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Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data

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The phylogenetic relationships of orbiniid taxa were reconstructed based on sequence data of the mitochondrial 16S rRNA and nuclear 18S rRNA genes. Both genes were analysed separately and in combination using maximum likelihood, Bayesian inference and maximum parsimony. Regardless of the method used, a clade consisting of the investigated Orbiniidae, *Methanoaricia dendrobranchiata* and *Questa* was strongly supported by the 18S dataset. The analysis of the combined dataset suggests inclusion of *M. dendrobranchiata* within the Orbiniidae with close relationships to species of *Orbinia* and *Phylo,* rather than as a sister taxon to all other orbiniids. Evidence is given for the paraphyletic status of *Leitoscoloplos*, *Naineris*, *Orbinia*, *Phylo* and *Scoloplos*, which represent the most species-rich genera of the Orbiniidae. It is thus reasoned that the morphological characters presently used for genus diagnosis are not informative for cladistic analysis. No support is found for the hypothesis that taxa of the Protoariciinae represent juveniles of Orbiniinae. Instead, in the case of *Protoaricia oerstedi*, strong support for a progenetic origin is found.

ADDITIONAL KEYWORDS: 16S – 18S – *Methanoaricia denrobranchiata* – phylogeny– progenesis – Questidae.

INTRODUCTION

Numerous specimens of a large polychaete have been found in association with hydrocarbon cold seeps in the Gulf of Mexico. First reported by MacDonald *et al.* (1990), these so called 'seepworms' bear an unusual combination of characters which makes it difficult to place them into a known polychaete family. Blake (2000) described the species as *Methanoaricia dendrobranchiata* (Fig. 1B) and included it in the Orbiniidae, a classification which has since been questioned.

The Orbiniidae comprise a group of deposit-feeding polychaetes, with a world-wide distribution. Approximately 150 species have been described in 18 genera (Glasby, 2000). The taxonomic history of the taxon was extensively reviewed by Hartman (1957), who established the classification of orbiniid worms in Protoariciinae and Orbiniinae. Protoariciinae are characterized as small and slender, and as possessing two (or more) peristomal rings (Fig. 1A), whereas most of the Orbiniinae are medium to large, with only one peristomal ring (Fig. 1C). Development and larval morphology are only known for a few orbiniid species: *Phylo foetida* was described by Eisig (1914), *Scoloplos armiger* and *S. simplex* (in this paper referred to as *Haploscoloplos fragilis*) by Anderson (1959, 1961), and *Leitoscoloplos pugettensis* and *S. acmeceps* by Blake (1980).

All these investigations are concordant with an early establishment of a single peristomal ring during ontogenetic development in the Orbiniinae. Blake (see Blake & Hilbig, 1990) was the first to report that there is evidence that some species, e.g. *Naineris laevigata* (see Giangrande & Petraroli, 1991) show two achaetous rings in early development, whereas the transition to a single ring occurs later. These observations gave rise to the hypothesis that many of the currently assigned Protoariciinae might be juveniles of taxa of the Orbiniinae (Blake, 1996). The alternative hypothesis would be to assume heterochronic evolution in the Protoariciinae. A progenetic origin of *Protoaricia oerstedi* was first hypothesized by Eisig (1914), who observed that the ventral pharyngeal organ (see Pur-

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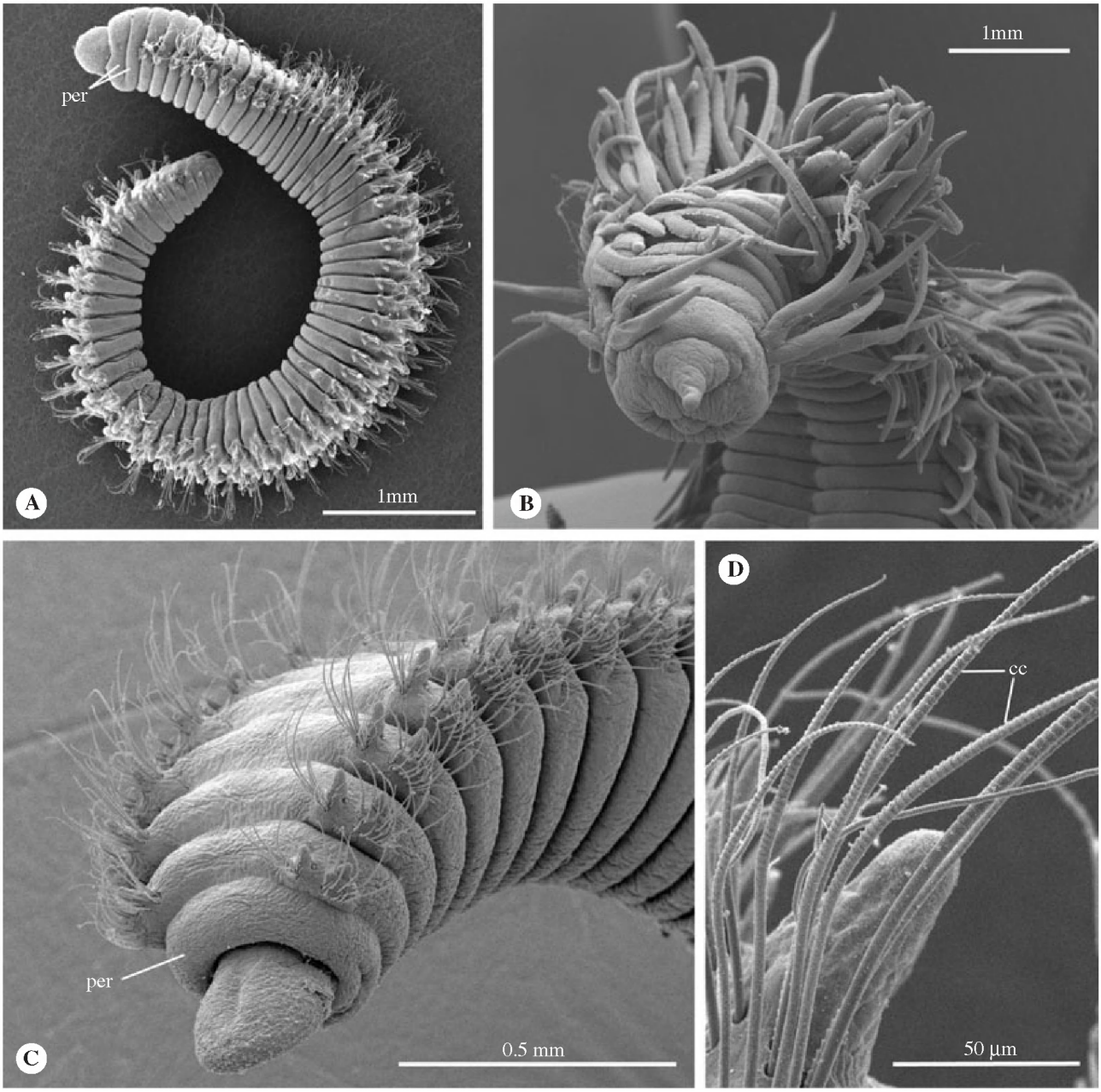

Figure 1. A, *Protoaricia oerstedi*, lateral view. B, *Methanoaricia dendrobranchiata*, anterior end. C, *Naineris dendritica*, anterior end. D, *Naineris dendritica*, notopodium with camerated chaetae. *Abbreviations:* cc, camerated chaetae; per, peristomal ring.

schke, 1988), the pygidial cirri and the shape of the thoracic neuropodia show a high similarity to the corresponding structures in juvenile specimens of *Naineris* or *Phylo*.

In a recent cladistic investigation of the phylogenetic interrelationships of the genera of Orbiniidae (Blake, 2000), characters relating to the number of peristomal rings were excluded and a data matrix consisting of 23 morphological absent/present characters was analysed. According to this analysis, *Methanoaricia dendrobranchiata* (the deep sea orbiniid described in the paper) is the sister taxon of all other orbiniids. Furthermore, Blake reclassified the Orbiniidae into the Microrbiinae (*Microorbinia*, *Orbiniella*, *Falklandiella*, and *Proscoloplos*) and a new combined Orbiniinae (the remaining genera), which included many genera of the former Protoariciinae (e.g. *Protoaricia*). The presence of distinct body regions was assessed as an

autapomorphy for the Orbiniinae, whereas their absence characterizes the Microrbiinae. However, the support for these clades is very weak and the monophyly of some of the genera is doubtful.

The Questidae, another taxon with uncertain affinities, comprise a group of interstitial species with 'oligochaetoid morphology' (Giere & Riser, 1981) and are, by some authors, regarded as representing the sister group of the Clitellata (Almeida *et al.*, 2003). This is contradicted by both molecular (Bleidorn, Vogt & Bartolomaeus, 2003a, b), and morphological (Rouse & Fauchald, 1997) studies, which both recover a closer relationship to the Orbiniidae.

The present study attempts to reconstruct orbiniid ingroup relationships (including the Questidae), as well as resolve the question of the phylogenetic position of *Methanoaricia*, using mitochondrial 16S rRNA and nuclear 18S rRNA gene sequences. Several studies have shown that these genes are suitable for unravelling ingroup relationships of annelid taxa (Dahlgren *et al.*, 2001; Jamieson *et al.*, 2002; Nygren & Sundberg, 2003; Borda & Siddall, 2004).

MATERIAL AND METHODS

Choice of taxa

The investigated orbiniid taxa (Table 1) represent a variety of all the major taxonomic groups. Outgroups (Table 1) represent putative sister taxa and have been chosen on the basis of hypotheses derived from morphological (Rouse & Fauchald, 1997) and molecular data (Bleidorn *et al.*, 2003a). Representatives of all scolecid families and the Parergodrilidae are included. The errant polychaete *Eunice pennata* is used to root all the obtained trees.

The 18S sequence of *Phylo foetida* was previously erroneously published as that of *Orbinia latreillii* (Bleidorn *et al.*, 2003b).

DNA extraction, PCR amplification and sequencing

DNA extraction was performed using the Qiagen DNeasy Tissue Kit, according to the manufacturer's instructions. PCR amplification of a ~1800 bp section of the 18S rRNA gene was performed using primer pair F19 + R993, or in two overlapping fragments using primer pairs F19 + R993 and F439 + R1843 (Table 2). A ~500 bp section of mitochondrial 16S rRNA gene was amplified using primer pair 16SarL and 16SbrH (Table 2). All amplifications were carried out on an Eppendorf Mastercycler and Eppendorf Mastercycler gradient. The PCR temperature reaction for the 18S was 94 °C for 2 min, 34 cycles at 94 °C for 30 s, 56 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 7 min. For the 16S the following file was used: 94 °C for 3 min, 34 cycles at 94 °C for 45 s, 50 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 7 min.

All products were purified with the Qiaquick PCR Purification Kit (Qiagen). Sequencing reactions were performed with a dye terminator procedure and loaded on capillary automatic sequencer CEQTM 8000 (Beckman Coulter, Fullerton CA, USA) following the recommendations of the manufacturer. The primers used in the sequencing reaction are listed in Table 2. All sequences (19 of the 18S rRNA gene and 22 of the 16S rRNA gene) were submitted to GenBank (for accession numbers see Table 1).

Alignment and data analysis

Sequences were aligned with CLUSTAL W (Thompson, Higgins & Gibson, 1994) using the default parameters for gap opening and gap penalty and subsequently manually edited by eye using BioEdit (Hall, 1999). Gap positions and regions that could not be aligned unambiguously were excluded from the analysis. The alignments, as well as several trees, have been submitted to TreeBASE. (http://www.treebase.org).

All phylogenetic analyses were carried out using PAUP v. 4.0b10 (Swofford, 2001) and MrBayes 3.0B4 (Huelsenbeck & Ronquist, 2001). A chi-square test of homogeneity of base frequencies across taxa was used to estimate the frequency distribution of observed number of substitutional changes per character for each gene. An ILD test (Farris *et al.*, 1995) was conducted using the partition homogeneity test in PAUP with 1000 replicates to test the congruence between the genes.

Unweighted parsimony with 1000 random addition replicates, heuristic search option with tree-bisection-reconnection (TBR) branch swapping, holding one tree per step and keeping all most parsimonious trees, was conducted for all datasets. Clade support was assessed with nonparametric bootstrap (Felsenstein, 1985) as implemented in PAUP (heuristic search, 500 replicates, TBR branch swapping, and simple addition sequence).

For estimating the appropriate model of sequence evolution, a hierarchical likelihood ratio test (hLRT) was carried out as implemented in MrModeltest v. 1.1b, a simplified version of Modeltest 3.06 (Posada & Crandall, 1998, 2001).

Maximum likelihood (ML) analysis was performed under the likelihood settings suggested for the given dataset by the result of the modeltest (see Table 3) using the heuristic search option with TBR branch swapping and ten random sequence addition replicates. Clade support was assessed with 500 bootstrap

Table 1. List of taxa used in this study with source and GenBank accession numbers (in bold text for newly sequenced taxa)

Taxa	Source	Accession nos. 18S	Accession nos. 16S
Eunice pennata (O.F. Müller, 1776) (Eunicidae)	GenBank	AY040684	AF321418
Scalibregma inflatum Rathke, 1843 (Scalibregmatidae)	Helgoland, Germany (coll. B. Hausam)	AF448163	**AY532331**
Cirrophorus furcatus Hartmann, 1957 (Paraonidae)	Santa Monica Bay, CA, USA (coll. C. Bleidorn)	**AY532349**	**AY532330**
Aricidea wassi Pettibone, 1965 (Paraonidae)	Santa Monica Bay, CA, USA (coll. C. Bleidorn)	**AY532351**	–
Arenicola marina (L., 1758) (Arenicolidae)	Arcachon, France (coll. C. Bleidorn)	AF508116	**AY532328**
Metasychis disparidentata (Moore, 1904) (Maldanidae)	Santa Monica Bay, CA, USA (coll. C. Bleidorn)	**AY532327**	**AY532352**
Dasybranchus caducus (Grube, 1846) (Capitellidae)	GenBank	AF448153	–
Thalassema thalassemum (Pallas, 1766) (Echiura)	Concarneau, France (coll. T. Bartolomaeus)	**AY532354**	–
Ophelia bicornis Savigny, 1818 (Opheliidae)	GenBank	AF508122	–
Sternaspis scutata (Ranzani, 1817) (Sternaspidae)	Adrian Sea, Croatia (coll. C. Bleidorn)	**AY532329**	**AY532353**
Cossura candida Hartman, 1955 (Cossuridae)	Santa Monica Bay, CA, USA (coll. C. Bleidorn)	**AY532350**	–
Parergodrilus heideri Reisinger, 1925 (Parergodrilidae)	GenBank	AJ310504	–
Stypocapitella subterranea Knöllner, 1934 (Parergodrilidae)	GenBank	AF412810	–
Questa paucibranchata Giere & Erséus, 1998 (Questidae)	GenBank	AF209464	–
Leitoscoloplos fragilis (Verrill, 1873)	Little Buttermilk Bay, MA, USA (coll. T. Dahlgren)	**AY532360**	**AY532341**
Leitoscoloplos pugettensis (Pettibone, 1957)	Santa Monica Bay, CA, USA (coll. C. Bleidorn)	**AY532365**	**AY532342**
Methanoaricia dendrobranchiata Blake, 2000	Brine Pool NR-1, Lousiana Slope, Gulf of Mexico, USA (coll. S. Hourdez)	**AY532333**	**AY532357**
Naineris dendritica (Kinberg, 1867)	Malibu, CA, USA (coll. C. Bleidorn)	**AY532358**	**AY532345**
Naineris laevigata (Grube, 1855)	GenBank	AY040696	–
Naineris quadricuspida (Fabricius, 1780)	Cattle Point, WA, USA (coll. K.M. Halanych)	**AY532361**	**AY532346**
Orbinia bioreti (Fauvel, 1919)	Concarneau, France (coll B. Hausam)	AF448158	**AY532334**
Orbinia latreillii (Audouin & Milne Edwards, 1833)	Roscoff, France (coll. C. Bleidorn)	**AY532355**	**AY532335**
Orbinia cf. *swani* Pettibone, 1963	Southern New England, MA, USA (coll. T. Dahlgren)	**AY532363**	**AY532336**
Orbiniella plumisetosa Buzhinskaja, 1992	Bering Island, Russia (coll. G. Mayer)	**AY532364**	**AY532348**
Phylo foetida (Claparède, 1870)	Arcachon, France (coll. C. Bleidorn)	**AY532356**	**AY532337**
Phylo michaelseni (Ehlers, 1897)	Southern New England, MA, USA (coll. A. Nygren)	**AY532362**	**AY532338**
Pettibonella multiuncinata Solis-Weiss & Fauchald, 1989	Twin Cayes, Belize (coll. C. Bleidorn)	**AY532359**	**AY532339**
Proscoloplos cygnochaetus Day, 1954	Roscoff, France (coll. H. Hausen)	AF448162	**AY532340**
Protoaricia oerstedi (Claparède, 1864)	Collioure, France (coll. C. Bleidorn)	AF508123	**AY532347**
Protoariciella uncinata Hartmann-Schröder, 1962	Buenos Aires, Argentina (coll. R. Elias)	AF508124	–
Scoloplos acmeceps Chamberlin, 1919	Morro Bay, CA, USA (coll. C. Bleidorn)	**AY532366**	**AY532344**
Scoloplos armiger (O.F. Müller, 1776)	GenBank	U50972	–
Scoloplos armiger (O.F. Müller, 1776)	Sylt, Germany (coll. T. Bartolomaeus)	**AY532367**	**AY532343**
Scoloplos (Leodamas) johnstonei Day, 1934	Cape Town, South Africa (coll. B. Hausam)	AF508126	**AY532332**

Table 2. Primers used for PCR and sequencing

Primer name	Sequence 5′–3′	Reference
18S		
F19	ACCTGGTTGATCCTGCCA	Turbeville *et al.* (1994)
R427	TCAGGCTCCCTCTCCGG	C. Lüter (pers. comm.)
F439 (3F)	GTTCGATTCCGGAGAGGGA	Giribet *et al.* (1996)
R993 (5R)	CTTGGCAAATGCTTTCGC	Giribet *et al.* (1996)
F1012 (5F)	GCGAAAGCATTTGCCAAGMA	Giribet *et al.* (1996)
R1372	GAGTCTCGTTCGTTATCGGA	C. Lüter (pers. comm.)
F1502	CAGGTCTGTGATGCCC	C. Lüter (pers. comm.)
R1825	CGGAAACCTTGTTACGAC	C. Lüter (pers. comm.)
R1843	GGATCCAAGCTTGATCCTTCTGCAGGTTCACCTAC	Elwood *et al.* (1985)
16S		
16SarL	CGCCTGTTTAACAAAAACAT	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)

Table 3. Models of sequence evolution used in the different analyses and the appropriate program settings

Dataset	Model	ML settings PAUP*	ML settings MrBayes
18S	SYM + I + Γ	Lset Base = equal Nst = 6 Rmat = (1.2021 2.4468 1.0467 0.9498 3.9515) Rates = gamma Shape = 0.5665 Pinvar = 0.3038;	lset nst = 6 rates = invgamma; prset RevMatPr = dirichlet(1.0,1.0,1.0,1.0,1.0,1.0) StateFreqPr = fixed(equal) ShapePr = uniform(0.05,50.0) PinVarPr = uniform(0.0,1.0);
16S	GTR + Γ	Lset Base = (0.3693 0.2141 0.1805) Nst = 6 Rmat = (623.1858 857.2402 770.7150 17.8028 3562.4211) Rates = gamma Shape = 0.3522 Pinvar = 0;	lset nst = 6 rates = gamma; prset RevMatPr = dirichlet(1.0,1.0,1.0,1.0,1.0,1.0) StateFreqPr = dirichlet(1,1,1,1) ShapePr = uniform(0.05,50.0);
18S +16S	GTR + I + Γ	Lset Base = (0.2725 0.2321 0.2634) Nst = 6 Rmat = (1.9304 2.7460 2.1765 0.9072 6.2102) Rates = gamma Shape = 0.5783 Pinvar = 0.2636;	lset nst = 6 rates = invgamma; prset RevMatPr = dirichlet(1.0,1.0,1.0,1.0,1.0,1.0) StateFreqPr = dirichlet(1,1,1,1) ShapePr = uniform(0.05,50.0) PinVarPr = uniform(0.0,1.0);

replicates using NNI branch swapping and simple addition sequence.

Bayesian analysis of the data set was conducted by using MrBayes 3.0B4 (Huelsenbeck & Ronquist, 2001). All priors were set according to the models as specified in Table 3. Four Markov chains, three heated (MCMCP temp = 0.3) and one cold, were started from a random tree and all four chains ran simultaneously for 500 000 generations, with trees being sampled every 250 generations for a total of 2001 trees. After the likelihood of the trees of each chain converged, the first 101 trees were discarded as burn-in. The majority-rule consensus tree containing the posterior probabilities of the phylogeny was determined from 1900 trees.

RESULTS

18S DATASET

After the exclusion of 575 ambiguous sites, the alignment contains 1571 positions, of which 864 are constant, 250 are variable and 457 are parsimony informative. The chi-square test of homogeneity of base frequencies across taxa resulted in no significant *P*-values ($\chi^2 = 97.0404$, d.f. = 99, $P = 0.537$). It can be assumed that compositional bias has no effect on the recovery of phylogenetic signal.

ML analysis and Bayesian inference revealed trees with the same topology (Fig. 2). The most likely tree of the ML analysis has a –ln likelihood value of 12459.92047. The chains of the Bayesian analysis

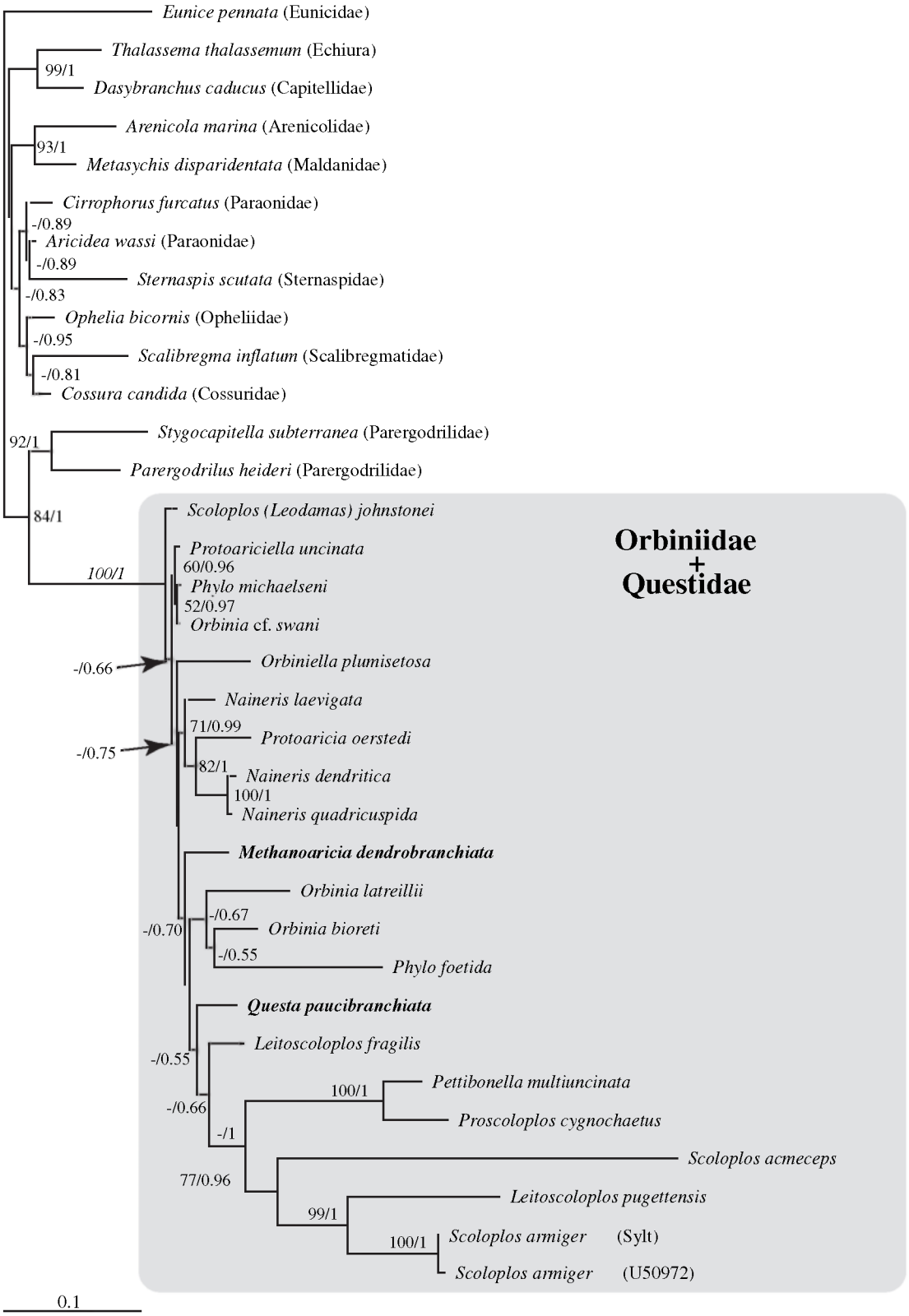

Figure 2. Maximum likelihood tree of the 18S rRNA gene dataset based on the SYM + I + Γ model of sequence evolution (–lnL = 12459.92047). The first value at each node represents the ML bootstrap support, the second the Bayesian posterior probability. Taxa which are discussed in detail in the discussion are in bold type.

reached equilibrium at no later than 25 250 generations. Bayesian posterior probabilities for each clade were derived from the remaining 1900 trees. Two equally parsimonious trees are recovered by the unweighted MP analysis (tree length = 2178; CI = 0.5197). The topology (results not shown) of the strict consensus differs slightly from the ML and Bayesian trees.

Regardless of the method used, a well supported clade [ML bootstrap (LBT) 100%, Bayesian posterior probabilities (BPP) 1.0, MP bootstrap (PBT) 100%] – consisting of the orbiniid taxa and *Questa* is recovered. Rather than a close relationship to one of the other scolecid taxa, a sister-group relationship between Orbiniidae + *Questa* and Parergodrilidae (LBT 84%, BPP 1.0, PBT 77%) is supported. In the ML and Bayesian analyses within the outgroup, the Paraonidae appear paraphyletic with regard to *Sternaspis*. However, this is not supported in the MP analysis (PBT 83% for a monophyletic Paraonidae). A close relationship between Maldanidae and Arenicolidae (LBT 93%, BPP 1.0, PBT 85%) as well as Capitellidae and Echiura (LBT 99%, BPP 1.0, PBT 99%) receives high support.

The orbiniid ingroup relationships are poorly resolved and are in most cases characterized by short branch lengths. Clades which are well supported by all methods are *Pettibonella* + *Proscoloplos* (LBT 100%, BPP 1.0, PBT 100%), *Scoloplos acmeceps* + (*Leitoscoloplos pugettensis* + *Scoloplos armiger*) (LBT 77%, BPP 0.96, PBT 90%), and *Naineris laevigata* + (*Protoaricia oerstedii* + (*Naineris dendritica* + *N. quadricuspida*) (LBT 71%, BPP 0.99, PBT 68%). All orbiniid genera that are represented by more than one taxon (*Leitoscoloplos*, *Naineris*, *Orbinia*, *Phylo*, and *Scoloplos*) appear paraphyletic in all analyses regardless of the method used. *Methanoaricia* and *Questa* are always recovered as orbiniid ingroup taxa. *Scoloplos* (*Leodamas*) *johnstonei* appears to be the most basal orbiniid (BPP 0.66) but this finding is not well supported.

16S DATASET

After excluding 193 ambiguous sites, the alignment of the 16S dataset contains 382 characters, of which 149 are constant, 44 are variable and 189 are parsimony informative. The chi-square test of homogeneity of base frequencies across taxa resulted in no significant *P*-values ($\chi^2 = 27.8839$, d.f. = 66, $P = 0.999$).

The ML tree (– lnL = 3943.65274) is illustrated in Figure 3 and ML bootstrapping values and Bayesian posterior probabilities are given at the nodes. Three equally parsimonious trees (results not shown) are recovered by MP analysis (tree length = 904, CI = 0.4306).

A monophyletic orbiniid clade is substantiated in all analyses (LBT 95%, BPP 1.0, PBT 95%). Well supported clades of the 18S analysis are also recovered in these analyses: *Protoaricia* + (*Naineris dendritica* + *N. quadricuspida*) (LBT 95%, BPP 1.0, PBT 95%), *Pettibonella* + *Proscoloplos* (LBT 80%, BPP 1.0, PBT 80%), *Scoloplos acmeceps* + (*Leitoscoloplos pugettensis* + *Scoloplos armiger*) (LBT 98%, BPP 1.0, PBT 98%). A close relationship of *Leitoscoloplos fragilis* to the latter clade also receives support, though less strongly (LBT 68%, BPP 0.93, PBT 68%). *Methanoaricia* appears in the ML and Bayes analysis as an orbiniid ingroup taxon and the same holds true for all of the three equal most parsimonious trees.

COMBINED ANALYSIS

The pairwise ILD-test for the two genes was not significant ($P = 0.233$), indicating that combining the data would be meaningful. After the exclusion of 773 sites the combined data matrix includes 1949 unambiguously aligned characters, of which 1054 are constant, 301 are variable and 594 are parsimony informative. The chi-square test of homogeneity of base frequencies across taxa resulted in no significant *P*-values ($\chi^2 = 59.5036$, d.f. = 66, $P = 0.7$).

The resolution of the tree is clearly improved with the combination of the two datasets. Heuristic search found a single most parsimonious tree (Fig. 4) in the unweighted MP analysis (tree length = 2641, CI = 0.5388). One tree (– lnL = 14756.30445) is obtained in the ML analysis (Fig. 5). The chains of the Bayesian analysis reached equilibrium at no later than 25 250 generations and the posterior probabilities derived from the remaining trees (1900) are mapped on the ML tree (Fig. 5).

All well-supported groups of the separate analyses received equal or better support from the combined analysis. The monophyly of the Orbiniidae is strongly supported (LBT 100%, BPP 1.0, PBT 100%) regardless of the method used and the same holds true for clades consisting of *Protoaricia* + (*Naineris dendritica* + *Naineris quadricuspida*) (LBT 100%, BPP 1.0, PBT 100%), *Pettibonella* + *Proscoloplos* (LBT 100%, BPP 1.0, PBT 100%) and *Scoloplos acmeceps* + (*Leitoscoloplos pugettensis* + *Scoloplos armiger*) (LBT 98%, BPP 1.0, PBT 98%). The topology of the trees obtained by ML/Bayes and MP differs slightly in the position of *Leitoscoloplos fragilis*. Whereas in the former a close relationship to a clade consisting of (*Pettibonella* + *Proscoloplos*) + (*Scoloplos acmeceps* + (*Leitoscoloplos pugettensis* + *Scoloplos armiger*)) is supported (LBT 56%, BPP 0.98), the latter recovers a relationship to all other orbiniids.

A clade consisting of *Phylo michaelseni* + *Orbinia* cf. *swani* (LBT 92%, BPP 1.0, PBT 88%) is recovered by

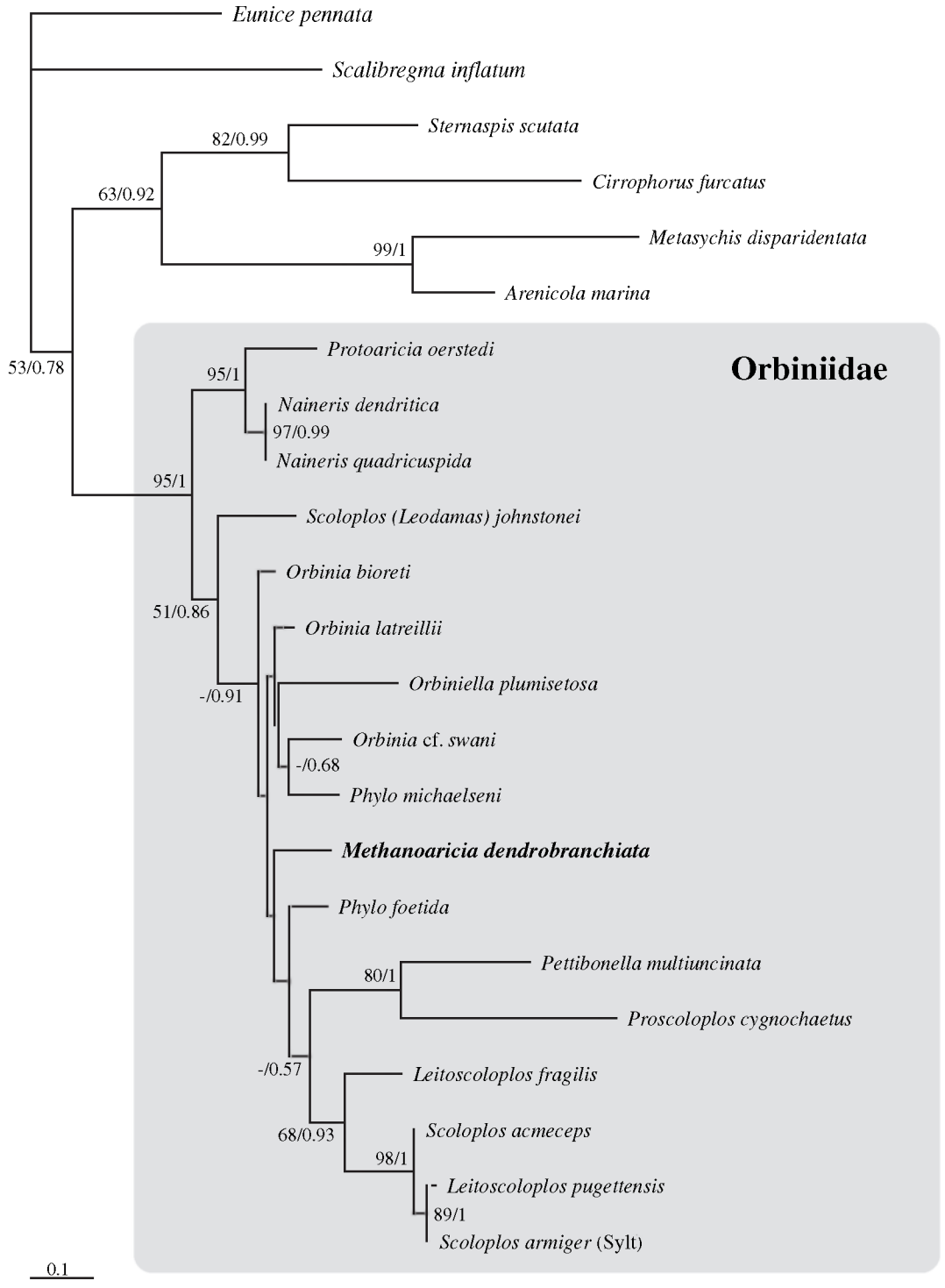

Figure 3. Maximum likelihood tree of the mitochondrial 16S rRNA gene dataset based on the GTR + Γ model of sequence evolution (–lnL = 3943.65274). The first value at each node represents the ML bootstrap support, the second the Bayesian posterior probability. Taxa which are discussed in detail in the discussion are in bold type.

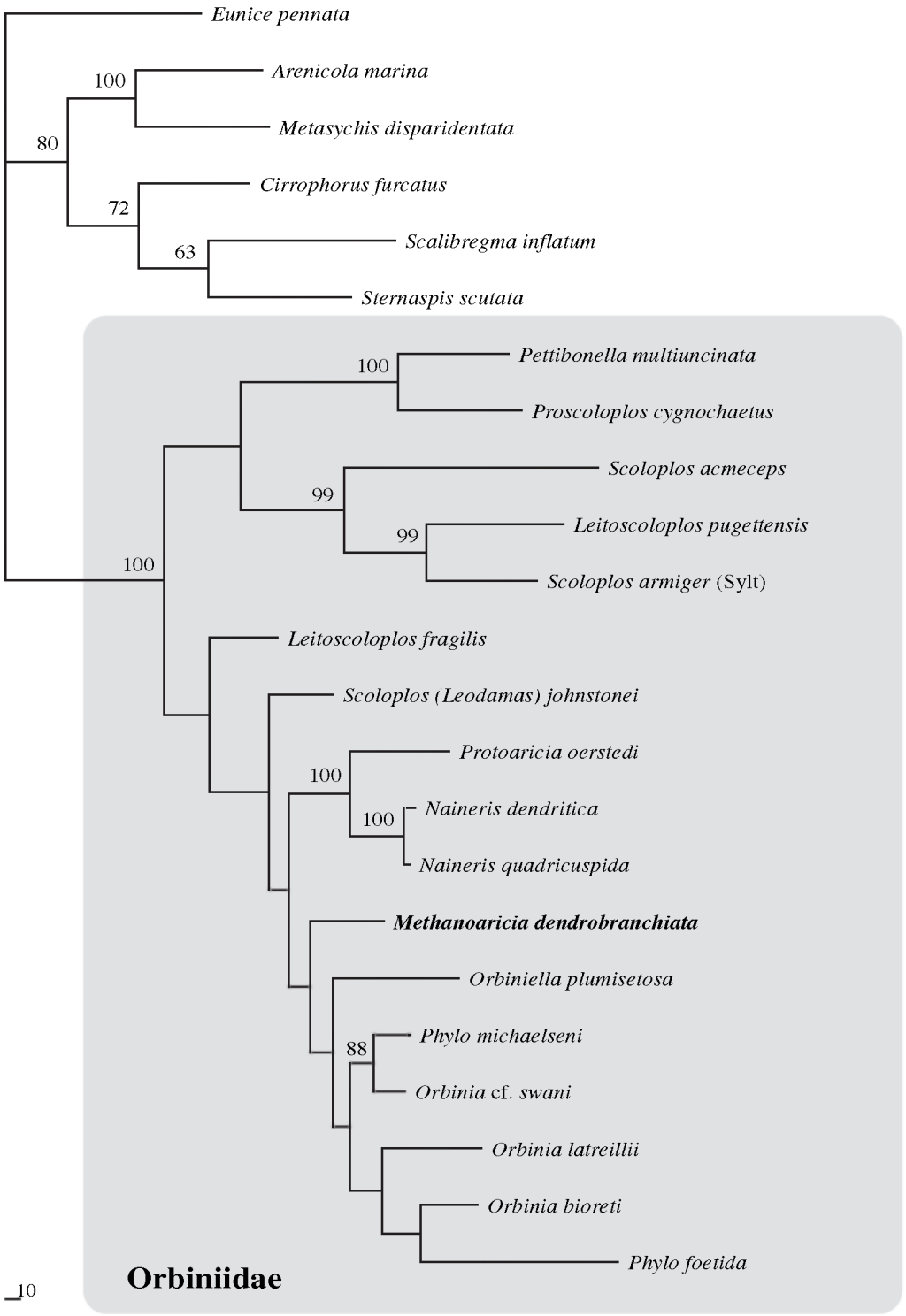

Figure 4. Most parsimonious tree (tree length = 2641, CI = 0.5388) of the maximum parsimony analysis of the combined dataset. The values at each node represent the MP bootstrap support. Taxa which are discussed in detail in the discussion are in bold type.

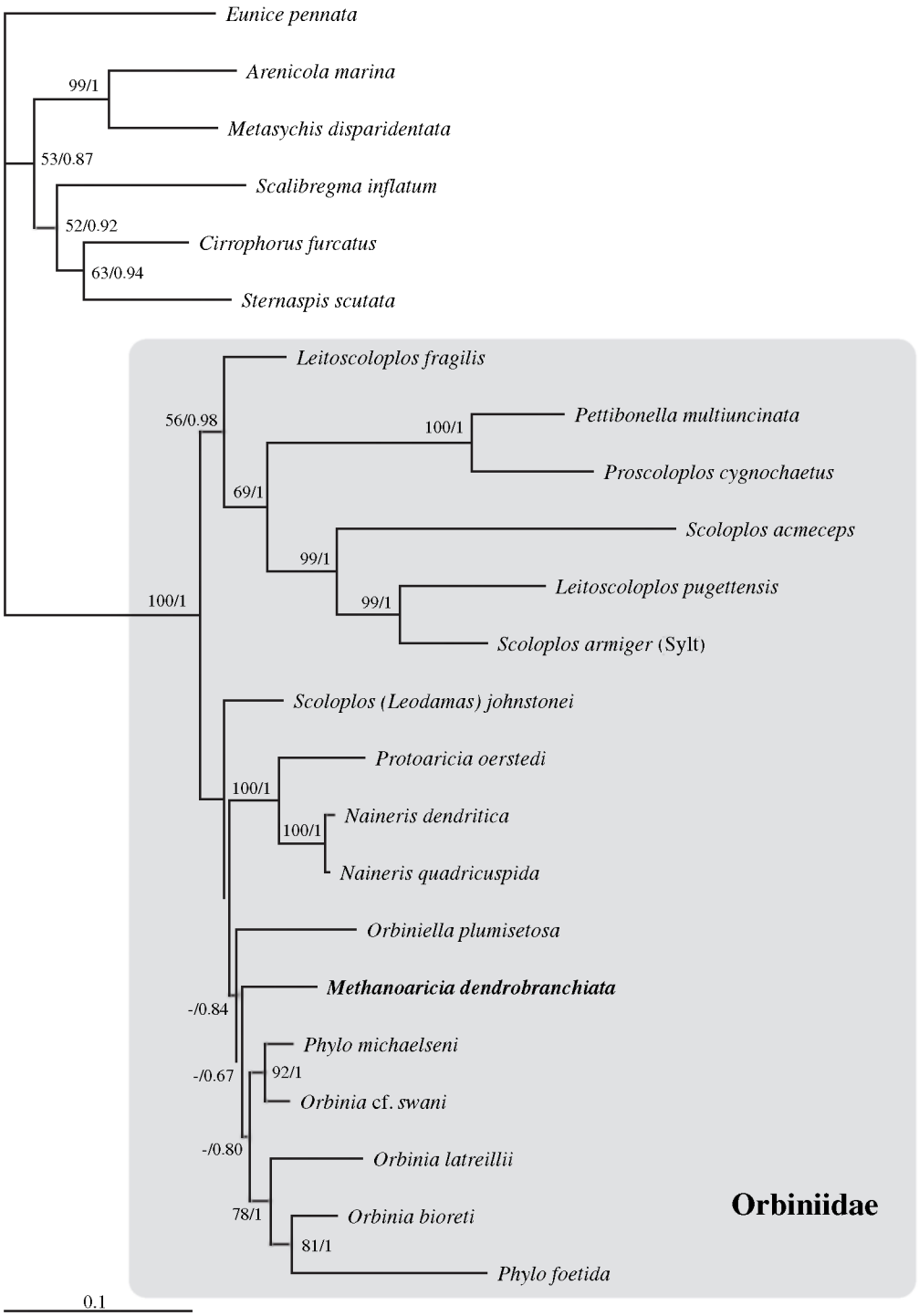

Figure 5. Maximum likelihood tree of the combined dataset based on the GTR + I + Γ model of sequence evolution (–lnL = 14756.30445). The first value at each node represents the ML bootstrap support, the second the Bayesian posterior probability. Taxa which are discussed in detail in the discussion are in bold type.

all methods, whereas *Phylo foetida* clusters with *Orbinia latreillii* and *O. bioreti* (LBT 78%, BPP 1.0). *Methanoaricia* appears as the sister taxon to all *Phylo* and *Orbinia* taxa in the Bayesian (BPP 0.84) and ML analysis. The position of *Scoloplos* (*Leodamas*) *johnstonei* remains uncertain, but appears to be more basal.

DISCUSSION

Orbiniid monophyly and the phylogenetic position of the questids

Analysis of the 18S dataset strongly supports the monophyly of a clade consisting of the orbiniids, *Methanoaricia* and *Questa*. The position of the enigmatic Questidae has remained controversial since their discovery by Hartman (1966). This family comprises a group of interstitial polychaetes that superficially resemble marine oligochaetes. Like them they are annelids with gonads limited to a few body segments (Giere & Riser, 1981), while their glandular epidermis, which forms a cocoon, is sometimes hypothesized as homologous to the clitellum of the Clitellata (Almeida *et al.*, 2003).

However, the presence of nuchal organs, the prostomial position of the supraoesophageal ganglia and the absence of an acrosomic tube in the spermatozoa are typical polychaete characters (Jamieson & Webb, 1984; Rouse & Fauchald, 1997; Giere & Erséus, 1998). Furthermore, phylogenetic analyses of large 18S datasets including many clitellate taxa always recover a well supported orbiniid-questid clade (Erséus, Prestegaard & Källersjö, 2000; Rota, Martin & Erséus, 2001; Bleidorn *et al.*, 2003a, b). The present analysis suggests that the questids are an orbiniid ingroup taxon, so that the peculiarities concerning the similarities in their reproductive biology to marine oligochaetes should be interpreted as due to convergent evolution.

One morphological character that is frequently proposed as a possible autapomorphy for the substantiation of orbiniid monophyly is the dorsal shifting of the parapodia in the abdomen (Fauchald & Rouse, 1997). This regionalization of the body in a dorsoventrally compressed 'thorax' and more fragile 'abdomen' arises from the general organization of the body musculature (Glasby, 2000). While in medium to large sized taxa like *Leitoscoloplos*, *Naineris*, *Orbinia*, *Phylo* and *Scoloplos* a distinct transition between thorax and abdomen is conspicuous, the transition is only weak or not observable in small sized taxa (e.g. *Orbiniella*, *Proscoloplos*). Such a transition is also absent in *Questa* and *Methanoaricia*.

A character which supports the monophyly of an orbiniid–questid clade is the presence of camerated (sometimes termed crenulated) chaetae (Fig. 1D). The formation of these characteristic crenulations is achieved by rings of microvilli and is described in detail by Hausam & Bartolomaeus (2001). This type of chaetae, typical of Orbiniidae (Rouse & Pleijel, 2001) is also found in *Methanoaricia* (Blake, 2000) and *Questa* species (Giere & Erséus, 1998). All these chaetae can differ in their appearance and show a great variability across orbiniid and questid taxa. Nevertheless, with the molecular data currently available it is more parsimonious to assume a common origin of these chaetae in an ancestor of questids and orbiniids. The lack of this type of chaetae in the newly discovered *Periquesta canariensis* (Brito & Nunez, 2002) is interpreted as a derived condition. Internally chambered chaetae are present in some taxa of the Nephtyidae (Rouse & Pleijel, 2001). My own SEM investigations of *Nephtys hombergi* have shown that these chaetae lack the typical regular pattern of the camerated chaetae that are unique to the taxa mentioned above. Ultrastuctural differences between the crenulations of the orbiniid chaetae and the dentition of brachiopod setae are discussed in Hausam & Bartolomaeus (2001).

Phylogenetic position of *Methanoaricia dendrobranchiata*

Since the discovery of the seepworm (MacDonald *et al.*, 1990) and its description as *Methanoaricia dendrobranchiata* by Blake (2000), a handful of research papers have investigated its biology. Hourdez *et al.* (2001, 2002) described the functional respiratory anatomy and investigated its respiratory adaptation to the strongly hypoxic and sulphidic environment which it inhabits. Eckelbarger & Young (2002) noted its modified sperm morphology, while Menon *et al.* (2003) described its epidermal ultrastructure in detail.

However, while *M. dendrobranchiata* has been closely studied, its phylogenetic position is far from being satisfactorily resolved. An unusual combination of characters led to the problem of identifying its systematic position. Although camerated chaetae and vascular branchiae are typical orbiniid characters, the nature of the prostomium, the early beginning of the branchiae, the organization of the parapodia as well as the absence of distinct body regions clearly distinguish this species from other large orbiniids.

The separate 18S and 16S datasets, as well as the combined dataset, do not support the hypothesis that *Methanoaricia* is 'a separate and distinct sister taxon' of the orbiniids (Blake, 2000). Instead, they suggest its inclusion as an orbiniid ingroup taxon. The combined dataset suggests a close relationship between it and *Orbinia* and *Phylo* spp., as together they represent orbiniids with a large body size. The derived mor-

phology of *Methanoaricia* could thus be interpreted as an adaptation to its unique biology and suggest that it has evolved due to the hypoxic and sulphidic environment in which it lives. This has been already suggested for the numerous branchiae by Blake (2000), who interpreted them as an adaptation to a low oxygen environment.

Ingroup relationships

A remarkable result of the phylogenetic analysis of the molecular data is the non-monophyly of all genera which have been included with more than one species. The genera involved (*Leitoscoloplos*, *Naineris*, *Orbinia*, *Phylo* and *Scoloplos*) are the most species-rich taxa in the Orbiniidae. *Leitoscoloplos* was reviewed by Mackie (1987), who distinguished five morphological groups and posited a possible polyphyletic origin of species referred to this taxon. *Scoloplos* is usually divided into two subgenera: *Leodamas*, comprising species with an early appearance (in respect to the anterior end) of branchiae, is mainly distributed in the southern hemisphere, whereas *Scoloplos sensu stricto*, which comprises the species with a later beginning of the branchiae, is more common in the northern hemisphere (Blake, 1996).

The main difference between *Leitoscoloplos* and *Scoloplos* species is that only the latter bear stout, ribbed chaetae in the thoracic neurosetae. Kruse, Reusch & Schneider (2003) suggest that *S. armiger* actually represents at least two sibling species: one with a direct, holobenthic development from egg cocoons, which inhabits intertidal zones and another with pelagic larvae preferring subtidal habitats.

The specimen of *S. armiger* investigated in the present study was also collected from the intertidal zone, and the molecular data strongly support a closer relationship to *L. pugettensis* (which also develops from egg cocoons) than to *Scoloplos acmeceps*, which produces pelagic larvae and no egg cocoons. The phylogenetic position of *L. fragilis*, another species which develops from egg cocoons, depends on the choice of method and gene. It seems that at least one of these modes of reproduction can be easily achieved convergently within orbiniids, although it appears questionable whether the characters used for species and genera diagnosis in *Scoloplos* and *Leitoscoloplos* are also informative for cladistic analysis. The investigated species of *Leodamas* might be a basal orbiniid taxon, but this is only poorly supported by the molecular data. The result that the former *Scoloplos* subgenera *Scoloplos s.s.* and *Leodamas* are distinct taxa which do not constitute sister groups is congruent with the findings of Blake (2000). This suggests that a revision of the taxonomy of the taxa assigned to *Scoloplos* and *Leitoscoloplos* is overdue.

While the paraphyly of *Orbinia* with regard to *Phylo* has long been suspected, resulting in the latter becoming a subgenus of the former (Pettibone, 1957), the finding that *Phylo* is itself paraphyletic is surprising. Species of *Phylo* are unique in possessing lanceolate spines on some posterior neuropodia; this can be seen as a strong autapomorphy. The combined molecular data support a close relationship of those *Orbinia* and *Phylo* species that overlap regionally. Thus, *O. latreillii*, *O. bioreti* and *P. foetida*, each collected from the French Atlantic coast, are supported as a monophyletic clade and the same applies to *Orbinia* cf. *swani* and *P. michaelseni*, both collected from the North American east coast. A clade consisting of all considered *Orbinia* and *Phylo* species is only poorly supported by the molecular data.

The paraphyly of *Naineris* with regard to *Protoaricia* is strongly supported by the analysis of the 18S dataset. In several of the collected specimens of *Protoaricia oerstedi* one could see eggs through the body wall. This observation corresponds with that of Augener (1936) and clearly demonstrates that *Pr. oerstedi* is a valid taxon and not a juvenile of *Naineris*. Instead, progenetic evolution, as hypothesized by Eisig (1914), appears to represent the best explanation for the similarities between *Protoaricia* and juveniles of *Naineris*.

In accordance with the results of Solis-Weiss & Fauchald (1989) all analyses of the molecular data recover a well supported *Pettibonella* + *Proscoloplos* clade. Both taxa are unique in possessing swan-shaped hooks. The modus of reproduction for *Pettibonella* is unknown; asexual reproduction is reckoned for *Proscoloplos* (Kelaher & Rouse, 2003), but this has to be confirmed in further investigations.

The relationships of *Protoariciella uncinata* (only represented in the 18S dataset) and *Orbiniella plumisetosa* remain unclear. Both are small orbiniids with a rounded prostomium and two peristomal rings. Like them, *Naineris* also possesses a round prostomium and to follow the hypothesis of Blake (1996) – that both taxa might represent different juvenile stages of *Naineris* species – it should be expected that they fall into a clade with *Naineris* or that their sequence data are identical with one of the investigated *Naineris* species. However, this is not the case. Analysis of the present data suggests that all taxa of the former Protoariciinae investigated in this study (*Orbiniella plumisetosa, Pettibonella multiuncinata, Protoaricia oerstedi, Protoariciella uncinata* and *Proscoloplos cygnochaetus*) represent valid species.

It is clear that the results of this molecular study stand in contrast to both the traditional view of Hartman (1957) and to the morphological cladistic analysis by Blake (2000). The differences in the phylogenetic position of *Methanoaricia* have been discussed above.

Blake's (2000) analysis splits the remaining orbiniids into two groups: the first comprises all the simple organized forms which lack body regionalization (Microrbiniinae) and the second includes most of the larger forms that show a distinct body regionalization (Orbiniinae). Support is given neither to his new combined Orbiniinae nor to the Microrbiniinae. Instead, it has to be concluded that the taxa assigned to the latter gained their simplification (= loss of characters) independently. Looking for reasons which explain these discrepancies, the fact that Blake's analysis was at genus level, where he used the characters of the type species (when available) for his data matrix, must be taken into account. The molecular data strongly indicate that most of the currently assigned orbiniid genera represent paraphyletic assemblages. Thus it can be reasoned that the characters which are presently used for genus diagnosis are not useful for the cladistic analysis.

Yet another problem for cladistic analysis can be the number of secondary loss of characters (e.g. Purschke, Hessling & Westheide, 2000). This might, for example, have been achieved by progenetic evolution, which is assumed to have occurred in many annelid taxa (e.g. Westheide, 1987), although in most cases an evolutionary scenario is posited. However, a phylogenetic hypothesis of the relevant taxa is necessary in order to make assumptions about heterochronic evolution (Fink, 1988), rather than vice versa. This is demonstrated by *Protoaricia oerstedi*, where progenesis – maturation at smaller size (McKinney, 1988) – represents the best explanation for the presence of larval structures in the adult.

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