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Phylogeny of Eunicida (Annelida) and Exploring Data Congruence Using a Partition Addition Bootstrap Alteration (PABA) Approach

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Abstract.—Even though relationships within Annelida are poorly understood, Eunicida is one of only a few major annelid lineages well supported by morphology. The seven recognized eunicid families possess sclerotized jaws that include mandibles and a maxillary apparatus. The maxillary apparatuses vary in shape and number of elements, and three main types are recognized in extant taxa: ctenognath, labidognath, and prionognath. Ctenognath jaws are usually considered to represent the plesiomorphic state of Eunicida, whereas taxa with labidognath and prionognath are thought to form a derived monophyletic assemblage. However, this hypothesis has never been tested in a statistical framework even though it holds considerable importance for understanding annelid phylogeny and possibly lophotrochozoan evolution because Eunicida has the best annelid fossil record. Therefore, we used maximum likelihood and Bayesian inference approaches to reconstruct Eunicida phylogeny using sequence data from nuclear 18S and 28S rDNA genes and mitochondrial 16S rDNA and cytochrome *c* oxidase subunit I genes. Additionally, we conducted three different tests to investigate suitability of combining data sets. Incongruence length difference (ILD) and Shimodaira-Hasegawa (SH) test comparisons of resultant trees under different data partitions have been widely used previously but do not give a good indication as to which nodes may be causing the conflict. Thus, we developed a partition addition bootstrap alteration (PABA) approach that evaluates congruence or conflict for any given node by determining how bootstrap scores are altered when different data partitions are added. PABA shows the contribution of each partition to the phylogeny obtained in the combined analysis. Generally, the ILD test performed worse than the other approaches in detecting incongruence. Both PABA and the SH approach indicated the 28S and COI data sets add conflicting signal, but PABA is more informative for elucidating which data partition may be misleading at a given node. All our analyses indicate that the monophyly of the labidognath/prionognath taxa and even a labidognath clade (i.e., a "Eunicidae"/Onuphidae/Lumbrineridae clade) is significantly rejected. We show that the definition of both the labidognath and ctenognath jaw type does not address adequately the variation within Eunicida and thus misleads our current evolutionary understanding. Based on the presented results a symmetric maxillary apparatus with a carrier and four to six maxillae is most likely the plesiomorphic condition for Eunicida. [COI; conflicting data; fossil record; ILD; Jaw Evolution; molecular phylogeny; rDNA; SH test.]

Eunicida is a diverse group of annelids found from intertidal to abyssal depths and is characterized by possessing a set of sclerotized jaws. The clade comprises seven recognized annelid families ("Dorvilleidae," "Eunicidae," Hartmaniellidae, Histriobdellidae, Lumbrineridae, Oenonidae, and Onuphidae) and contains over 900 nominal species in 100 genera (Rouse and Pleijel, 2001). Although the term Eunicida was not used until the 1960s, species of all families, except Hartmaniellidae, were described in the late 18th and early 19th century, and thus members of Eunicida have a long scientific history. Ranging in size from the largest known polychaetes (*Eunice*, up to 6 m in length) to small interstitial forms (e.g., *Neotenotrocha sterreri* at 250 μm, Eibye-Jacobsen and Kristensen, 1994), the group displays a wide variety of life history strategies. Some eunicids are commercially or culturally important. For example, epitokes (i.e., a sexually mature stage filled with gametes) of *Palola viridis* ("Eunicidae") are collected as food by Polynesian natives and *Diopatra aciculata* (Onuphidae) supports a substantial commercial fishery as bait.

Within Annelida, our understanding of phylogeny is wanting and there is currently strong support for only a few nodes deep in the annelid tree (e.g., Clitellata: see Purschke, 2002; Struck et al., 2002a). Interestingly, Eunicida, which is usually recognized as an order, is one of the best morphologically defined higher taxonomic groups. The primary synapomorphic character defining the clade is a ventral pharyngeal organ with a complex jaw apparatus consisting of mandibles and rows of maxillary pieces with or without a carrier (e.g., Orensanz, 1990). This jaw apparatus has only been lost in few interstitial dorvilleid species (e.g., *Parapodrilus*, Westheide, 1965) and the parasitic oenonid *Biborin* (see Hilbig, 1995). In their cladistic analysis, Rouse and Fauchald (1997) also recovered monophyly of the eunicid taxa within Aciculata. However, in their final conclusion they extended Eunicida to include the sister taxon of these taxa, Amphinomida. However, this relationship is only based on the synapomorphic possession of a ventral hypertrophied stomodaeum and only obtained in analyses with absence/presence coding schemes using either successive or a priori weighting. In the multistate as well as unweighted analyses, Amphinomida is within Aciculata either part of a polytomy or the most basal taxon. Furthermore, the different stomodael organs are very likely not homologous (see Purschke and Tzetlin, 1996). Therefore, the inclusion of Amphinomida within Eunicida, either as sister taxon to all other taxa or as highly derived due to the lack of the jaw apparatus, is not supported and we will follow others (Rouse and Pleijel, 2001) in using the well-supported traditional clade. Nevertheless, an amphinomid was included as an outgroup taxon.

The jaw apparatus has been the main character used to infer phylogenetic history within Eunicida. Although the mandibles are relatively similar between taxa, the shape and complexity of the maxillary apparatus have been

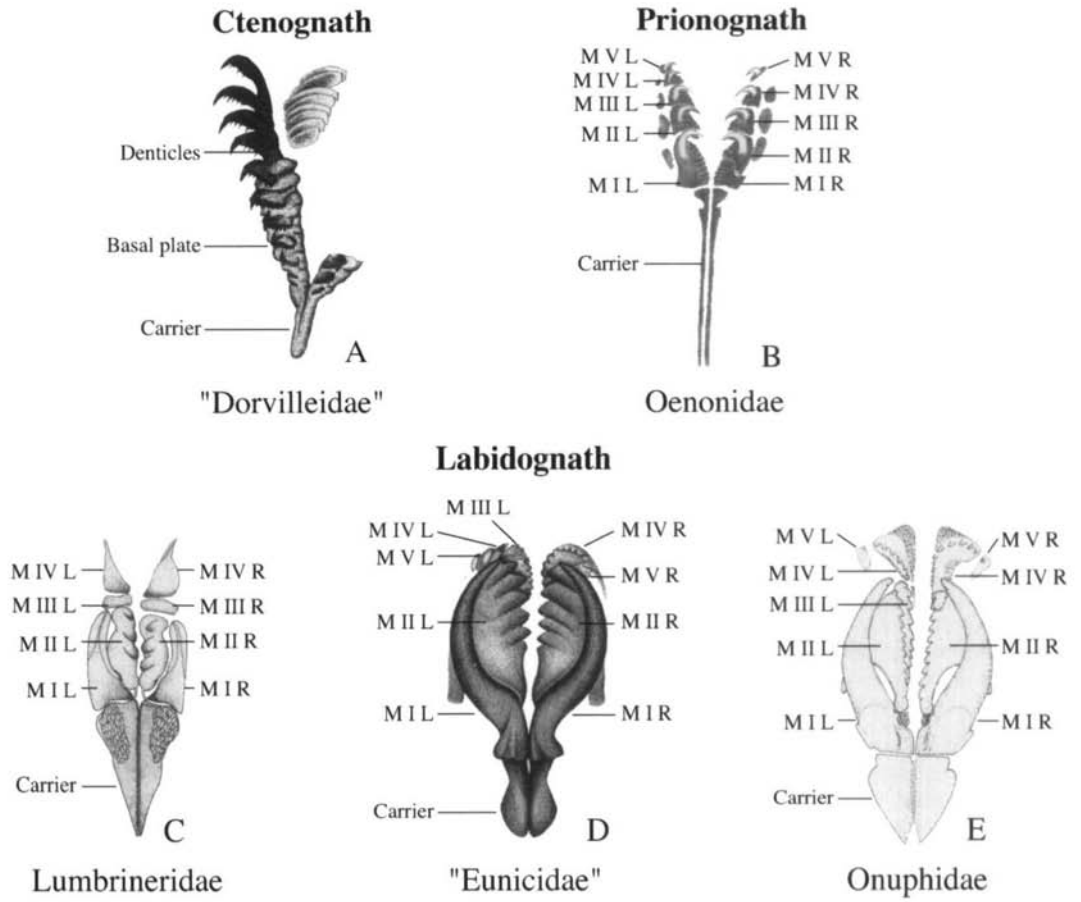

FIGURE 1. Different maxillary apparatuses of Eunicida. (A) Ctenognath: *Schistomeringos nigridentata* ("Dorvilleidae"). (B) Prionognath: *Oenone fulgida* (Oenonidae). (C to E) Labidognath: (C) *Lumbrineris nonatoi* (Lumbrineridae); (D) *Eunice cirrobranchiata* ("Eunicidae"); (E) *Onuphis eremita* (Onuphidae). Abbreviations: M = Maxillae; I–V = 1st–5th; R = right; L = left. Drawings modified after: (A, C, D) Rouse & Pleijel (2001); (B) http://www.nhm.ac.uk/zoology/taxinf/browse/genera/oenone.htm; (E) Paxton (1986).

used to designate three major types of jaw morphologies: ctenognath, prionognath, and labidognath (shown on Fig. 1). Typically, the architectural types labidognath and prionognath, first introduced by Ehlers (1864, 1868), possess four to six pairs of maxillae with a carrier. In the labidognath maxillary apparatuses of "Eunicidae," Lumbrineridae, and Onuphidae, the maxillae are arranged in semicircles and the short carrier is broadly attached to the first maxillae. In contrast, in the prionognath type of Oenonidae and Histriobdellidae with parallel arranged maxillae the carrier is long and slender (see Orensanz, 1990, and literature therein; Rouse and Pleijel, 2001). Tzetlin (1980), however, referred to the jaws of Histriobdellidae as ctenognath. Whether Hartmaniellidae possess a labidognath or prionognath jaw is currently unclear (Orensanz, 1990; Rouse and Fauchald, 1997), but a recent redescription showed similarities with the labidognath type (Carrera-Parra, 2003). The ctenognath type in "Dorvilleidae" is defined by relatively large basal maxillae and symmetrical arranged rows of numerous anterior denticles in longitudinal series without carriers (Rouse and Pleijel, 2001). Nevertheless, some genera (e.g., *Dorvillea*) with a ctenognath jaw also possess carriers. However, it is unlikely that these are homologous with the labidognath/prionognath carriers (Paxton, 2004).

In contrast to most soft-bodied annelids, hardened jaw apparatuses of Eunicida are found in the fossil record. These fossilized annelid jaw parts are usually referred to as scolecodonts and all three recent eunicid types are known from the fossil record as well as two additional eunicid types, xenognath and placognath, which show resemblance to the ctenognath type (see Szaniawski, 1996). The first occurrence of scolecodonts, supposedly ctenognath, is in the late Cambrian (H. S. Williams, personal communication in Eriksson et al., 2004). The first certain ctenognath type is early Ordovician (Tremadoc 495 to 485 Mya). Most other types appear by 485 to 470 Mya (Underhay and Williams, 1995) and are rare until the Middle Ordovician (~460 Mya) when an abundant record of eunicid jaws is found (Orensanz, 1990; Szaniawski, 1996; Hints et al., 2004). Because eunicid jaw elements offer the best record of the otherwise sparse annelid fossil record, studies of timing and abundance of these fossils may help to elucidate early evolutionary events in Annelida such as their assumed rapid radiation (e.g., McHugh, 2000), or the effect of mass or cyclic extinctions (e.g., Raup and Sepkoski, 1982; Rohde and Muller,

2005). Furthermore, phylogenetic studies addressing the evolution of recent taxa help to reveal the evolutionary plasticity of their jaws and thus will lead to a better assessment of fossil diversity.

Over the past century several authors presented phylogenetic hypotheses addressing the Eunicida (Hartman, 1944; Kielan-Jaworowska, 1966; Kozur, 1970; Tzetlin, 1980; Orensanz, 1990). Although the presence of intermediaries between the different types of jaw apparatuses, and thus potential homoplasy, was acknowledged (e.g., Orensanz, 1990; Szaniawski, 1996), the conclusions regarding the phylogeny of Eunicida were mainly based on the jaw elements (e.g., Fig. 7 in Orensanz, 1990). Orensanz (1990) used both neontology and fossil information to propose an evolutionary scheme of Eunicida. He concluded, based on the fossil record and a hypothesized ontogenetic progression from a ctenognath-like–bearing juvenile to labidognath-bearing adults in Onuphidae (Paxton, 1986), that the ctenognath apparatus is a symplesiomorphy for Eunicida and that "Dorvilleidae" is the most basal recent taxon. Given Orensanz's (1990) arguments, the basal placement for "Dorvilleidae," also proposed by others (Hartman, 1944; Kielan-Jaworowska, 1966; Kozur, 1970), implies that there is not a single known synapomorphy that defines "Dorvilleidae" as monophyletic (Struck et al., 2002b).

Within Eunicida a clade of all labidognath- and prionognath-bearing taxa ("Eunicidae," Hartmanniellidae, Histriobdellidae, Lumbrineridae, Oenonidae, and Onuphidae) is generally accepted due to the possession of a maxillary apparatus with a carrier and five pairs of maxillary pieces (Hartman, 1944; Kielan-Jaworowska, 1966; Kozur, 1970; Orensanz, 1990). However, within this clade it remains unclear if the prionognath taxa are the sister group to all labidognath taxa (Hartman, 1944; Kielan-Jaworowska, 1966; Kozur, 1970) or derived within them (Orensanz, 1990). In addition, it is not clear whether or not labidognath types are homologous (Orensanz, 1990). Although maxillary apparatuses of Lumbrineridae, "Eunicidae," and Onuphidae are usually termed as labidognath, clear differences between jaws of Lumbrineridae and "Eunicidae"/Onuphidae are exhibited. Ehlers (1864, 1868), who coined the terms labidognath and prionognath, regarded the lumbrinerids as connecting intermediaries. The labidognath jaws of "Eunicidae" and Onuphidae both show a *Paulinites*-theme jaw (i.e., they are asymmetrical due to the loss of a right maxillary element), but Lumbrineridae as well as prionognath Oenonidae show a *Rhamphoprion*-theme jaw (i.e., symmetrical; Edgar, 1984). Also in the latter theme, asymmetry can occur but only due to size differences between the left and right maxillae. Furthermore, "Eunicidae" and Onuphidae mineralize their jaw elements with aragonite, whereas Lumbrineridae incorporates calcite. "Dorvilleidae" and Oenonidae generally do not mineralize their jaws (Colbath, 1986). The situation in Hartmanniellidae and Histriobdellidae is unknown. Therefore, the homology of the different so-called labidognath jaws is controversial and thus the position of Lumbrineridae within a clade comprising all taxa with so-called labidognath or prionognath types. Despite the differences some have treated all labidognaths as homologous (Hartman, 1944; Kielan-Jaworowska, 1966; Kozur, 1970; Tzetlin, 1980).

A different phylogenetic scheme was proposed by Tzetlin (1980), in which Oenonidae, with a prionognath jaw, was considered as most basal. Dorvilleid species of *Ophryotrocha* with K-type maxillae I were regarded as a transitional stage from ctenognath to labidognath. This view was modified by Lu and Fauchald (2000) to suggest that species of *Ophryotrocha* have a transitional stage from ctenognath to labidognath-prionognath. Both hypotheses render "Dorvilleidae" paraphyletic.

Furthermore, a clade comprising "Eunicidae" and Onuphidae is supported by several synapomorphies, including possession of the *Paulinites*-theme type of labidognath jaw (Edgar, 1984; Orensanz, 1990). Contrary to Onuphidae, neither morphological nor molecular synapomorphies characterizing "Eunicidae" are known. Therefore, we regard "Dorvilleidae" and "Eunicidae" as likely to be paraphyletic and in need of further investigation.

Despite the general agreement in major aspects of Eunicida phylogeny based on morphological and fossil data, different hypotheses have never been rigorously tested by morphological cladistic analysis or by independent molecular data. Only a few molecular analyses of polychaete phylogeny based on 18S rDNA exist that include species from "Dorvilleidae," "Eunicidae," Lumbrineridae, and Onuphidae (Struck et al., 2002a, 2002b; Hall et al., 2004). Generally, studies fail to strongly support monophyly of Eunicida, but some recover a clade exclusively comprising eunicid taxa. A close relationship of "Eunicidae" and Onuphidae is significantly supported (Struck et al., 2002a, 2002b; Hall et al., 2004). However, other relationships within Eunicida have not been resolved, as judged by low bootstrap support (<50) on single gene trees of 18S data. Struck et al. (2002b) suggest that "Dorvilleidae" is paraphyletic due to the placement of *Pettiboneia urciensis* away from other dorvilleids, and not *Ophryotrocha* as mentioned above.

To determine the relationships within Eunicida, and by extension evolution of jaw types, we employed data from four different genes (nuclear 18S rDNA and 28S rDNA, and mitochondrial 16S rDNA and cytochrome *c* oxidase subunit I), which have already been used to address phylogenetic aspects in Annelida and other lophotrochozoan taxa (e.g., Nygren and Sundberg, 2003; Jördens et al., 2004; Passamaneck et al., 2004). The genes were treated as four data partitions, and all possible combinations of partitions were analyzed with both maximum likelihood and Bayesian inference. Because we were particularly interested in understanding which recovered nodes were well supported, our analyses lead us to explore congruence among the data partitions.

Identifying Congruence or Incongruence

Three general approaches to analyzing multiple data sets (i.e., partitions) have been proposed: analyze each

partition separately and build a consensus tree (e.g., Miyamoto and Fitch, 1995), combine partitions prior to analysis (e.g., Kluge, 1989), and combine partitions only after certain conditions are met (e.g., Bull et al., 1993; Huelsenbeck et al., 1996). The first two approaches disregard either congruence or incongruence between the data sets, respectively. For the purposes of our study, we were not interested in whether or not to combine data, but were more interested in the agreement or disagreement for particular clades across data partitions. Perhaps the most popular method for assessing congruence in phylogenetic signal across data sets is an incongruence length difference (=ILD) test (Farris et al., 1995). Readers interested in the pros and/or cons of ILD tests are referred to the recent review by Hipp et al. (2004). Another recent approach testing for congruence employs the Shimodaira and Hasegawa (1999; SH) test wherein likelihood scores of trees obtained by one partition(s) are evaluated against the best tree produced by a specified partition or combination of partitions (e.g., Nygren and Sundberg, 2003; Hipp et al., 2004; Passamaneck et al., 2004). This approach allows for reciprocal tests. Namely, a given partition(s) can be used to test topologies of other partitions, and these other partitions can be used to test the topology of the first partition. Thus, further insight into the source of conflict can be gained. Generally, congruence can be assumed when the topology of the combined data set is not rejected by any of the data sets (Hipp et al., 2004).

Both the ILD and the SH approach are global in that they test the overall topology and do not reveal which taxa or nodes cause incongruence. Thus, the tests may be prone to rejecting congruence between partitions even though the conflicting signal is restricted to a limited number of nodes or taxa (e.g., Nygren and Sundberg, 2003). Therefore, a refinement for both tests has been suggested (Nygren and Sundberg, 2003; Hipp et al., 2004) in which each taxon is deleted in turn and the analysis repeated to determine how specific taxa influence *P* values. This successive taxon deletion approach may be useful for detecting conflict due to a particular terminal node, but is not guaranteed to find conflict at internal nodes.

Therefore, we developed a partition addition bootstrap alteration (PABA) approach that allows the node and/or data partition causing the incongruence to be easily and more precisely identified. The approach is based on a methodological investigation of the alteration of bootstrap support at a given node as different data partitions are added. The general idea is that addition of congruent, or incongruent, data will cause bootstrap support for the node(s) of interest to increase or decrease, respectively. Although this idea of alterations of bootstrap support has been used to identify the source of congruent or incongruent signal by several authors (Barrett et al., 1991; Chippindale and Wiens, 1994; Olmstead and Sweere, 1994; Huelsenbeck et al., 1996; Mason-Gamer and Kellogg, 1996; Cunningham, 1997b; Halanych, 1998), investigations, in general, are not systematically conducted and only nodes or partitions already suspected of conflict or of a priori interest are considered. The PABA approach attempts to overcome these shortcomings and allows congruent or conflicting signal to be identified on a node-by-node and/or partition-by-partition basis. However, it does not suggest based on a test statistic if data sets should be combined or not.

Material and Methods

Collection of Molecular Data

Table 1 lists the taxa employed in this study, which genes (i.e., data partitions) we obtained for which taxa, and GenBank accession numbers. Upon collection, samples were preserved in >70% nondenatured EtOH or frozen at −80°C. Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) according to manufacturer's instructions. Amplification and sequencing of the four genes (nuclear 18S and 28S rDNA, and mitochondrial 16S rDNA and cytochrome *c* oxidase subunit I [COI]) used primers in Table 2 and the protocols listed below (all used a HotStart-PCR protocol):

18S (~1800 bp), 25-μl reaction. Prerun: 3 min 94°C; application of polymerase; 1 cycle: 3 min 94°C; 40 cycles: 1 min 94°C, 1 min 30 s 40°C, 2 min 30 s 72°C; 1 cycle: 7 min 72°C. Reaction-mix: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, ~1 ng/μl genomic DNA, 0.4 mM dNTPs, 0.8 μM of each primer (18e/18R1843), 0.04 U/μl *Taq* DNA Polymerase (Promega).

28S (~2200–3200 bp), 50-μl reaction. Prerun: 3 min 94°C; application of polymerase; 1 cycle: 2 min 94°C; 7 cycles: 30 s 94°C, 30 s 55°C (−0.5°C at every step), 12 min 70°C; 35 cycles: 30 s 94°C, 30 s 52°C, 12 min 70°C; 1 cycle: 10 min 72°C. Reaction-mix 5 to 7 μl of each 10× LA PCR Buffer.II and 10 mM dNTPs, and 2 to 7 μl 25 mM $MgCl_2$ (Takara Bio Inc., Otsu, Japan), 1 μl of each 20 μM primer (28F63.2 or 28F5/28R3 or 28R3264.2) and 0.15 to 0.25 μl 5 U/μl TaKaRa LA *Taq* (Takara Bio. Inc.).

16S (~500 bp), 25-μl reaction. Prerun: 3 min 94°C; application of polymerase; 1 cycle: 2 min 94°C; 40 cycles: 30 s 94°C, 30 s 40°C, 1 min 72°C; 1 cycle: 7 min 72°C. Reaction-mix: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, ~1 ng/μl genomic DNA, 0.2 mM dNTPs, 0.4 μM of each primer (16SarL/16SbrH), 0.04 U/μl *Taq* DNA Polymerase (Promega).

COI (~460–1300 bp), 25-μl reaction. Prerun: 3 min 94°C; application of polymerase; 1 cycle: 2 min 94°C; 40 cycles: 30 s 94°C, 1 min 50°C, 2 min 72°C. 1 cycle: 7 min 72°C. Reaction-mix: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, ~1 ng/μl genomic DNA, 0.2 mM dNTPs, 0.8 μM of each primer (LCO1490 or COI3/CO1r), 0.04 U/μl *Taq* DNA Polymerase (Promega).

All products were verified on a 1% agarose gel and purified with the QIAquick PCR Purification Kit (Qiagen). If necessary PCR products were size selected on the agarose gels and/or cloned using the pGEM-T Easy

TABLE 1. List of taxa and genes used. GenBank accession numbers of determined sequences in bold. Voucher number and locality are provided with the GenBank file.

Taxon	Accession number			
	18S	28S	16S	COI
"Dorvilleidae"				
Dorvillea bermudensis Åkesson and Rice, 1992	AF412802			
Dorvillea erucaeformis (Malmgren, 1865)	**AY838846**	**AY838859**	**AY838827**	**AY838868**
Dorvillea similis Crossland, 1924	AF412803			
Microdorvillea sp. n.	**AY527051**			
Ophryotrocha labronica La Greca and Bacci, 1962	**AY838855**		AF321429	**AY838874**
Parapodrilus psammophilus Westheide, 1965	AF412800			
Parougia sp.	AF412798		**AY838841**	
Pettiboneia urciensis Campoy and San Martin, 1980	AF412801		**AY838842**	
Protodorvillea kefersteinii (McIntosh, 1869)	AF412799	AY732230	**AY838843**	**AY598738**
Schistomeringos rudolphi (Chiaje, 1828)	AF412804			**AY598741**
"Eunicidae"				
Eunice harassii Audouin and Milne-Edwards, 1833	**AY525620**			
Eunice pennata 1 (O. F. Müller, 1776)	AY040684			
Eunice pennata 2 (O. F. Müller, 1776)	**AY838848**			
Eunice pennata 3 (O. F. Müller, 1776)	**AY838849**		**AY838829**	**AY838870**
Eunice sp.	AF412791	AY732229		**AY598733**
Eunice tenuis (Treadwell, 1921)	**AY838850**			
Eunice torquata Quatrefages, 1865	**AY838851**			
Eunice vittata (Chiaje, 1828)	AF412790			
Lysidice ninetta Audouin and Milne-Edwards, 1834	AF412793		**AY838834**	
Marphysa bellii (Audouin and Milne-Edwards, 1834)	AF412789		**AY838835**	
Marphysa sanguinea (Montagu, 1815)	**AY525621**	**AY838861**	**AY838836**	**AY598736**
Nematonereis unicornis (Grube, 1840)	AF412792			
Lumbrineridae				
Lumbrineris funchalensis 1 (Kinberg, 1865)	AF412796		**AY838831**	**AY598735**
Lumbrineris funchalensis 2 (Kinberg, 1865)	AF412797			
Lumbrineris inflata (Moore, 1911)	**AY525622**	AY364864	**AY838832**	AY366520
Lumbrineris latreilli 1 Audouin and Milne-Edwards, 1834	**AY525623**	AY366512	**AY838833**	AY364855
Lumbrineris latreilli 2 Audouin and Milne-Edwards, 1834	AB106247			
Lumbrineris latreilli 3 Audouin and Milne-Edwards, 1834	AF519238			
Lumbrineris sp.	AB106248			
Ninoe nigripes Pettibone, 1982	**AY838852**	**AY838862**	**AY838837**	**AY838871**
Oenonidae				
Arabella iricolor (Montagu, 1804)	**AY525624**			**AY598731**
Arabella semimaculata (Moore, 1911)	**AY838844**	**AY838857**	**AY838825**	**AY838866**
Drilonereis longa Webster, 1879	**AY838847**	**AY838860**	**AY838828**	**AY838869**
Oenone fulgida Pettibone, 1982	**AY838853**	**AY838863**	**AY838838**	**AY838872**
Onuphidae				
Aponuphis bilineata (Baird, 1870)	AF412795		**AY838824**	
Diopatra aciculata Knox and Cameron, 1971	**AY838845**	**AY838858**	**AY838826**	**AY838867**
Hyalinoecia tubicola O.F. Müller, 1776	AF412794	AY732228	**AY838830**	**AY598734**
Mooreonuphis stigmatis (Treadwell, 1922)	**AY527055**			
Onuphis elegans (Johnson, 1901)	**AY838854**	**AY838864**	**AY838839**	**AY838873**
Onuphis similis (Fauchald, 1968)	**AY525625**			
Amphinomidae				
Paramphinome jeffreysi (Mcintosh, 1868)	**AY838856**	**AY838865**	**AY838840**	**AY838875**
Glyceridae				
Glycera dibranchiata Ehlers, 1868	**AY995208**	**AY995207**	**AY995209**	**AY995210**
Siboglinidae				
Riftia pachyptila Jones, 1981	AF168739	Z21534	AY741662	AY741662

Vector Systems (Promega) according to the manufacturer's protocol (this was mainly needed for larger 28S rDNA products). A CEQ 8000 Genetic Analysis System (Beckman Coulter) using CEQ dye terminator chemistry was used for bidirectional sequencing of all products. Up to five clones of recombinant products were sequenced.

Phylogenetic Analyses

Although the annelid phylogeny is poorly resolved, Eunicida is currently incorporated within Aciculata (Rouse and Pleijel, 2001). To address the uncertainty in annelid phylogeny, a phyllodocid (*Glycera dibranchiata*; Glyceridae), a nonphyllodocid Aciculata (*Paramphinome jeffreysi*; Amphinomidae) and a non-Aciculata taxon (*Riftia pachyptila*; Siboglinidae) were employed as outgroup taxa. Sequences were aligned with ClustalW using default settings (Thompson et al., 1994) and subsequently corrected by hand in GeneDoc (Nicholas and Nicholas, 1997). Ambiguous positions were excluded from the subsequent analysis (see Table 3). The alignments (accession no. S1354; 18S matrix with 43 taxa, accession no.

TABLE 2. Primer sequences used in amplification and sequencing. Positions correspond to residues of *Homo sapiens* (18S), *Platynereis dumerlii* (16S and COI), and *Nereis succinea* (28S). F = forward; R = reverse.

Name	Sequence (5′→ 3′)	Position	Direction	Reference
16S				
16SarL	CGCCTGTTTATCAAAAACAT	571–588	F	Palumbi et al., 1991
16SbrH	CCGGTCTGAACTCAGATCACGT	1055–1076	R	Palumbi et al., 1991
18S				
18e	CTGGTTGATCCTGCCAGT	3–21	F	Hillis and Dixon, 1991
18F509	CCCCGTAATTGGAATGAGTACA	548–569	F	Struck et al., 2002b
18L	GAATTACCGCGGCTGCTGGCACC	609–632	R	Hillis and Dixon, 1991
18R925D	GATCYAAGAATTTCACCTCT	955–974	R	Present study
18F997	TTCGAAGACGATCAGATACCG	1044–1065	F	Struck et al., 2002b
18r	GTCCCCTTCCGTCAATTYCTTTAAG	1191–1215	R	Hillis and Dixon, 1991
18F1435	AGGTCTGTGATGCCCTTAGAT	1489–1509	F	Struck et al., 2002b
18R1779	TGTTACGACTTTTACTTCCTCTA	1811–1834	R	Struck et al., 2002b
18R1843	GGATCCAAGCTTGATCCTTCTGCAGGTTCACCTAC	1843-1877	R	Modified from Cohen et al., 1998
28S				
F63.2	ACCCGCTGAAYTTAAGCATAT	1–21	F	Passamaneck et al., 2004
Po28F1	TAAGCGGAGGAAAAGAAAC	24–43	F	Present study
28F5	CAAGTACCGTGAGGGAAAGTTG	335–356	F	Passamaneck et al., 2004
28R6	CAACTTTCCCTCACGGTACTTG	335–356	R	Passamaneck et al., 2004
Po28F2	CGACCCGTCTTGAAACACGG	847–967	F	Present study
Po28R5	CCGTGTTTCAAGACGGGTCG	847–967	R	Present study
28F1_2	GGGACCCGAAAGATGGTGAAC	1049–1069	F	Passamaneck et al., 2004
Po28R4	GTTCACCATCTTTCGGGTCCCAAC	1046–1069	R	Present study
28ee	ATCCGCTAAGGAGTGTGTAACAACTCACC	1507–1525	F	Hillis and Dixon, 1991
28ff	GGTGAGTTGTTACACACTCCTTAGCGG	1509–1525	R	Hillis and Dixon, 1991
Po28R3	GCTGTTCACATGGAACCCTTCTCC	1756–1780	R	Present study
28F4	CGCAGCAGGTCTCCAAGGTGMACA GCCTC	2168–2196	F	Passamaneck et al., 2004
28R2	GAGGCTGTKCACCTTGGAGACCTG CTGCG	2168–2196	F	Passamaneck et al., 2004
28v	AAGGTAGCCAAATGYCTCGTCATC	2623–2647	F	Hillis and Dixon, 1991
28R3	GATGACGAGGCATTTGGCTACC	2625–2647	R	Passamaneck et al., 2004
Po28R2	CCTTAGGACACCTGCGTTA	3019–3037	F	Present study
28F6	CAGACCGTGAAAGCGYGGCCTATC GATCC	3115–3143	F	Passamaneck et al., 2004
Po28R1	GAACCTGCGGTTCCTCTCG	3404–3286	R	Present study
R3264.2	TWCYRMCTTAGAGGCGTTCAG	3488–3508	R	Passamaneck et al., 2004
COI				
LCO1490	GGTCAACAAATCATAAAGATATTGG	14–38	F	Folmer et al., 1994
HCO2198c	TGATTTTTTGGTCACCCTGAAGTTTA	697–722	F	Present study
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	697–722	R	Folmer et al., 1994
COI 3	GTNTGRGCNCAYCAYATRTTYACNGT	850–875	F	Kojima et al., 1997
COIr	CCDCTTAGWCCTARRAARTGTTG NGG	1270–1295	R	Modified from Nelson and Fisher, 2000

TABLE 3. Data and models used in analyses.

Data sets	No. of positions: Total	Included	Excluded	No. of distinct data patterns	ML model
Individual					
18S (43 taxa)	2609	1579	1030	536	TrN+I+Γ
16S (23 taxa)	586	311	275	187	GTR+Γ
COI (21 taxa)	446	393	53	238	TrN+Γ
28S (16 taxa)	2862	1787	1065	509	TrN+Γ
Combined (16 taxa)					
18S	2609	1579	1030	342	TIM+I+Γ
16S	586	311	275	155	TrN+Γ
COI	446	393	53	221	TrN+Γ
18S/16S	3195	1890	1305	478	TrNef+I+Γ
18S/COI	3055	1972	1083	551	TrN+I+Γ
18S/28S	5471	3376	2095	814	TrN+I+Γ
16S/COI	1032	704	328	365	TrN+I+Γ
16S/28S	3448	2108	1340	654	TrN+I+Γ
COI/28S	3308	2190	1118	725	TrN+I+Γ
18S/16S/COI	3641	2283	1358	684	TrN+I+Γ
18S/16S/28S	6057	3687	2370	949	TrN+I+Γ
18S/COI/28S	5917	3768	2148	1023	TrN+I+Γ
16S/COI/28S	3894	2501	1393	864	TrNef+I+Γ
18S/16S/COI/28S	6503	4080	2423	1155	TrN+I+Γ

M2394; 16S with 23 taxa, accession no. M2391; COI with 21 taxa, accession no. M2393; and combined data set with 16 taxa, accession no. M2392) are available at TREEBASE (www.treebase.org).

Individual maximum likelihood (ML) and Bayesian inferences (BI) analyses of each gene were conducted with all available taxa for that partition and a set of 16 taxa shared for all partitions (for 28S these are one in the same). Also, for the shared 16-taxon set, all 11 possible combinations of the data sets were analyzed by ML and BI (Table 3). Prior to all analyses, χ^2 tests for homogeneity of base frequencies across taxa were performed.

For ML analyses, appropriate models of sequence evolution for each of the 18 data sets were assessed by hierarchical likelihood-ratio tests (=hLRT) using ModelTest V 3.06 (Posada and Crandall, 1998, 2001). The most likely tree was reconstructed in PAUP*4.0b (Swofford, 2002) using tree-bisection-reconnection (TBR) branch swapping and 10 random taxon additions and the parameters indicated by ModelTest V 3.06. The reliability of phylogenetic nodes was estimated by 100 bootstrap (BS)

replicates with one random taxon addition and TBR branch swapping.

MrModelTest 1.1b (Nylander, 2002) was used to determine appropriate models of sequence evolution of each of the individual data sets for BI. MrBayes 3.0B4 (Huelsenbeck and Ronquist, 2001) was used for BI with prior probability distributions of the individual model parameters according to the model specified by MrModelTest results. In the case of the combined data analyses, each partition was assigned its individual model and prior probability distributions, and model parameters and branch lengths between the partitions were unlinked to implement a partitioned likelihood analysis. Each Markov chain, three heated and one cold, ran simultaneously for 5×10^5 generations, with trees being sampled every 100 generations for a total of 5001 trees. Based on convergence of likelihood scores the first 250 trees in each analysis were discarded as *burn in*. The majority-rule consensus tree containing posterior probabilities (PP) of the phylogeny was determined from the remaining 4751 trees.

Because PP are generally higher than BS values (see Figs. 1 and 2 and Huelsenbeck et al., 2002) and are less reliable measurements of support than BS values (e.g., Suzuki et al., 2002), the term "significant support" refers herein to a BS $\geq$ 95, or to results based on likelihood ratio tests with defined P values.

Hypotheses Testing

Significance tests were performed under the ML criterion for each data set to test the traditionally assumed monophyly of a labidognath/prionognath clade. However, a preliminary study of 18S data indicated nonmonophyly (Struck et al., 2002b). Therefore, two-tailed Kishino and Hasegawa (1989) tests (KH tests) with a RELL approximation were used to compare a priori hypotheses for and against monophyly of this clade. Additionally, the labidognath/prionognath monophyly hypothesis was compared against the best solution with a one-tailed SOWH test for each data set (Goldman et al., 2000). To carry out the SOWH test, we generated 100 parametric bootstrap data sets with Seq-Gen V. 1.2.7 (Rambaut and Grassly, 1997) using the best topology congruent with the tested hypothesis (i.e., monophyly of labidognath/prionognath taxa) as the model tree . For each of the 100 parametric bootstrap data sets, an RELL approximation as described by Goldman et al. (2000) was performed to accelerate the analysis without altering the results significantly. Therefore, parameters were optimized on the topology used as the model tree and congruent with the a priori hypothesis. Then these parameters were used in a heuristic search (TBR branch swapping and 10 random taxon additions) to recover the best solution. The test statistic, the difference in likelihood values of topologies supporting the a priori hypothesis and the best solution for the observed data, is considered as significantly different if it is $\geq$95% of differences measured in simulated data sets (i.e., $P \leq 0.05$).

Congruence and PABA Approach

Congruence of different data partitions (in this case genes) was tested with both the ILD test (Farris et al., 1995) and SH tests (Shimodaira and Hasegawa, 1999) as implemented in PAUP*4.0b. The ILD test was conducted using a heuristic search with 1000 replicates, TBR branch swapping, and simple taxon addition for all 11 combined data sets. In the case of SH tests, variance estimations of the difference in the likelihood values of given topologies to the best topology were used to test whether the topology produced by a given partition was accepted or rejected by different data partitions (Nygren and Sundberg, 2003; Passamaneck et al., 2004). Therefore, the best topologies obtained by the 15 different 16-taxon data sets were compared to each other based on each of these data sets using the SH test. RELL approximations with 1000 replicates and ML methods described above were conducted. Due to its multiple tree correction, the SH test tends to increase the confidence set with increasing number of trees, thus the SH test overestimates the confidence interval (Shimodaira, 2002; Strimmer and Rambaut, 2002). Furthermore, to produce the appropriate distribution of the test statistic, the credible set of trees has to contain all trees that could possibly be true (Shimodaira and Hasegawa, 1999; Goldman et al., 2000). However, even with our smallest, 16-taxon data set, such a credible set would comprise 25,515 possibly true trees assuming a priori monophyly of Eunicida, "Eunicidae"/Onuphidae, Lumbrineridae, Oenonidae, and Onuphidae. Nevertheless, in this approach, we use the SH test to indicate the possibility of a conflict between partitions and not to reject particular hypotheses. Therefore, it is more important to be at least internally consistent to invoke the same systematic error due to not using the complete credible set of trees. Therefore, the number of trees (i.e., the 15 best trees) in our analyses did not vary.

Furthermore to determine which taxa may cause incongruence, ILD and SH approaches as proposed by Nygren and Sundberg (2003) were carried out. For each data set, taxa were excluded in turn and the ILD or SH test repeated, resulting in 176 additional ILD and 240 SH tests.

Because we were not satisfied that either of these approaches sufficiently described the source of possible incongruence and its influence in the data set, we developed the partition addition bootstrap alteration (PABA) approach. This approach can expose incongruence by examining the alteration (δ) of bootstrap support (BS) values at a given node when additional data partitions are added. δ is examined under all possible combinations of partition addition (both number of partitions and order of addition) to elucidate how all partitions interact with each other. The rationale is that signal from additional data will increase BS value for a given node if the evolutionary history of the node is congruent in the partitions. In contrast, incongruent and/or conflicting evolutionary history between partitions at a given node will result in a decrease of BS. No alteration means that neither

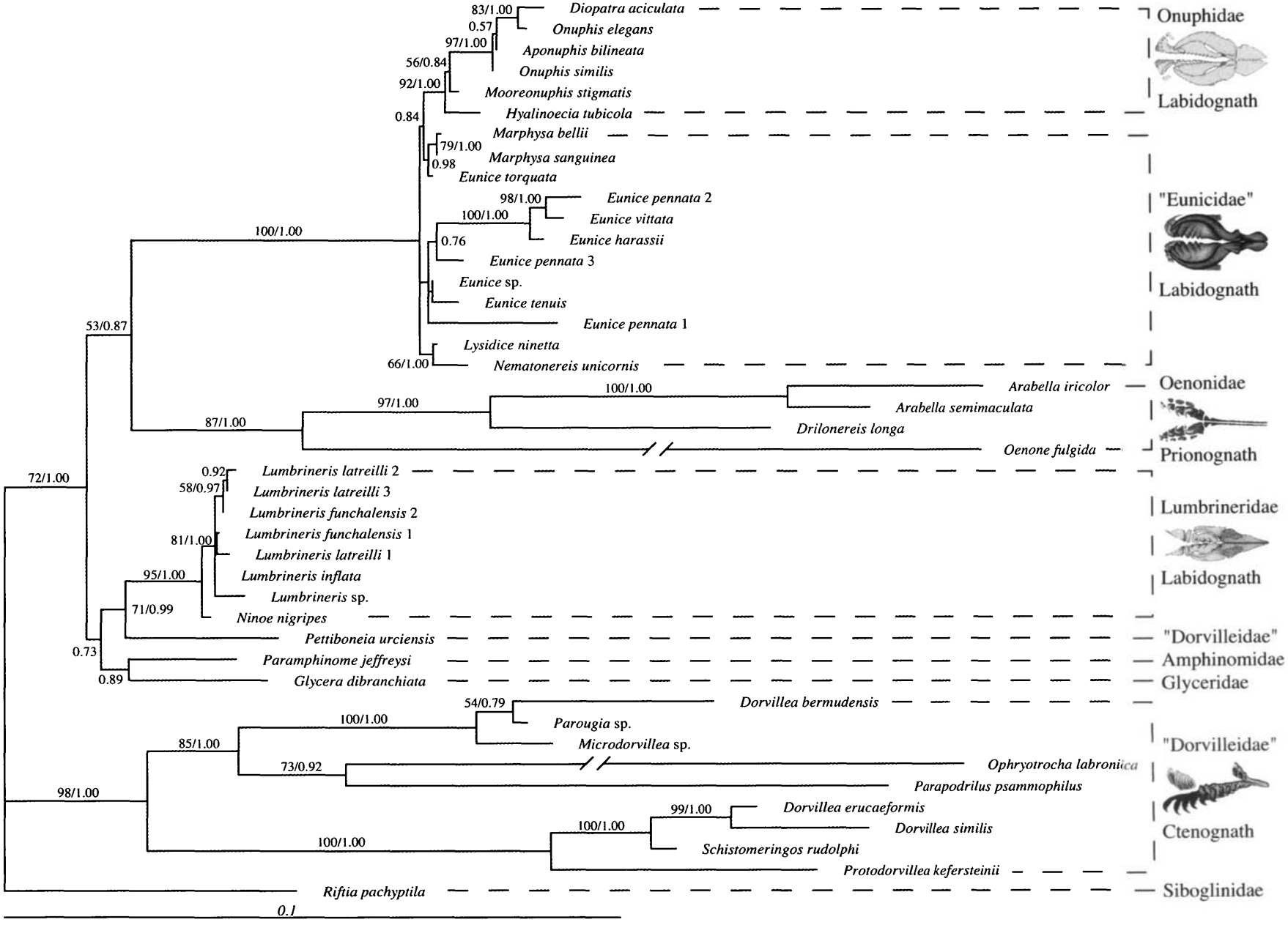

FIGURE 2. ML tree of the 18S analysis with 43 taxa (−ln L = 9690.59). BS values above 50 are shown above the branches on the left; PPs from BI are shown on the right or alone.

congruence nor incongruence between examined partitions for the particular node can be inferred. In the case of an already maximally supported node (BS = 100), further increase of BS value cannot be achieved, although the underlying phylogenetic signal may still change. Similarly, in the case of a minimally supported node (BS $\leq$ 5), further decrease can also not be achieved. However, because all possible combinations of partition addition are examined, there are multiple possibilities to examine δ unless all partitions support the node of interest with BS = 100 (in such cases a congruence test is usually a moot point). The general PABA approach is outlined below and a specific example follows in Results.

1. Build the combined data tree using the data partitions and taxa of interest and number the nodes of interest. Herein we chose the most taxonomically inclusive data set based on the largest number of molecular data available to reveal which nodes gather support by all partitions and thus are more likely to represent the species tree instead of only a gene tree.
2. Assuming taxa are the same across partitions; determine BS values of all nodes of interest for each partition and all possible combinations of partitions. We examined all nodes, but this could be one or a subset of nodes. Because the bipartitions table in PAUP shows only BS values of 5 or higher, all BS values $\leq$5 were set to 5 (thus the maximum of δ is 95).
3. For each given node, calculate the alteration, δ, of BS value when a partition is added to an existing data set for all possible combinations and orders of partition addition (e.g., add 18S as 2nd partition to the 16S data set or as 3rd to the 16S/COI data set).
4. Calculate for each given node and partition the mean δ at each possible position of addition (e.g., 18S as 2nd, 3rd, and 4th partition added). δ is not included in calculating the mean if and only if both the before and after BS value is either 100 ($\delta = 0$) or $\leq$5 ($\delta = 0$).
5. The mean δ values are tabulated and examined for trends in the data that correspond to a particular node or data partition.

This approach in general can be applied in several different ways. Although we employ only a maximum likelihood bootstrap approach here, the partition addition bootstrap addition can be used with distance, parsimony, or likelihood approaches. Similarly, it can be used with posterior probabilities or any other nodal support value. Concerning posterior probabilities in practice this may not work well as BI tends to give values of near 1.00 or below 0.5 with little in between. We focus on the combined tree as our starting topology, but any starting topology could be used (e.g., if a particular tree is favored for some reason).

RESULTS

Phylogenetic Analyses

Table 3 summarizes data set information including number of taxa, numbers of nucleotide positions, number of distinct data patterns, and the substitution model used. 28S had the most characters (1787 bp unambiguously aligned), followed by 18S (1579 bp), COI (393 bp), then 16S (311 bp) for the 16-taxon data sets. The χ^2 test showed that homogeneity of base frequencies across taxa was not rejected for any data set. For ML analyses, the hLRT indicated the Tamura and Nei model (or closely related variations) and a Γ distribution with or without a proportion of invariant sites. All models indicated by the hLRT for the BI were the general time reversible with a Γ distribution and in the 43-taxon 18S and 21-taxon COI data sets with an additional proportion of invariant sites. Thus all analyses employed similar models with, for the most part, the same number of parameters free to vary (i.e., degrees of freedom). For any given data set, topologies produced by ML and BI were very similar if not identical. However, the best trees differed among the 18 different data sets. The results of all ML analyses are shown in Figures 2 to 4. For space considerations, we present just ML trees. BI results are consistent with conclusions reached herein. Likelihood scores and numbers of best trees for both ML and BI are in Table 4 as appropriate.

We focus our discussion of organismal issues to analyses of the 43-taxon 18S data set (most taxa; Fig. 2) and the combined 18S/28S/16S/COI data set (most nucleotides; Fig. 3) for various reasons (e.g., space, amount of data, number of taxa, etc.). The results of all other ML analyses are shown in Figure 4 (only BS values above 50 are shown for graphical convenience). The 43-taxon 18S topology supports the monophyly of Onuphidae (BS: 92; PP: 1.00), Oenonidae (BS: 87; PP: 1.00), and Lumbrineridae (BS: 95; PP: 1.00) (Fig. 2). Furthermore, a monophyletic "Eunicidae"/Onuphidae is also corroborated (BS: 100; PP: 1.00), but "Eunicidae" is paraphyletic. Interestingly, the dorvilleid *Pettiboneia urciensis* is closely related to Lumbrineridae (BS: 71; PP: 0.99). A clade of all other dorvilleids (BS: 98; PP: 1.00) is basal and groups with the outgroup *Riftia pachyptila* (BS: 72; PP: 1.00). Thus, the ingroup is not monophyletic and rooting with the other two outgroup taxa would result in a basal clade comprising Lumbrineridae and *Pettiboneia urciensis*.

In the four-gene analyses (Fig. 3), Eunicida is monophyletic (BS: 63; PP: 0.98). This data set corroborates monophyly of all recognized eunicid families considered, but taxon sampling is limited. In contrast to the 43-taxon 18S analyses, "Dorvilleidae" groups with "Eunicidae"/Onuphidae (BS: 90; PP: 1.00; note *P. urciensis* was not included). The most basal eunicid taxon is Lumbrineridae and Oenonidae is the sister group of "Dorvilleidae"/"Eunicidae"/Onuphidae (BS: 84; PP: 1.00).

When considering all analyses (Fig. 4), a "Eunicidae"/Onuphidae clade as well as Oenonidae are usually found, and often with BS values of 100 (Fig. 3, nodes 10 and 6, respectively). The position of *P. urciensis* away from the other dorvilleids in analyses based on 16S alone is noteworthy. In analyses including 28S but not 18S (28S, 16S/28S, COI/28S, and 16S/COI/28S), the dorvilleid *Dorvillea erucaeformis* groups with the outgroup taxa

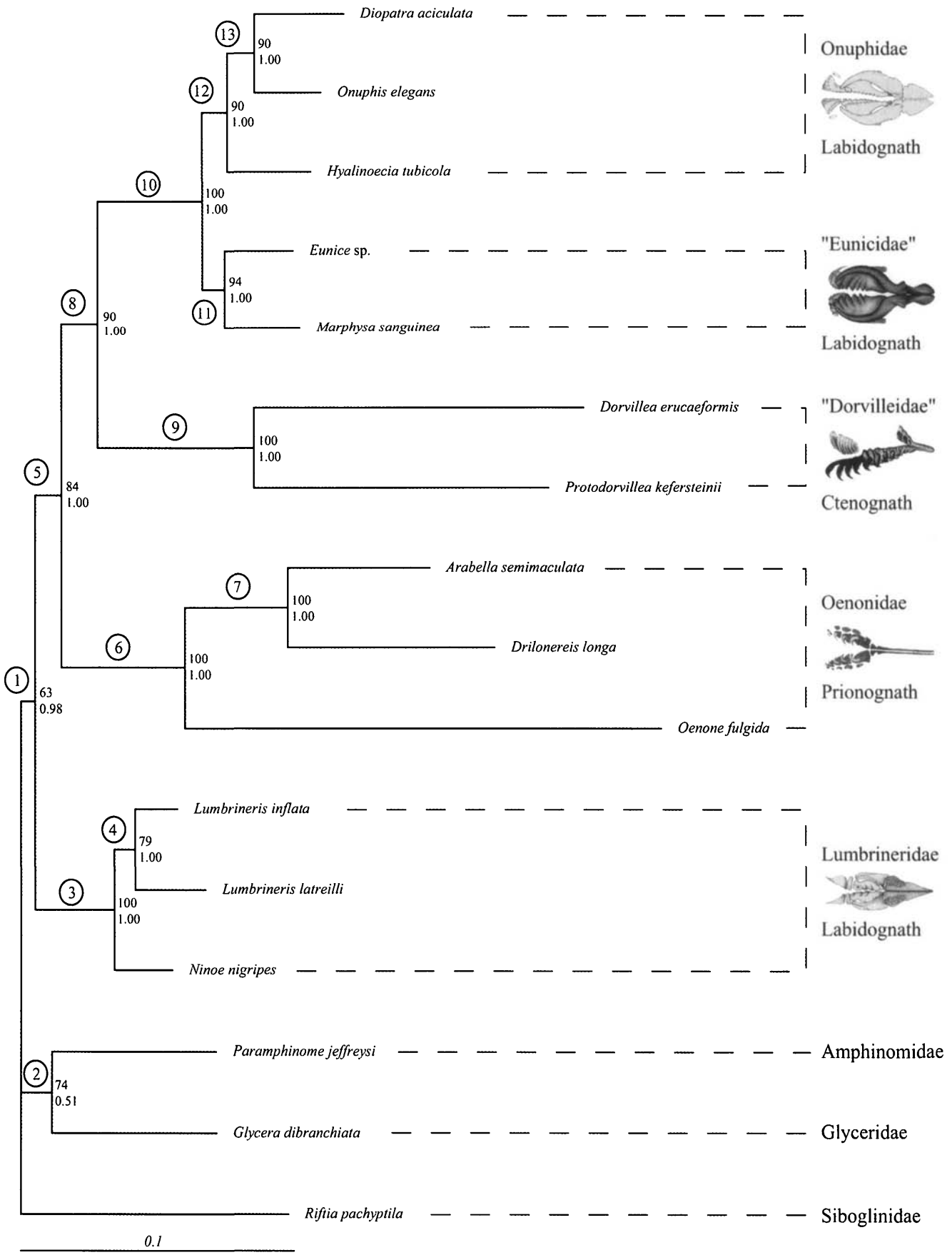

FIGURE 3. ML tree of the four-gene analysis with 16 taxa (−ln L = 21,337.51). BS values above 50 are shown above PP values from BI right to the node. Circled numbers refer to node numbers in the PABA approach.

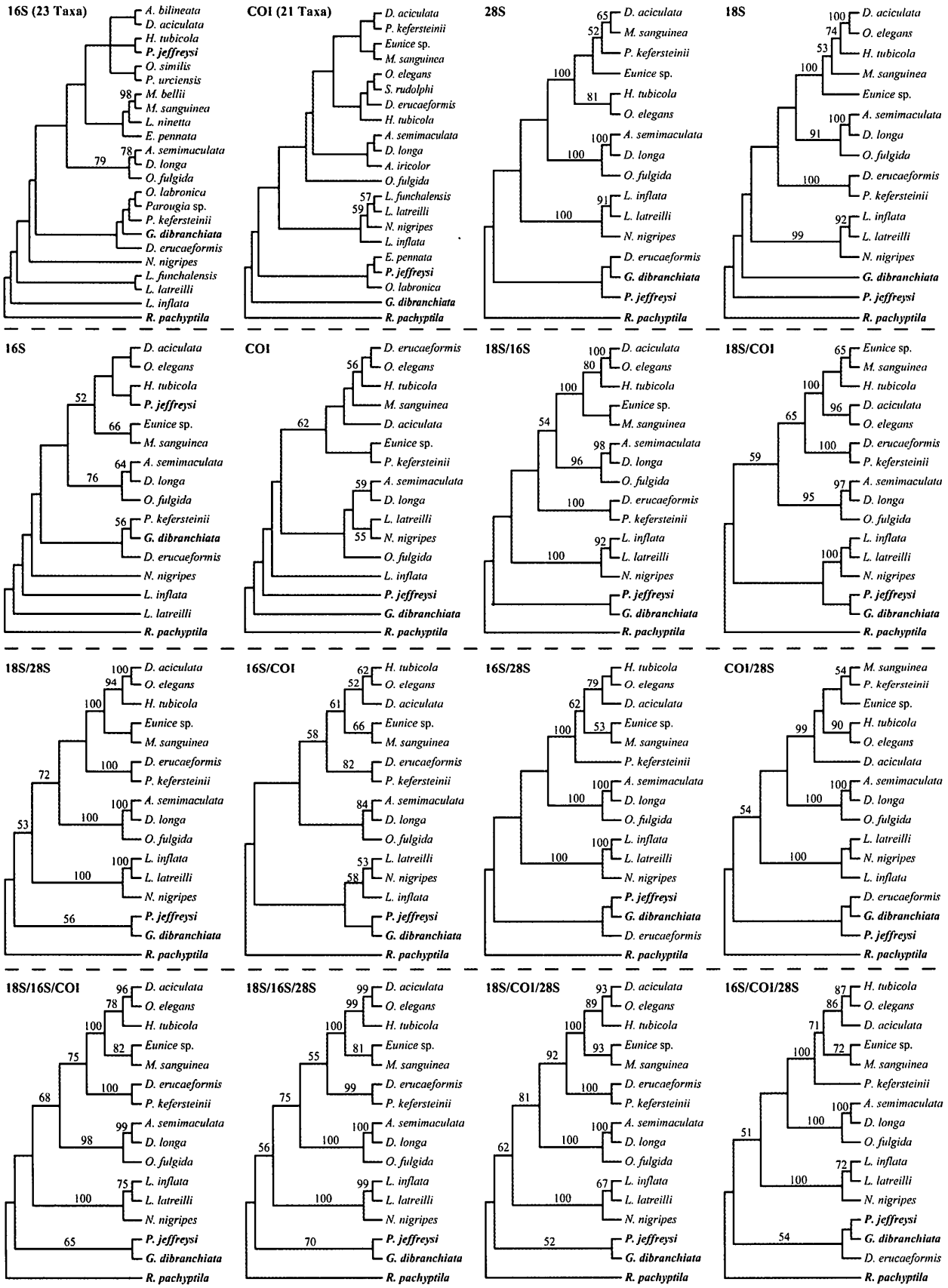

FIGURE 4. Strict consensus tree of two ML trees in the 16S analysis with 23 taxa as well as the cladograms of ML trees of all other analyses with 16 taxa and the COI analysis with 21 taxa (−ln L, see Table 4). Due to graphical convenience only BS values above 50 are shown, and each tree is rooted with *Riftia pachyptila*. Outgroup taxa are shown in bold.

TABLE 4. Results of phylogenetic analyses as well as significance tests for labidognath/prionognath clade. Significance values $P \leq 0.05$ in bold.

Data sets	No. of best trees	−ln L of ML	Mean −ln L of BI ± standard deviation	KH test	SOWH test
Individual					
18S (43 taxa)	1	9,861.24098	9,925.7376 ± 8.3723	0.238	**0.05**
16S (23 taxa)	2	3,112.93215	3,141.8081 ± 5.8815	0.220	**<0.01**
COI (21 taxa)	1	4,648.11000	4,677.0068 ± 6.8788	0.189	**<0.01**
28S (16 taxa)	1	8,117.82256	8,134.9494 ± 4.7766	**0.014**	**<0.01**
Combined (16 taxa)					
18S	1	6,326.95678	6,351.0296 ± 4.8340	0.728	**0.03**
16S	1	2,230.73446	2,222.8406 ± 4.5558	0.248	**<0.01**
COI	1	3,687.33941	3,713.3784 ± 5.7370	0.167	**<0.01**
18S/16S	1	8,895.08489	8,586.3018 ± 6.3147	0.448	**<0.01**
18S/COI	1	10,691.61388	10,077.9752 ± 7.2746	0.443	**<0.01**
18S/28S	1	14,654.61301	14,535.9599 ± 6.2416	0.168	**<0.01**
16S/COI	1	5,996.89766	5,956.0321 ± 6.7743	0.518	**<0.01**
16S/28S	1	10,597.77575	10,386.0004 ± 6.2334	**0.003**	**<0.01**
COI/28S	1	12,355.05749	11,872.4834 ± 7.4089	**0.009**	**<0.01**
18S/16S/COI	1	13,045.32219	12,314.1548 ± 8.2175	0.371	**<0.01**
18S/16S/28S	1	17,185.73135	16,771.4732 ± 7.3663	0.138	**<0.01**
18S/COI/28S	1	19,089.07941	18,260.4308 ± 8.0943	**0.050**	**<0.01**
16S/COI/28S	1	14,719.76462	14,120.8567 ± 8.1303	**0.003**	**<0.01**
18S/16S/COI/28S	1	21,482.30719	20,494.9128 ± 9.5563	0.072	**<0.01**

Glycera dibranchiata and *Paramphinome jeffreysi* (BS ≤ 55). In analyses based on COI alone, *D. erucaeformis* is part of a clade of "Dorvilleidae," "Eunicidae," and Onuphidae, and in all others, it is closely related to the dorvilleid *Protodorvillea kefersteinii* (maximum BS: 100). With regard to the position of these two dorvilleid taxa, all tests (ILD, SH, and PABA) indicate that the 28S sequences of these two taxa cause conflict in the data set (Tables 5 to 10). Furthermore, the PABA approach indicates that the derived position of the two dorvilleids shown in Figure 3 is supported by all four genes despite the conflict (nodes 5 and 8, Table 10). The 43-taxon 18S and the 23-taxon 16S analyses set *Ophryotrocha labronica* as derived within the "Dorvilleidae," but COI places it separate from other dorvilleids. Additionally, analyses of the 16S data alone fail to recover a monophyletic Lumbrineridae; a result not found in any other analysis but the 16-taxon COI-only analysis. Lumbrineridae is never closely related to "Eunicidae" and Onuphidae and instead is usually basal to a clade comprising Oenonidae, "Dorvilleidae," "Eunicidae," and Onuphidae (Fig. 3, node 5). In particular, all analyses resulting in a monophyletic Eunicida (Fig. 3, node 1) recover a basal lumbrinerid. Furthermore, in all other analyses (except for the 23-taxon 16S data set), constraining monophyly of Eunicida also results in basal lumbrinerids/*P. urciensis* (data not shown). Finally, although not well supported, the combination of the two mitochondrial genes (16S/COI) shows nearly the same relationships as the combination of both the two nuclear genes (18S/28S) and all four genes (Figs. 3 and 4). In the combination of the two mitochondrial genes, Lumbrineridae is more closely related to two outgroup taxa than to the other eunicids. Additional differences are only within Onuphidae as well as Lumbrineridae.

The position of the outgroup taxa seems to be problematic only in the 16S-alone analyses, in which the taxa spread out through the ingroup. In all other analyses, the taxa are in relative close proximity to each other.

TABLE 5. Results of the ILD test of congruence of data sets. Significant P values ≤ 0.05 in bold. All combinations of three or four genes result in $P \leq 0.015$ (in bold).

Taxa	18S/16S	18S/COI	18S/28S	16S/COI	16S/28S	COI/28S
All taxa	**0.008**	**0.004**	**0.001**	0.787	**0.001**	**0.001**
Excluded:						
A. semimaculata	0.060	**0.001**	**0.001**	0.652	**0.001**	**0.001**
D. aciculata	**0.003**	**0.030**	**0.001**	0.802	**0.001**	**0.001**
D. erucaeformis	**0.003**	**0.002**	0.153	0.630	**0.002**	**0.008**
D. longa	**0.007**	**0.001**	**0.001**	0.663	**0.001**	**0.001**
Eunice sp.	**0.003**	**0.001**	**0.001**	0.499	**0.001**	**0.001**
H. tubicola	**0.005**	**0.004**	**0.001**	0.607	**0.001**	**0.001**
L. inflata	**0.002**	**0.001**	**0.001**	0.362	**0.001**	**0.001**
L. latreilli	**0.004**	**0.012**	**0.001**	0.747	**0.001**	**0.001**
M. sanguinea	**0.009**	**0.001**	**0.001**	0.403	**0.001**	**0.001**
N. nigripes	**0.019**	**0.025**	**0.001**	0.879	**0.001**	**0.001**
O. fulgida	**0.001**	**0.003**	**0.001**	0.565	**0.001**	**0.001**
O. elegans	**0.012**	**0.002**	**0.001**	0.540	**0.001**	**0.001**
P. kefersteinii	**0.004**	**0.001**	0.298	0.636	**0.001**	**0.001**
P. jeffreysi	0.077	**0.001**	**0.001**	0.271	**0.001**	**0.001**
R. pachyptila	**0.006**	**0.004**	**0.001**	0.675	**0.001**	**0.001**
G. dibranchiata	0.347	**0.001**	**0.001**	0.510	**0.003**	**0.001**

Hypotheses Testing

None of the analyses in this study support a clade that exclusively comprises all taxa bearing a labidognath or prionognath jaw (Figs. 2 to 4), the a priori hypothesis of primary interest. KH tests reject monophyly of a labidognath/prionognath clade in all comparisons including 28S but not 18S and in the 18S/COI/28S analysis (Table 4). Furthermore, the P values of the KH tests decrease in all analyses including 18S if number of both taxa (e.g., 16-taxon → 43-taxon: 0.728 → 0.238) and characters (e.g., 18S → 18S/16S → 18S/16S/COI → 18S/16S/COI /28S: 0.728 → 0.448 → 0.371 → 0.072) is added. Thus, with adding more information the a priori hypothesis becomes less likely. Additionally, the more appropriate SOWH tests (Goldman et al., 2000)

TABLE 6. Results of the SH test of congruence of data sets. Significant P values ≤ 0.05 in bold.

	Topology														
Data set	18S	16S	COI	28S	18S/16S	18S/COI	18S/28S	16S/COI	16S/28S	COI/28S	18S/16S/ COI	18S/16S/ 28S	18S/COI/ 28S	16S/COI/ 28S	18S/16S COI/28S
18S	—	**<0.001**	**<0.001**	**<0.001**	0.880	0.778	0.892	0.109	**<0.001**	**<0.001**	0.892	0.892	0.892	**<0.001**	0.892
16S	0.159	—	**<0.001**	**0.010**	0.273	0.135	0.192	0.082	0.079	**0.005**	0.192	0.192	0.192	0.079	0.192
COI	**0.015**	**0.016**	—	**0.013**	**0.018**	0.254	0.320	0.486	**0.043**	0.086	0.320	0.320	0.320	**0.043**	0.320
28S	**0.003**	**<0.001**	**<0.001**	—	**0.004**	**0.004**	**0.004**	**0.004**	0.501	0.648	**0.004**	**0.004**	**0.004**	0.501	**0.004**
18S/16S	0.825	**<0.001**	**<0.001**	**<0.001**	—	0.794	0.965	0.241	**<0.001**	**<0.001**	0.965	0.965	0.965	**<0.001**	0.965
18S/COI	0.679	**<0.001**	**<0.001**	**<0.001**	0.835	—	0.970	0.592	**<0.001**	**<0.001**	0.970	0.970	0.970	**<0.001**	0.970
18S/28S	0.773	**<0.001**	**<0.001**	**0.003**	0.963	0.762	—	0.258	**0.033**	**<0.001**	1.000	1.000	1.000	**0.033**	1.000
16S/COI	0.164	0.124	0.103	**0.017**	0.278	0.632	0.763	—	0.167	**0.044**	0.763	0.763	0.763	0.776	0.763
16S/28S	**0.049**	**<0.001**	**<0.001**	0.740	0.123	**0.047**	0.103	0.056	—	0.640	0.103	0.103	0.103	1.000	0.103
COI/28S	**0.008**	**<0.001**	**<0.001**	0.620	**0.030**	**0.047**	0.073	0.095	0.851	—	0.073	0.073	0.073	0.851	0.073
18S/16S/COI	0.595	**<0.001**	**<0.001**	**<0.001**	0.880	0.845	1.000	0.607	**<0.001**	**<0.001**	—	1.000	1.000	**<0.001**	1.000
18S/16S/28S	0.730	**<0.001**	**<0.001**	**<0.001**	1.000	0.732	1.000	0.312	**0.048**	**<0.001**	1.000	—	1.000	**0.048**	1.000
18S/COI/28S	0.563	**<0.001**	**<0.001**	**<0.001**	0.828	0.849	1.000	0.625	0.078	**<0.001**	1.000	1.000	—	0.078	1.000
16S/COI/28S	**0.012**	**<0.001**	**<0.001**	0.520	0.051	0.055	0.136	0.136	1.000	0.715	0.136	0.136	0.136	—	0.136
18S/16S/COI/28S	0.519	**<0.001**	**<0.001**	**<0.001**	0.853	0.771	1.000	0.591	0.061	**<0.001**	1.000	1.000	1.000	0.061	—

exhibit significant differences in all analyses (Table 4). These tests were set up by constraining an Onuphidae/"Eunicidae"/Lumbrineridae/Oenonidae clade to be monophyletic and comparing against the best tree recovered.

ILD and SH Congruence Approaches

ILD tests show that most of the partitioned data sets contain conflicting signal (Table 5), with only the 16S/COI data sets considered congruent. In an attempt to further clarify the incongruence, each taxon was deleted in turn to determine if one or a few taxa were particularly problematic. However, only for the 18S/16S and the 18S/28S data sets did the taxa deletion approaches indicate taxa that potentially cause conflict (as judged by changes in P value across the significant threshold of 0.05). In the 18S/16S data set, three taxa caused such an effect: *Arabella semimaculata*, *Paramphinome jeffreysi*, and *Glycera dibranchiata*. The former two result only in a slight increase above 0.05, whereas *G. dibranchiata* clearly raised the P value. In 18S/28S the conflict seems to be due to *Dorvillea erucaeformis* and *Protodrovillea kefersteinii*.

Using the SH approach (Table 6), out of 210 possible comparisons, 132 resulted in a P value above 0.05, signaling that congruence was not rejected, whereas 78 comparisons rejected congruence ($P > 0.05$). The 28S data set rejects all topologies not obtained from data sets mainly based on 28S (28S, 16S/28S, COI/28S, and 16S/COI/28S). Conversely, all topologies obtained by data sets mainly based on 28S are rejected by most other data sets. Additionally, topologies of both 16S and COI data sets were rejected by all other data sets except for the 16S/COI data set. It is noteworthy that the presented topology of the four-gene analyses (Fig. 3) is only rejected by the 28S data set. We also performed deletion of taxa with the SH approach (sensu Nygren and Sundberg, 2003). Table 7 summarizes the change in SH scores above or below significance ($P < 0.05$) when a given taxon is deleted (summed across all data sets). To objectively

TABLE 7. Number of increased and decreased P values in SH test above or below the significance level, respectively, depending on deleted taxon. Possible maximal changes: decreasing: 132; increasing: 78. Taxa with values beyond standard deviation from the mean in bold.

Deleted taxon	Increase	Decrease
A. semimaculata	4	4
D. aciculata	14	2
D. erucaeformis	**25**	5
D. longa	2	1
Eunice sp.	3	**17**
H. tubicola	10	1
L. inflata	3	**15**
L. latreilli	4	9
M. sanguinea	7	2
N. nigripes	8	3
O. fulgida	4	3
O. elegans	11	4
P. kefersteinii	**41**	7
P. jeffreysi	5	6
R. pachyptila	4	6
G. dibranchiata	8	3
Mean ± standard deviation	9.56 ± 10.16	5.50 ± 4.66

TABLE 8. Bootstrap values in all data sets with 16 taxa corresponding to the nodes (bipartitions) of combined analyses of all four genes. Node label see Figure 3.

	Nodes												
Data sets	1	2	3	4	5	6	7	8	9	10	11	12	13
28S	16	12	100	91	21	100	100	5	5	22	5	15	8
18S	42	26	99	92	39	91	100	19	100	100	39	74	100
16S	5	9	8	25	5	76	64	5	40	24	66	22	28
COI	10	17	17	5	5	6	59	62	5	5	38	6	5
18S/16S	35	69	100	92	49	96	98	22	100	100	43	80	100
18S/COI	33	37	100	38	59	95	97	65	100	100	65	47	96
18S/28S	53	56	100	100	72	100	100	50	100	100	76	94	100
16S/COI	5	35	58	14	14	49	84	58	82	61	66	52	10
16S/28S	20	50	100	100	25	100	100	5	5	62	53	79	42
COI/28S	17	11	100	33	17	100	100	19	5	41	40	52	5
18S/16S/COI	30	65	100	75	68	98	100	75	100	100	82	78	96
18S/16S/28S	56	70	100	99	75	100	100	55	99	100	81	99	99
18S/COI/28S	62	52	100	67	81	100	100	92	100	100	93	89	93
16S/COI/28S	27	44	100	72	39	100	100	29	5	71	72	86	10
18S/16S/COI/ 28S	63	74	100	79	84	100	100	90	100	100	94	90	90

TABLE 9. Detailed example of the determination of the alteration of bootstrap support δ depending on the order a particular partition is added shown for node 5 (see Fig. 3).

	BS				BS				BS				BS		
Add 18S	Before	After	δ	Add 16S	Before	After	δ	Add COI	Before	After	δ	Add 28S	Before	After	δ
							As 2nd								
16S	5	49	44	18S	39	49	10	18S	39	59	20	18S	39	72	33
COI	5	59	54	COI	5	14	9	16S	5	14	9	16S	5	25	20
28S	21	72	51	28S	21	25	4	28S	21	17	−4	COI	5	17	12
Mean			50	Mean			8	Mean			8	Mean			22
							As 3rd								
16S/COI	14	68	54	18S/COI	59	68	9	18S/16S	49	68	19	18S/16S	49	59	26
16S/28S	25	75	50	18S/28S	72	75	3	18S/28S	72	81	9	18S/COI	59	81	22
COI/28S	17	81	64	COI/28S	17	39	22	16S/28S	25	39	14	16S/COI	14	39	25
Mean			56	Mean			11	Mean			14	Mean			24
							As 4th								
16S/COI/28S	39	84	45	18S/COI/28S	81	84	3	18S/16S/28S	75	84	9	18S/16S/COI	68	84	16

determine which taxa cause the potential conflict, the mean and the standard deviation of the changes in SH scores above as well as below the significance level were estimated. Only values beyond the one standard deviation margin were considered suspect. Four taxa in particular (*Dorvillea erucaeformis*, *Protodorvillea kefersteinii*, and *Eunice* sp., *Lumbrineris inflata*) have a large effect on the significance of *P* values depending on their inclusion in the data set. The two dorvilleids seemed to account for most of the incongruent signal in the 28S partition in that *P* values increase for comparisons using either 28S-based topologies or the 28S data set when one of them was excluded. On the other side, the deletion of *Eunice* sp. results in a decrease of congruent signal for comparisons using the 16S/COI, 16S/28S, and 16S/COI/28S topologies. The same is true for *L. inflata* and comparisons of both 16S/28S and 16S/COI/28S topologies and data sets. Thus, across partitions, these two taxa have a net effect of promoting congruence when they are included.

PABA Approach

As mentioned above, we developed a novel approach to understand the potential of support or conflict of data partitions at a given node (or set of nodes). Results of the PABA approach are given in Tables 8 to 10. For clarity, we will first walk through the calculations in line with the description in Material and Methods and then more generally discuss the results.

1. The combined tree is shown in Figure 3. In order to more broadly understand signal in the data, we examined all the nodes of that tree. For illustrative purposes and because it is central to the hypothesis of labidognath/prionognath clade monophyly, we focus specifically on node 5 in explaining PABA.
2. The BS value for each node under each partition or combination of partitions is shown in Table 8.
3. The alteration (δ) at a given node was calculated for each combination of partitions added in all possible

TABLE 10. Alteration of bootstrap support δ to nodes in Figure 3 as data partitions are added. Interesting alterations in bold. n.a. = not applicable due to alteration from 100 to 100.

		Gene											
		18S as			16S as			COI as			28S as		
Node	BS value	2nd	3rd	4th	2nd	3rd	4th	2nd	3rd	4th	2nd	3rd	4th
1	63	30	35	36	−3	3	1	−4	4	7	11	24	33
2	74	43	30	30	34	25	22	12	−5	4	23	8	9
3	100	88	42	n.a.	21	n.a.	n.a.	26	n.a.	n.a.	59	42	n.a.
4	79	36	31	7	6	25	12	**−41**	**−26**	**−20**	37	31	4
5	84	50	56	45	8	11	3	8	14	9	22	24	16
6	100	55	49	n.a.	24	3	n.a.	−12	2	n.a.	42	20	2
7	100	36	15	n.a.	12	2	n.a.	9	1	n.a.	39	7	1
8	90	22	47	61	−1	8	−2	38	40	35	**−6**	**10**	**15**
9	100	83	69	95	77	−1	n.a.	42	n.a.	1	**−35**	**−39**	**n.a.**
10	100	83	45	29	48	30	n.a.	28	9	n.a.	37	10	n.a.
11	94	25	32	22	27	18	1	20	25	13	9	24	12
12	90	59	28	4	39	23	1	**13**	**0**	**−9**	41	32	12
13	90	85	77	81	20	1	−3	**−8**	**−14**	**−9**	**14**	**−1**	**−6**
Average over all nodes		53	43	41	24	12	4	10	5	3	23	15	10
Average over nodes 1–3, 5–7, 10–11		51	38	32	21	13	7	11	7	8	30	20	12

orders. Table 9 shows the δ values calculated for node 5. Values for other nodes are not shown. For example, the BS value for node 5 with just 16S data was ≤ 5, but when the 18S partition was added it increased to 49 (Tables 8 and 9). Thus, δ is 44. When 18S was added as the 3rd partition to the COI/28S data, the BS value increased from 17 to 81, a $\delta = 64$. Note that for node 5, BS values only decreased once, when COI was added as second to 28S ($\delta = -4$).

4. Table 9 also shows the means in δ calculated for each possible order of addition for all partitions for node 5. When the 18S was added as the second data set it caused a mean alteration of BS value (δ) of 50, and as a third data set a mean δ of 56. The values of $\delta = 0$ (no alteration) due to an already maximally or minimally supported node (BS: 100 or 5, respectively) are excluded from the calculation of the mean to prevent bias.
5. Mean δ values from all nodes of interest were compiled in Table 10. For example, the row corresponding to node 5 includes the BS value of the combined tree in Figure 3 (for comparison) and the mean δ values obtained from Table 9. Table 10 is essentially the result of the PABA approach. For some nodes the calculation of the mean was not applicable due to already maximally supported nodes and no alteration of BS values for all possible combinations at the order of addition (e.g., node 3: 18S added as 4th to 16S/COI/28S or 16S added as 3rd to 18S/COI, 18S/28S, or COI/28S; Tables 8 and 10). Although the other possibility of a minimally supported node and no alteration is the case for individual combinations (e.g., node 1: COI added as 2nd to 16S; Table 8), it cannot be observed in calculating the mean for any of the nodes of Table 10.

General results of the PABA approach are consistent with observations made by many molecular phylogenetic studies. As more data or data partitions are added, bootstrap values tend to increase (i.e., there is more phylogenetic signal). This is evidenced in Tables 9 and 10 by the fact that δ is usually positive. Also, this approach reveals that incongruence in phylogenetic signal is usually due to a particular data partition or group of taxa (node in this case). For example, the 28S partition is solely responsible for the conflict regarding the monophyly of the two dorvilleid taxa, *Dorvillea erucaeformis* and *Protodrovillea kefersteinii* (node 9). δ values for this node are very high (−35 or −39, respectively) when the 28S partition is added as 2nd or 3rd. However, when added as 4th, the 28S partition is not able to overwhelm the signal in the other partitions. Interestingly, at node 8, the closest internal node to node 9, the 28S partition has a slightly negative impact when added 2nd, but with an increasing amount of data (i.e., added as 3rd and 4th), the 28S adds signal suggesting a closer relationship of "Eunicidae," Onuphidae, and "Dorvilleidae." In contrast, at node 13 the 28S data adds conflicting signal with an increasing amount of data. Whereas the 18S and 16S do not seem to be problematic, the COI data also has a negative influence on node 13 and possibly on node 12. Additionally, all of the conflict at node 4 is due to the COI partition.

Lastly, the four partitions do not show the same contribution to phylogenetic signal across the tree. If one averages the mean δ across all nodes (Table 10), the 18S has the largest positive influence on nodal support as judged by BS values, and COI has the smallest. The magnitude of influence of a given partition is also related to when it is added to the data set because it is a different percentage of the total amount of data. In other words, δ will be larger when a partition is added as the second as compared as the fourth.

DISCUSSION

Eunicida Phylogeny

The current understanding of Eunicida phylogeny, which holds that "Dorvilleidae" is basal and that taxa possessing either labidognath or prionognath jaws are monophyletic, was not supported by any analysis herein. "Dorvilleidae" is traditionally regarded as the most basal taxon within Eunicida. Their ctenognath jaws are thought to be plesiomorphic because they are less complex than labidognath and prionognath jaws and may represent earlier ontogenetic stages (Orensanz, 1990). Although a few analyses favor a more basal position of some dorvilleids (e.g., 28S), all analyses resulting in monophyletic Eunicida support the labidognath-bearing Lumbrineridae as the basal Eunicida taxon (Figs. 3 and 4). Furthermore, lumbrinerids are basal in all other analyses, except for the 23-taxon 16S analysis, when the outgroup taxa are constrained to be outside the ingroup. In the 43-taxon 18S analysis, this clade also comprises *P. urciensis* besides Lumbrineridae. Thus, the molecular analyses generally substantiate the derived position of some dorvilleids as well as a basal position of Lumbrineridae. Conceivably, other dorvilleids not included here may still come out basal due to paraphyly of "Dorvilleidae."

We never observed support for a clade of taxa with labidognath jaws (Hartman, 1944; Kielan-Jaworowska, 1966; Tzetlin, 1980). Thus, the molecular data support the hypothesis of a possible independent origin of Lumbrineridae and "Eunicidae"/Onuphidae (Orensanz, 1990). However, both the basal position of Lumbrineridae and the highly derived position of some dorvilleids refutes the hypothesis of Orensanz (1990) and other authors corroborating a derived monophyletic eunicid group of all taxa possessing either a labidognath or prionognath apparatus (Hartman, 1944; Kielan-Jaworowska, 1966; Kozur, 1970; Lu and Fauchald, 2000). This hypothesis is also significantly rejected by all SOWH tests preformed. Thus, our results are consistent with previous weakly supported (BS < 50) taxon-limited topologies based solely on 18S data that show "Eunicidae" and Onuphidae closer to "Dorvilleidae" than to Lumbrineridae (Struck et al., 2002a, 2002b; Hall et al., 2004). Furthermore, our findings are inconsistent with Oenonidae as the most basal recent eunicid taxon (Tzetlin, 1980).

Several interesting organismal issues have also been raised by some of our analyses. Although Onuphidae

and "Eunicidae" form a well-supported clade, the relationship between these taxa is not clear. In the limited 16-taxon tree (Fig. 3) they are sister taxa, but in 43-taxon 18S tree Onuphidae forms a clade nested within "Eunicidae." The synapomorphies of onuphid taxa are distinct frontal lips (formerly referred to as "frontal palps") and well-developed ceratophores at the base of palps and antennae (Paxton, 1986). "Eunicidae," however, lacks synapomorphies (e.g., Rouse and Pleijel, 2001); this is one of the reasons we question the monophyly of this clade.

Based on similarities in jaw morphology and the fossil record, Orensanz (1990) revived the old hypothesis that Oenonidae includes Arabellidae rendering the latter to a junior synonym. A close relationship of former arabellid species (*Arabella* and *Drilonereis*) and *Oenone fulgida* is supported by nearly all analyses and Oenonidae in the sense of Orensanz (1990) is usually well supported. Histriobdellidae and Hartmaniellidae are two other groups not sampled here that may be related to Oenonidae. Members of the former are generally considered to have prionognath jaws (Rouse and Fauchald, 1997, but see Tzetlin, 1980) and are commensal/parasitic, like many species of oenonids. As for Hartmaniellidae, the situation is more complicated. They are poorly studied and their jaws have been identified as both prionognath (Rouse and Fauchald, 1997) and labidognath (Orensanz, 1990). However, a recent redescription of *Hartmaniella* was able to show strong similarities with the labidognath type of "Eunicida" and Onuphidae (Carrera-Parra, 2003).

The recognition of "Dorvilleidae" is made problematic by *Pettiboneia urciensis*, which falls out separate from other dorvilleids (see also Struck et al., 2002b). Unfortunately, we were not able to obtain the 28S and COI from *P. urciensis* due to a shortage of genomic DNA. 18S and 16S data suggest different phylogenetic positions, but both away from the other dorvilleids included. The placement of *Dorvillea bermudensis* as sister to *Parougia* sp. is also interesting confirming recent morphological data that *D. bermudensis* might be misplaced and actually belongs to the genus *Parougia* (H. Paxton, personal communication).

Jaw Evolution

A strict parsimonious interpretation of the Figure 3 topology suggests that the last common Eunicida ancestor possessed a labidognath jaw apparatus or at least an apparatus with a carrier and four to six pairs of maxillary elements. Thus, prionognath and ctenognath jaw types are inferred to be derived conditions. However, as several authors have pointed out, eunicid jaw evolution is more variable than the widely used jaw type scheme suggests (Ehlers, 1864, 1868; Edgar, 1984; Colbath, 1986; Orensanz, 1990; Szaniawski, 1996). The ctenognath type and the Lumbrineridae labidognath type were of special concern herein. The latter has been regarded as either intermediate between labidognath and prionognath (e.g., Ehlers, 1864, 1868) or as independently evolved within a prionognath/labidognath clade (Orensanz, 1990). The clear differences between the labidognath apparatuses found in Lumbrineridae and "Eunicidae"/Onuphidae can be demonstrated by the mineralization of their jaw elements (Colbath, 1986) as well as by jaw symmetry (Edgar, 1984). Based on these differences and our molecular results, it is most likely that these two jaw types are indeed not homologous as implied by the naming and use in literature. However, the recognition of four jaw types makes the delineation of a plesiomorphic state from Figure 3 impossible, because all possibilities of jaw evolution are equal parsimonious.

Orensanz (1990) placed the ctenognath type as basal based on the fossil record and developmental data. Although the earliest known fossil jaws are of the ctenognath type, most other types can be found just 10 million years later (Underhay and Williams, 1995). Even though larval stages of onuphids are observed to possess a ctenognath-like jaw apparatus (e.g., Paxton, 1986; Orensanz, 1990), the maxillary apparatus has an additional single forceps-like element, which is unusual for ctenognath jaws (H. Paxton, personal communication). The definition of ctenognath-type jaws (Kielan-Jaworowska, 1966; Szaniawski, 1996; Rouse, 2001) is violated by many dorvilleid species. For example, *Pettiboneia urciensis* possesses a maxillary apparatus with two denticle rows (Campoy and San Martin, 1980) that are clearly distinguishable in shape and size from the denticles and basal plates found in species of *Dorvillea*, *Protodorvillea*, and *Schistomeringos*. In Eibye-Jacobsen and Kristensen's (1994) cladistic analysis of "Dorvilleidae," the above taxa have only two of the nine characters of the maxillary apparatus in common and additionally these two characters are not independent from each other (the presence of free denticles and their number). In general, the maxillary characters are identified as some of the most homoplastic by their analysis. Thus, as in the case of the labidognath jaw type, it is most likely that the different ctenognath-like jaws are not homologous. Although our analyses generally favor Lumbrineridae as sister to all other eunicids, other dorvilleids may still come out basal due to paraphyly of "Dorvilleidae" and thus a ctenognath-like jaw might still be plesiomorphic. However, it still has to be considered with regard to both Figures 2 and 3 that an apparatus with a carrier and four to six pairs of maxillary elements may have evolved several times independently from such a ctenognath-like jaw, in contrast to the traditional view of jaw evolution assuming a single evolutionary event. Summarizing these findings, it may be more informative to drop these categories and instead focus more on identifying homologous elements in jaw complexes, especially in regard to the fossil record.

Another interesting aspect in jaw evolution is jaw symmetry. In the taxa of the *Rhamphoprion*-theme (Oenonidae, Lumbrineridae), the jaws are usually symmetrical with regard to the number of maxillae, whereas in the taxa of *Paulinites*-theme ("Eunicidae," Onuphidae) the jaws are asymmetrical (Edgar, 1984; Orensanz, 1990). The symmetrical/asymmetrical distinction is usually

not applied to ctenognath-bearing organisms (i.e., "Dorvilleidae"), but in general they can be considered symmetrical. Thus and based on the presented results, symmetry of the jaw apparatus appears to be plesiomorphic for Eunicida.

Some *Ophryotrocha* species (specifically ones with K-type jaws, see Dahlgren et al., 2001) have been hypothesized to possess a transitional stage from a ctenognath jaw to a labidognath and/or prionognath type (Tzetlin, 1980; Lu and Fauchald, 2000). However, based on the 18S and 16S analyses presented, a hypothesized transitional stage can be rejected. *O. labronica* is highly derived within a clade of dorvilleids that do not possess K-type jaws. Note that *O. labronica*'s position in the COI tree is suspect as the COI analyses fail to recover monophyly of recognized families well supported by other data partitions. Our molecular results support Paxton's (2004) hypothesis that the forceps part of *Ophryotrocha*'s apparatus is not homologous to the labidognath maxillae I. Considering the demonstrated independent evolution of Lumbrineridae and "Eunicidae"/Onuphidae, forceps-like maxillae have also evolved several times showing the evolutionary plasticity of jaw elements within Eunicida. Finally, Westheide and Riser's (1983) hesitant suggestion of a relationship between *Ophryotrocha* and *Parapodrilus* (based on the possession of a median unpaired caudal appendage at the pygidium) is corroborated by the analysis of the 18S data set.

Congruence and PABA

In this four-gene analysis, the novel PABA approach was more useful than an ILD or SH approach for detecting the phylogenetic signal present at given nodes within different partitions. The ILD test has been widely used to assess if data partitions should be combined, but it appears to be too conservative because it rejects combining the data with even a modest amount of incongruence (Table 5; also see Hipp et al., 2004). The ILD test fails to show congruence among most partitions in the overall approach. Using the taxon deletion approach, ILD indicates that the two dorvilleids, *Dorvillea erucaeformis* and *Protodrovillea kefersteinii*, are problematic in the 18S/28S comparison. The problematic nature of these taxa with regard to the 28S data set was also clearly shown by the SH and PABA approaches. Using ILD, *Glycera dibranchiata* also introduces incongruence in the 18S/16S comparison. However, the PABA approach shows that the 16S data set contributes strongly to node 2 uniting *G. dibranchiata* and *Paramphinome jeffreysi* (Table 10; Fig. 3). Furthermore, PABA investigations of the conflicting nodes in the 16S-alone trees (Fig. 4) reveal that adding any of the three other partitions to the 16S-alone data set decreases the BS value below 7 and the 16S-alone data set does not induce any increase when added to any of them (data not shown). Simulation studies show that the ILD test is strongly affected by incongruence in substitution parameters (Dolphin et al., 2000; Yoder et al., 2001; Barker and Lutzoni, 2002; Darlu and Lecointre, 2002; Dowton and Austin, 2002; Hipp et al., 2004), but more appropriate reconstruction models in parsimony are able to reduce this effect (Cunningham, 1997a).

The SH and PABA approaches appeared roughly equal in ability to determine which partitions were conflicting, but PABA outperforms the SH approach by more precisely pinpointing how given nodes are impacted by conflicting partitions. For example, even using the taxon-deletion SH approach, it is difficult to detect the problems at nodes 4 and 13 on the preferred topology (Fig. 3) because the taxa near those nodes do not seem to influence the SH results beyond the standard deviation (Table 7). Generally the taxon-deletion approaches are more affected by conflicts resulting from more than one node, especially stronger ones. However, the conflict in signal at those nodes is clearly evident using the PABA approach (Table 10). Nygren and Sundberg (2003) also commented that the SH approach can fail to pinpoint the cause of the conflict. After taxon deletion, their 16S data set did not reject their 18S topology, but their 18S data set still rejected their 16S topology.

The contribution of each partition to overall signal in the combined data can be gauged with the PABA approach. The mean bootstrap alteration values (δ) in Table 10 suggest that the 18S partition contributed the most signal (in general) followed by 28S, 16S, and then COI. This same rank order by mean δ values held for all nodes that were generally supported by all partitions. Moreover, as one might expect, this measure correlated with several other indicators of phylogenetic signal, including consistency (or homoplasy) index for the singular data sets on the Figure 3 topology (CI-18S = 0.730; 28S = 0.691; 16S = 0.469; COI = 0.408) and percentage of variable positions (18S: 30.5%; 28S: 33.4%; 16S: 46.6%; COI: 54.2%). Thus, the influence of a partition on topology and nodal support is correlated with the homoplasy of a partition.

In terms of this multipartition Eunicida study, several interesting instances of congruence and incongruence were noted. Our example of PABA was developed around node 5 because it was central to the issue of prionognath/labidognath monophyly. All partitions increase the support for this node even when BS values by individual partitions were low (Fig. 4 and Table 9). In contrast, judging solely from the BS values on the combined tree (Fig. 3), one would not realize that the 28S data set was a significant source of conflict at node 9 (BS value 100). However, when added as 4th partition, the conflicting signal in the 28S sequences is overwhelmed by the signal in the other three partitions (note no change in BS value from 100 to 100). A similar effect can be observed at the neighboring node 8. When added second, the 28S data set induces a slight conflict, but with additional data, an increasing support for this node can also be demonstrated from the 28S partition (Table 10).

The ability to assess the congruence on a node-by-node basis can be a significant advantage over global approaches (e.g., ILD and SH) of detecting congruence, because the PABA approach allows the simultaneous examination of nodes and data partition. The 28S and COI partitions appeared to introduce considerable conflict

into combined analyses. However, that conflict was not a global attribute of the data sets and for some nodes they provide considerable support. For example, both COI and 28S add congruent signal to nodes 3, 5, 7, 10, and 11. The ILD, and to some degree the SH, approach cannot distinguish congruent regions of the tree from incongruent areas. Partitions that conflicted are nonetheless interesting because if any conflict had been expected a priori, a division between mitochondrial and nuclear genes might seem the most likely. Instead, the nuclear 18S is more congruent with the mitochondrial 16S than the nuclear 28S, which is part of the same locus.

Conflict between data sets can be due to different reasons. Paralogy in a gene can lead to the recovery of gene duplication effects rather than speciation. However, in neither of the ribosomal genes has paralogy been observed. The variability of the 28S could be either too high or too low (i.e., too variable or too conservative) for the particular phylogenetic reconstruction at hand, but the percentage of variable positions in both the 18S and 28S data set is nearly the same. Furthermore, both genes have been used to address questions within Metazoa as well as within lophotrochozoan taxa like Mollusca (e.g., Mallatt and Winchell, 2002; Passamaneck et al., 2004). Finally, the rate variation between lineages is notable in the two nuclear partitions, 18S (Fig. 2) and 28S (data not shown). Specifically, taxa with labidognath jaws tend to have lower rates of nucleotide substitution than oenonids and dorvilleids. There is no obvious reason why labidognath-bearing individuals would have lower rates, other than dorvilleids and oenonids tend to be either small and/or parasitic and thus may have shorter generation times. Rate variation has been long known as causing instability in 18S analyses (e.g., Huelsenbeck, 1997; Peterson and Eernisse, 2001). Thus, instability due to the long branches in the dorvilleids is most likely the reason for the conflict between the 28S and the other partitions. However, the PABA approach also shows that with additional data the conflict can be overcome. Furthermore, it is noteworthy that when more data partitions are added the variation in rates is less pronounced (Fig. 3).

Combined analysis of all four partitions provides strong support for most of the nodes on the tree. Only at node 13 was more than one partition incongruent. We were encouraged that signal across different partitions could be identified and that, in general, bootstrap values and likelihood-ratio tests increased support for nodes as more data were added. This situation holds promise for obtaining better resolution deep in the annelid tree as more data are gathered.

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