inference), character reconstruction on the resultant trees supported epigamy as the ancestral reproductive mode in Syllidae, with the independent evolution of schizogamy in Syllinae and Autolytinae (Nygren & Sundberg, in press). Because the relationships among three clades of the syllid *Autolytus* were unresolved, the evolution of reproductive modes within Autolytinae remains ambiguous.

Rooting the Annelida

In phylogenetic reconstruction of any organismal group, an outgroup is usually used to root the tree. The outgroup is often the sister group of the ingroup, or multiple outgroups may be used. Unfortunately, there is no undisputed sister group known for Annelida, and inclusion of

multiple presumed outgroups has resulted in their inclusion within Annelida in the molecular analyses undertaken so far. Rouse & Pleijel (2001) highlight the rooting of the polychaete tree as a major problem in annelid systematics, and discuss the alternative hypotheses that have been proposed and their implications for the body form of the basal annelids. One possible solution to the lack of an obvious sister group to root the Annelida tree comes from the molecular phenomenon of gene duplication.

If a gene duplicated prior to the divergence of a group of extant organisms, then a combined analysis of both gene copies for the same taxa will produce an unrooted network of two subtrees that basically mirror each other (Page & Holmes, 1998; Mathews & Donoghue, 1999) (Fig. 2a). By placing the root of this network at the point at which the fewest gene duplications are required to explain the data, the root of each subtree will be identified (Fig. 2b, c). Thus, no outgroup is required. This approach has been used to identify the root of the tree of life (Doolittle & Brown, 1995) and the root of the angiosperms (Mathews & Donoghue, 1999). It is

possible that the root of annelid subtrees for copies of a duplicated gene can also be identified. The first task is to recognize a gene duplication event in the line leading to the Annelida. Some potential candidate genes include members of the actin (e.g., Carlini et al., 2000), tyrosine kinase (e.g., Miyata & Suga, 2001), and Delta/Serrate/LAG-2 (e.g., Lissemore & Starmer, 1999) gene families, among others. Extensive preliminary work would be required to determine which, if any genes, are appropriate. There are many possible pitfalls to rooting trees using duplicated genes that must be recognized and avoided if it is to be successful. For example, gene conversion, recombination between the two gene copies, and very unequal evolutionary rates of the two gene copies could yield misleading data. However, given the lack of alternatives, it is worth pursuing this method, albeit cautiously.

Other future directions

Several recent molecular studies of polychaete relationships have used maximum parsimony to

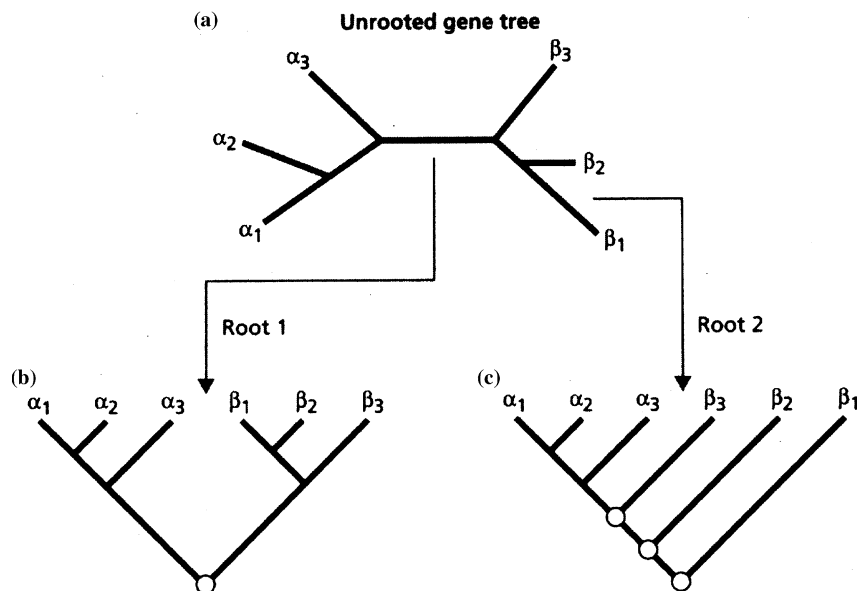


Figure 2. Using gene duplications to root a tree (from Page & Holmes, 1998). By minimizing the number of gene duplications that must be invoked to explain the relationships among sequences from two copies of a gene for the same taxa, the root of the tree for those taxa can be identified. (a) Simultaneous analysis of two gene copies (α and β) from three species (1, 2, and 3) results in an unrooted network of the α_1 , α_2 , α_3 , β_1 , β_2 , and β_3 sequences. (b) Placing the root of the tree on the branch that separates the α and β gene sequences requires one duplication event (denoted by open circle). (c) Placing the root anywhere else requires more than one duplication event (denoted by three open circles in this case).

analyze equally weighted characters (e.g., Colgan et al., 2001; Dahlgren et al., 2001; Rota et al., 2001). This restriction is unfortunate and difficult to justify, because it overlooks the great deal that is known about molecular evolution, which can be incorporated as explicit substitution models in maximum likelihood analyses or as weighting schemes in maximum parsimony analyses, for example. Substitution models and weighting schemes are based on evidence for different rates of substitution along a molecule (e.g., stems versus loops in rRNA genes), and for different rates of accrual of distinct character changes (e.g., transversions versus transitions (Struck et al., 2002a; Bleidorn et al., 2003) (see Page & Holmes, 1998). Future molecular systematic studies of polychaete relationships should fully explore the data by integrating such models and schemes.

Beyond full analyses of molecular data, critical assessment of tree topology is another important step in any systematic study. Critical assessment of node support on trees of polychaete relationships is usually done by getting bootstrap proportions for each node. Basically, bootstrap proportions represent the percentage of 1000 analyses of pseudoreplicates of the original data matrix that support nodes also found in the tree based on the analysis of the original data matrix. These proportions are easy to evaluate; they indicate how well the nodes on a tree are supported by the data matrix. Bootstrap proportions of greater than 70 are associated with well-supported nodes in simulations (Hillis & Bull, 1993). Another measure of node support has been used in some of the most recent studies of polychaete relationships, i.e., posterior probabilities from Bayesian inference (e.g., Bleidorn et al., 2003). However, as mentioned in Bleidorn et al. (2003), a number of authors have found that posterior probabilities do not correlate well with bootstrap proportions (e.g., in Leaché & Reeder, 2002, posterior probabilities of 95% were found for nodes with bootstrap proportions less than 50). Thus, high Bayesian posterior probability values must be interpreted with caution (see Huelsenbeck et al., 2002; Suzuki et al., 2002 for detailed discussion).

As of August 2002, there were only 2103 Annelida sequences available on GenBank, of which approximately 540 were for polychaetes, and almost half of these were ribosomal gene se-

quences. The heavy reliance on 18S rRNA sequences for molecular analyses of polychaete relationships continues, because it allows authors to build on the richest molecular database thus far for the annelids. A few sister group relationships are supported in the recent 18S rRNA analyses, e.g., Orbinidae + Questidae (Rota et al., 2001; Struck et al., 2002a; Bleidorn et al., in press), but beyond that analyses based on these data have been inconclusive or contradictory regarding polychaete relationships and monophyly of Annelida.

Further exploration of the phylogenetic usefulness of 28S rRNA, a large-subunit rRNA gene, especially in combination with the small-subunit 18S rRNA gene, for resolution of annelid relationships is warranted. Mallatt & Winchell (2002) recently presented combined analyses of these two genes for protostomes and showed that together they provide higher support for Ecdysozoa and Lophotrochozoa than 18S rRNA alone. In addition, the combined data set supports a sister relationship between the polychaete and the echiurid in the analyses, and monophyly of Annelida. While these analyses included very few taxa (16), they nonetheless illustrate the benefits of combining 28S rRNA and 18S rRNA sequences, i.e., 28S rRNA can be easily sequenced and addition of 28S rRNA adds phylogenetic signal (Mallatt & Winchell, 2002).

Both 18S rRNA and 28S rRNA, as structural genes, present some difficulties for alignment of sequences from diverse taxa. In most cases, authors have used the secondary structure model of 18S rRNA to align annelid sequences and/or they have "manually edited" the alignments; ambiguous regions in the alignments are usually excluded from the analyses (e.g., Colgan et al., 2001; Struck et al., 2002b). It is difficult to avoid the subjectivity involved in doing this, and the analyses cannot be replicated. These alignments (and those used in any published analysis) should be made immediately and publicly available to all readers for further investigation. "Alignments available from the author" is not satisfactory and the deposition of all published alignments in the EMBL ALIGN (as done by Rota et al., 2001) or TREEBASE databases is urged. Given the likelihood that many annelid relationships will remain unresolved until combined analyses of

multiple genes are undertaken, it is particularly important to facilitate the use of all published data in future studies.

Several highly conserved nuclear protein-coding genes have been used in previous analyses of annelid relationships. These genes have the advantage of being easily aligned, but in some cases they have not been very useful. For example, given the highly conserved nature of the Histone H3 gene, it does not provide many parsimony informative sites for analysis of polychaete relationships (McHugh, 2000). Previous studies that include multiple data sets include those by Brown et al. (1999) and Colgan et al. (2001), both of which use the nuclear genes U2 snRNA, Histone H3, and 28S, and neither of which yielded resolved, well-supported basal nodes. There was also a lack of support for basal nodes in a combined analysis of elongation factor-1 α , U2 snRNA, Histone H3, and 28S sequences from terebellidan, sabellidan, and spionidan polychaetes (McHugh, 2001). However, all of these studies were limited to 25 or fewer polychaeta.

Additional genes that are potentially informative for polychaete systematic studies include enolase, Na⁺, K⁺-ATPase (Friedlander et al., 1994), and myosin heavy chain type II. The latter of these has recently been used to hypothesize a basal bilaterian position of the small and simple acoel and nemertodermatid flatworms (Ruiz-Trillo et al., 2002). The polychaete, two clitellates, and echiurid included in that analysis formed a weakly supported monophyletic annelid clade; the availability of these sequences allows the design of annelid-specific primers for the myosin heavy chain type II gene. RNA polymerase II and elongation factor-2, as well as elongation factor-1 α are other nuclear coding genes that are currently being sequenced for more than 100 polychaetes as part of an ongoing collaborative project on annelid evolution (see <https://www.fastlane.nsf.gov/servlet/showaward?award=012064>). This project also includes analysis of mitochondrial gene order and the complete mitochondrial genome sequences for the same taxa. Mitochondrial genomic-level data support inclusion of the siboglinids within Annelida (Boore & Brown, 2000), and indicate that Annelida and Sipuncula form a monophyletic clade to the exclusion of the Mollusca and Brachiopoda (Boore & Staton, 2002).

From the relatively few molecular studies of polychaete systematics, it appears that a combination of sequence data from several conserved genes, genomic-level data, and morphological characters for a sample of taxa that fully represents the great diversity of polychaetes and other annelids may be necessary for a robust, stable phylogenetic hypothesis for Annelida. While this may seem insurmountable, phylogenetic analyses at lower taxonomic levels can provide a good start (Bleidorn et al., in press), provided they draw on the same molecular data that can ultimately be combined for a complete analysis of annelid relationships.

Acknowledgements

I am very grateful to Günter Purschke (Universität Osnabrück) and Thomas Bartolomeus (Freie Universität Berlin) for inviting me to participate in the symposium on “Morphology, Molecules, Evolution and Phylogeny in Polychaeta and Related Taxa” at Haus Ohrbeck in Osnabrück, Germany. I appreciate the helpful comments of two anonymous reviewers of this manuscript. I also thank Barbara Hoopes (Colgate University) for making it possible for me to attend the symposium, Nancy Schult (Colgate University) for her comments on the manuscript, and Laura Katz (Smith College) for discussion of gene duplication. I gratefully acknowledge the financial support of the US National Science Foundation (Grant No. 0120646 EAR BIOCOMPLEXITY).

References

- Bleidorn, C., L. Vogt & T. Bartolomeus, 2003. A contribution to sedentary polychaete phylogeny using 18S rRNA sequence data. *Journal of Zoological Systematics and Evolutionary Research* 41: 186–195.
- Boore, J. L. & W. M. Brown, 2000. Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate the Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. *Molecular Biology and Evolution* 17: 87–106.
- Boore, J. L. & J. L. Staton, 2002. The mitochondrial genome of the sipunculid *Phascolopsis gouldii* supports its association with Annelida rather than Mollusca. *Molecular Biology and Evolution* 19: 127–137.
- Brown, S., G. Rouse, P. Hutchings & D. Colgan, 1999. Assessing the usefulness of histone H3, U2 snRNA and 28S

- rDNA in analyses of polychaete relationships. *Australian Journal of Zoology* 47: 499–516.
- Carlini, D. B., K. S. Reece & J. E. Graves, 2000. Actin gene family evolution and the phylogeny of coleoid cephalopods (Mollusca: Cephalopoda). *Molecular Biology and Evolution* 17: 1353–1379.
- Colgan, D. J., P. A. Hutchings & S. Brown, 2001. Phylogenetic relationships within the Terebellomorpha. *Journal of the Marine Biological Association of the United Kingdom* 81: 765–773.
- Dahlgren, T. G., B. Akesson, C. Schander, K. M. Halanych & P. Sundberg, 2001. Molecular phylogeny of the model annelid *Ophryotrocha*. *The Biological Bulletin* 201: 193–203.
- Dahlgren, T. G., J. Lundberg, F. Pleijel & P. Sundberg, 2000. Morphological and molecular evidence of the phylogeny of Nereidiform polychaetes (Annelida). *Journal of Zoological Systematics and Evolutionary Research* 38: 249–253.
- De Rijk, P. & R. De Wachter, 1993. DCSE, an interactive tool for sequence alignment and secondary structure research. *Computational Applications in Bioscience* 9: 735–740.
- Desbruyères, D. & L. Laubier, 1980. *Alvinella pompejana* g. et sp. nov., Ampharetidae aberrant des sources hydrothermales de la ride Est-Pacifique. *Oceanologica Acta* 3: 267–274.
- Doolittle, W. R. & J. R. Brown, 1995. Root of the universal tree of life based on ancient aminoacyl-transfer-RNA synthetase gene duplications. *Proceedings of the National Academy of Sciences of the United States of America* 92: 2441–2445.
- Eernisse, D. J., 1997. Arthropod and annelid relationships re-examined. In Fortey, R. A. & R. H. Thomas (eds), *Arthropod Relationships, Systematics*. Association Special Volume Series 55. Chapman and Hall, London: 43–56.
- Fauchald, K., 1977. The polychaete worms. Definitions and keys to the orders, families and genera. *Natural History Museum Los Angeles County, Science Series* 28: 1–188.
- Friedlander, T. P., J. C. Regier & C. Mitter, 1994. Phylogenetic information content of five nuclear gene sequences in animals: initial assessment of character sets from concordance and divergence studies. *Systematic Biology* 43: 511–525.
- Hessling, R., 2002. Metameric organisation of the nervous system in developmental stages of *Urechis caupo* (Echiura) and its phylogenetic implications. *Zoomorphology* 121: 221–234.
- Hessling, R. & W. Westheide, 2002. Are Echiura derived from a segmented ancestor? Immunohistochemical analysis of the nervous system in developmental stages of *Bonellia viridis*. *Journal of Morphology* 252: 100–113.
- Huelsbeck, J. P., B. Larget, R. E. Miller & F. Ronquist, 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* 51: 673–688.
- Kim, C. B., S. -Y. Moon, S. Gelder & W. Kim, 1996. Phylogenetic relationships of annelids, molluscs, and arthropods evidenced from molecules and morphology. *Journal of Molecular Evolution* 43: 207–215.
- Leaché, A. D. & T. W. Reeder, 2002. Molecular systematics of the Eastern Fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology* 51: 44–68.
- Lissemore, J. L. & W. T. Starmer, 1999. Phylogenetic analysis of vertebrate and invertebrate Delta/Serrate/LAG-2 (DSL) proteins. *Molecular Phylogenetics and Evolution* 11: 308–319.
- Mallatt, J. & C. J. Winchell, 2002. Testing the new animal phylogeny: first use of combined large-subunit and small-subunit rRNA gene sequences to classify the protostomes. *Molecular Biology and Evolution* 19: 289–301.
- Martin, P., 2001. On the origin of the Hirudinea and the demise of the Oligochaeta. *Proceedings of the Royal Society of London B* 268: 1089–1098.
- Mathews, S. & M. J. Donoghue, 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286: 947–950.
- McHugh, D., 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. *Proceedings of the National Academy of Sciences of the United States of America* 94: 8006–8009.
- McHugh, D., 2000. Molecular phylogeny of the Annelida. *Canadian Journal of Zoology* 78: 1873–1884.
- McHugh, D., 2001. Molecular phylogenetic analyses indicate a rapid radiation of polychaete annelids. *American Zoologist* 41: 1520–1521.
- Miyata, T. & H. Suga, 2001. Divergence pattern of animal gene families and relationship with the Cambrian explosion. *BioEssays* 23: 1018–1027.
- Moon, S. Y., C. B. Kim, S. R. Gelder & W. Kim, 1996. Phylogenetic positions of the aberrant branchiobdellidans and aphanoneurans within the Annelida as derived from 18S ribosomal RNA gene sequences. *Hydrobiologia* 324: 229–236.
- Nygren, A. & P. Sundberg, 2003. Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida). *Molecular Phylogenetics and Evolution* 29: 235–249.
- Nylander, J. A. A., C. Erseus & M. Kallersjo, 1999. A test of monophyly of the gutless Phalloporinae (Oligochaeta, Tubificidae) and the use of a 573-bp region of the mitochondrial cytochrome oxidase I gene in analysis of annelid phylogeny. *Zoologica Scripta* 28: 305–313.
- Orensanz, J. M., 1990. The eunicemorph polychaete annelids from Antarctic and Subantarctic Seas. *Antarctic Research Series* 52: 1–183.
- Page, R. D. M. & E. C. Holmes, 1998. *Molecular Evolution. A Phylogenetic Approach*. Blackwell Science, Oxford, 346 pp.
- Purschke, G., 2002. On the ground pattern of Annelida. *Organisms, Diversity and Evolution* 2: 181–196.
- Purschke, G., R. Hessling & W. Westheide, 2000. The phylogenetic position of the Clitellata and the Echiura – on the problematic assessment of absent characters. *Journal of Zoological Systematics and Evolutionary Research* 38: 165–173.
- Rota, E., P. Martin & C. Erseus, 2001. Soil-dwelling polychaetes: enigmatic as ever? Some hints on the phylogenetic relationships as suggested by a maximum parsimony analysis of 18S rRNA gene sequences. *Contributions to Zoology* 70: 127–138.
- Rouse, G. W., 1999. Trochophore concepts: ciliary bands and the evolution of larvae in spiralian Metazoa. *Biological Journal of the Linnean Society* 66: 411–464.
- Rouse G. W. & K. Fauchald, 1997. Cladistics and polychaetes. *Zoologica Scripta* 26: 139–204.

- Rouse, G. W. & F. Pleijel. 2001. *Polychaetes*. Oxford University Press, New York, 354 pp.
- Rousset, V., G. W. Rouse, J.-P. Féral, D. Desbruyères & F. Pleijel. 2003. Molecular and morphological evidence of Alvinellidae relationships (Terebelliformia, Polychaeta, Annelida). *Zoologica Scripta* 32: 185–197.
- Ruiz-Trillo, I., J. Paps, M. Loukota, C. Ribera, U. Jondelius, J. Bagaña & M. Riutort. 2002. A phylogenetic analysis of myosin heavy chain type II sequences corroborates that Acoela and Nemertodermatida are basal bilaterians. *Proceedings of the National Academy of Sciences of the United States of America* 99: 11246–11251.
- Schulze, S. R., S. A. Rice, J. L. Simon & S. A. Karl. 2000. Evolution of poecilogony and the biogeography of North American populations of the polychaete *Streblospio*. *Evolution* 54: 1247–1259.
- Siddall, M. E., K. Apakupakul, E. M. Burreson, K. A. Coates, C. Erséus, S. R. Gelder, M. Källersjö & H. Trapido-Rosenthal. 2001. Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of oligochaetes. *Molecular Phylogenetics and Evolution* 21: 346–351.
- Struck, T. H., R. Hessling & G. Purschke. 2002a. The phylogenetic position of the Aeolosomatidae and Parergodrilidae, two enigmatic oligochaete-like taxa of the “Polychaeta”, based on molecular data from 18S rDNA sequences. *Journal of Zoological Systematics and Evolutionary Research* 40: 155–163.
- Struck, T. H., W. Westheide & G. Purschke. 2002b. Progenesis in Eunicida (“Polychaeta,” Annelida) – separate evolutionary events? Evidence from molecular data. *Molecular Phylogenetics and Evolution* 25: 190–199.
- Suzuki, Y., G. V. Glazko & M. Nei. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences of the United States of America* 99: 16138–16143.
- Thompson, J. D., D. G. Higgins & T. J. Gibson. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.
- Westheide, W., D. McHugh, G. Purschke & G. Rouse. 1999. Systematization of the Annelida: different approaches. *Hydrobiologia* 402: 291–307.
- Winnepenninckx, B., T. Backeljau & R. De Wachter. 1995. Phylogeny of protostome worms derived from 18S rRNA sequences. *Molecular Biology and Evolution* 12: 641–649.
- Winnepenninckx, B. M. H., Y. Van de Peer & T. Backeljau. 1998. Metazoan relationships on the basis of 18S rRNA sequences: A few years later... *American Zoologist* 38: 888–906.