

Phylogenetic relationships of Serpulidae (Annelida: Polychaeta) based on 18S rDNA sequence data, and implications for opercular evolution

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Abstract

Phylogenetic relationships of (19) serpulid taxa (including Spirorbinae) were reconstructed based on 18S rRNA gene sequence data. Maximum likelihood, Bayesian inference, and maximum parsimony methods were used in phylogenetic reconstruction. Regardless of the method used, monophyly of Serpulidae is confirmed and four monophyletic, well-supported major clades are recovered: the Spirorbinae and three groups hitherto referred to as the *Protula*-, *Serpula*-, and *Pomatoceros*-group. Contrary to the taxonomic literature and the hypothesis of opercular evolution, the *Protula*-clade contains non-operculate (*Protula*, *Salmacina*) and operculate taxa both with pinnulate and non-pinnulate peduncle (*Filograna* vs. *Vermiliopsis*), and most likely is the sister group to Spirorbinae. Operculate Serpulinae and poorly or non-operculate Filograninae are paraphyletic. It is likely that lack of opercula in some serpulid genera is not a plesiomorphic character state, but reflects a special adaptation.

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Introduction

Serpulids are common members of marine hard-substratum communities with a worldwide distribution (Rouse and Pleijel 2001). Currently, there are approximately 343 species which are assigned to 74 genera (H.A. ten Hove, unpublished data). Serpulidae have

distinctive calcareous tubes and bilobed tentacular crowns, each with numerous radioles that bear shorter secondary branches (pinnules) on the inner side. It is common for one radiole (rarely two) to be modified into an operculum (Thomas 1940; Segrove 1941; Orrhage 1980) (Fig. 1A, B). The operculum is used to block the tube in case of danger from predators or desiccation (ten Hove 1984). The structure of the operculum and its peduncle in the adult stage was historically used to divide the Serpulidae into three subfamilies: Serpulinae, Filograninae, and Spirorbinae (Chamberlin 1919; Rioja 1923; Fauvel 1927).

The Serpulinae bear opercula that never have pinnules on their stalks in the adult stage, whereas the Filograninae

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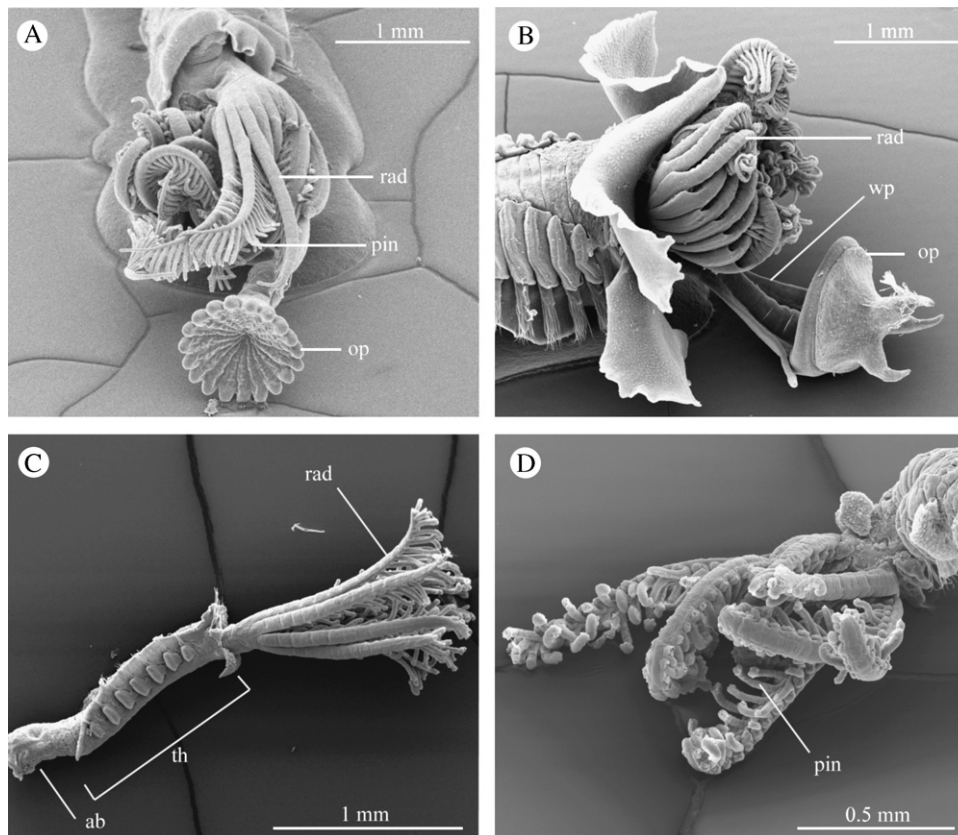


Fig. 1. (A) *Serpula concharum*, ventral view. (B) *Pomatoceros triqueter*, lateral view. (C) *Salmacina* sp. (D) *Salmacina* sp., tentacular crown, pinnules with swollen tips. Abbreviations: ab = abdomen, op = operculum, pin = pinnule, rad = radiole, th = thorax, wp = winged peduncle.

are defined by an either absent (Figs. 1C and D) or poorly developed operculum born on a pinnulated stalk that retains its pinnules even in adult stage. Like Serpulinae, the Spirorbinae have opercula on a modified stalk. Many spirorbins use their opercula as specialized brood chambers (Bailey 1969; Pillai 1970). They have distinctive coiled tubes and corresponding asymmetric bodies. Because of their unique morphology, Spirorbinae are considered by several authors to be a separate family, presumably the sister-group to serpulids (Pillai 1970; Fauchald 1977; Knight-Jones 1978; Uchida 1978; Bianchi 1979). This classification is supported by the observation that spirorbin opercula are derived from different branchial radioles than in other serpulids.

But even within Serpulidae the operculum and its development vary. It proceeds either directly or indirectly (ten Hove 1984). Direct development occurs when the operculum develops on a smooth peduncle without pinnules as observed in *Pomatoceros triqueter* (Linnaeus, 1758) (Segrove 1941), in *Spirobranchus* species and all Spirorbinae (e.g. Nott 1973). In contrast, indirect development is characteristic of operculate filigranin taxa (*Apomatus*, *Josephella*, *Filigrana*), and of some Serpulinae (*Vermiliopsis*, *Serpula*, *Hydroides*, *Crucigera*). Here the operculum develops on a pinnulated peduncle. In the

Filigraninae, the stalk retains its pinnules during development and into the adult stage. In the indirect-developing Serpulinae, the pinnules are lost during development (Müller 1864). Indirect development is thought to be plesiomorphic because of Müller's (1864) observation that juvenile individuals of *Serpula* species originally do not possess an operculum, and subsequently pass through a stage in which their opercula have pinnulated stalks prior to loss of their pinnules. Direct opercular development could thus be regarded as apomorphic (ten Hove 1984).

Starting from Müller's (1864) ontogenetic perspective as well as from functional viewpoints (Zeleny 1905; Ludwig 1957), ten Hove (1984) proposed an evolutionary scenario for serpulid phylogeny based on a transformation series of the branchial crown. This series begins with non-operculate forms (mostly filigranin serpulids) and leads to highly modified operculate genera (Serpulinae and Spirorbinae).

While division of Serpulidae into Spirorbinae, Filigraninae and Serpulinae is a widely used classification scheme (Fauvel 1927; Bianchi 1981; Hobson and Barse 1981; Hartmann-Schröder 1996; Hayward and Ryland 1996), several authors question the status of these subfamilies and whether they are reflective of true phylogenetic relationships within the Serpulidae (e.g. ten

Hove 1984; Smith 1991; Kupriyanova and Jirkov 1997). Questions regarding this classification have arisen because it is based on variable morphological characters. These include the number and structure of thoracic chaetigers and the structure of the operculum itself (ten Hove and Jansen-Jacobs 1984). Since the development of the operculum can differ within the subfamilies (Nogueira and ten Hove 2000), the Filograninae and Serpulinae are regarded as paraphyletic by some authors (ten Hove 1984; Smith 1991; Kupriyanova and Jirkov 1997). The status of the spirorbins as a monophyletic serpulid ingroup is generally accepted today (ten Hove 1984; Fitzhugh 1989; Smith 1991; Kupriyanova 2003; Macdonald 2003).

The present study is an attempt to assess the phylogenetic relationships within the Serpulidae by using 18S rDNA sequence data. The 18S rRNA gene is suitable for discerning relationships among annelid taxa at this taxonomic level (Nygren and Sundberg 2003; Borda and Siddall 2004; Bleidorn 2005; Bleidorn et al. 2005) and should increase our understanding of this difficult group. The resulting phylogenies will also be used to re-evaluate ten Hove's (1984) evolutionary scenario.

Material and methods

18S rRNA gene sequence data for a total of 19 serpulid taxa were drawn from specimens collected or were obtained from GenBank (Table 1). A sister-group relationship between Serpulidae and Sabellidae has been hypothesized in morphological studies (ten Hove 1984; Fitzhugh 1989; Rouse and Fauchald 1997; Kupriyanova 2003). Therefore, two sabellid species are included as outgroup taxa, together with representatives of the Sabelliidae, the Oweniidae and the Terebellidae. All trees obtained were rooted with the sequences of the errant polychaete *Eunice pennata* (Müller, 1776) (Eunicidae).

Samples for DNA extraction were preserved in 100% ethanol and stored at -20°C . Specimens of all examined species are deposited in the collection of the Zoological Museum of the University of Amsterdam (ZMA) or in the collection of the South Australian Museum (SAM, Table 1). Genomic DNA was extracted using a Qiagen DNeasy Tissue Kit following the manufacturer's instructions. PCR amplification of a ~ 1800 bp region of the 18S rDNA gene was done using primer pairs F19 + R1843. Additional primers were used for sequencing (see Bleidorn et al. 2005 for primer names and protocols). Using Eppendorf Hot Start *Taq* polymerase, all amplifications were carried out on an Eppendorf Mastercycler or Eppendorf Mastercycler Gradient with the following PCR temperature profile: 94°C for 2 min; 34 cycles at 94°C for 30 s, 56°C for 1 min and 72°C for

2 min; final extension at 72°C for 10 min. After detection by gel electrophoresis the products were purified with the Qiaquick Gel Extraction Kit (Qiagen). Sequencing reactions were performed with a dye terminator procedure and loaded on a capillary automatic sequencer CEQ 8000 (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's recommendations. All sequences were submitted to GenBank (for accession numbers see Table 1). Sequences were aligned with CLUSTAL W (Thompson et al. 1994) using default parameters, and subsequently manually edited by eye using BioEdit (Hall 1999). Ambiguously aligned regions were excluded from the analysis. The alignment and trees have been submitted to TreeBASE (www.treebase.org).

For estimating the appropriate model of sequence evolution, a hierarchical likelihood ratio test was carried out as implemented in the program MrModeltest version 3.04 (Posada and Crandall 1998, 2001). The test criteria indicate that the substitution model of Tamura and Nei (1993), with equal base frequencies, invariant sites and gamma distribution (TrNef + I + Γ), is the optimal model.

The phylogenetic signal in the data was assessed using TreePuzzle 5.0 to conduct a likelihood-mapping analysis (Strimmer and von Haeseler 1997). This test was performed under the Tamura Nei substitution model (Tamura and Nei 1993), with gamma distribution and four categories. The probabilities were calculated for three topologies of a total of 10,000 quartets.

Maximum parsimony and likelihood analyses were done using PAUP* version 4.0b10 (Swofford 2001). Maximum likelihood analysis was performed under the likelihood settings suggested by Modeltest; the heuristic search options were tree-bisection-reconnection (TBR) branch swapping, and 10 random sequence addition replicates. Bootstrap support values (Felsenstein 1985) were determined from 1000 replicates subject to full heuristic searches with simple sequence addition and NNI branch swapping.

Maximum parsimony analyses were performed with equal weighting. Maximum parsimony searches were run with 100 random addition replicates, heuristic searches, and TBR branch swapping. Bootstrap values were determined from 1000 replicates subject to full heuristic searches with 10 random taxon addition and TBR branch swapping.

Bayesian analyses were conducted using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). All priors were set according to the model: 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). Four Markov chains, three heated and one cold, were started from a random tree and all four chains ran

Table 1. Taxa (newly sequenced, unless obtained from GenBank) and material studied

Taxon	Source	Accession no.	
<i>Crucigera zygophora</i> (Johnson, 1901) (Serpulinae)	Barkley Sound, BC, Canada (coll. T. Macdonald)	DQ242543	E3503 ^b
<i>Ditrupa arietina</i> Müller, 1776 (Serpulinae)	Collioure, France (coll. C. Bleidorn)	DQ140401	ZMA V.Pol. 5238 ^a
<i>Filograna implexa</i> Berkeley, 1835 (Filograninae)	Vadholmen, Hitra, Norway (coll. T. Bakken; 63°39.0'N 08°44.0'E)	DQ140402	ZMA V.Pol. 5239 ^a
<i>Ficopomatus enigmaticus</i> (Fauvel, 1923) (Serpulinae)	GenBank	AY577889	–
<i>Galeolaria caespitosa</i> Lamarck, 1818 (Serpulinae)	GenBank	AB106257	–
<i>Hydroides pseudouncinatus</i> Zibrowius, 1968 (Serpulinae)	Banyuls, France (coll. C. Bleidorn)	DQ140403	ZMA V.Pol. 5240 ^a
<i>Pomatoceros lamarckii</i> (Quatrefages, 1865) (Serpulinae)	Banyuls, France (coll. J. Lehrke)	DQ140404	ZMA V.Pol. 5241 ^a
<i>Pomatoceros triqueter</i> (Linnaeus, 1758) (Serpulinae)	Banyuls, France (coll. J. Lehrke)	DQ140405	ZMA V.Pol. 5242 ^a
<i>Protula</i> sp.1 (Filograninae)	GenBank	AY611453	–
<i>Protula</i> sp. 2 (Filograninae)	Banyuls, France (coll. J. Lehrke)	DQ140406	ZMA V.Pol. 5243 ^a
<i>Pseudochitinopoma occidentalis</i> (Bush, 1905) (Serpulinae)	Wizard Island, Barkley Sound, BC, Canada (coll. T. Macdonald)	DQ242542	E 3501 ^b
<i>Salmacina</i> sp. (Filograninae)	Banyuls, France (coll. J. Lehrke)	DQ140407	ZMA V.Pol. 5244 ^a
<i>Serpula concharum</i> Langerhans, 1880 (Serpulinae)	Banyuls, France (coll. J. Lehrke)	DQ140408	ZMA V.Pol. 5245 ^a
<i>Serpula vermicularis</i> Linnaeus, 1767 (Serpulinae)	Banyuls, France (coll. J. Lehrke)	DQ140409	ZMA V.Pol. 5246 ^a
<i>Spirobranchus corniculatus</i> (Grube, 1872) (Serpulinae)	Aquarium Berlin, probably from Indo- Pacific region (coll. J. Lehrke)	DQ140410	ZMA V.Pol. 5247 ^a
<i>Vermiliopsis infundibulum</i> (Philippi, 1844) (Serpulinae)	Banyuls, France (coll. J. Lehrke)	DQ140411	ZMA V.Pol. 5248 ^a
<i>Circeis armoricana</i> Saint-Joseph, 1894 (Spirorbinae)	Bamfield, BC, Canada (coll. T. Macdonald)	DQ242545	E 3476 ^b
<i>Spirorbis bifurcatus</i> Knight-Jones (1978) (Spirorbinae)	Bamfield, BC, Canada (coll. T. Macdonald)	DQ242569	E 3489 ^b
<i>Spirorbis spirorbis</i> (Linnaeus, 1758) (Spirorbinae)	GenBank	AY577887	–
<i>Sabella spallanzanii</i> (Gmelin, 1791) (Sabellidae)	GenBank	AY436350	–
<i>Sabella pavonina</i> Savigny, 1820 (Sabellidae)	GenBank	U67144	–
<i>Sabellaria alveolata</i> (Linnaeus, 1767) (Sabellariidae)	Arcachon, France (coll. C. Bleidorn)	DQ140412	–
<i>Gunnarea capensis</i> (Johansson, 1927) (Sabellariidae)	GenBank	AY577892	–
<i>Owenia fusiformis</i> Delle Chiaje 1841 (Oweniidae)	GenBank	AF448160	–
<i>Myriochele</i> sp. (Oweniidae)	GenBank	AY340437	–
<i>Amphitritides gracilis</i> (Grube, 1860) (Terebellidae)	GenBank	AF508115	–
<i>Eunice pennata</i> (Müller, 1776) (Eunicidae)	GenBank	AY040684	–

^aVouchers are deposited in the Zoological Museum, Amsterdam or

^bin the South Australian Museum, Adelaide.

simultaneously for 500,000 generations, with trees being sampled every 500 generations for a total of 1001 trees. After the likelihood of the trees of each chain converged,

the first 101 trees were discarded as burn-in. Posterior probabilities were determined from a majority rule consensus of 900 trees.

Finally, “approximately unbiased” (AU) and non-scaled bootstrap probability (NP) tests of a tree topology selection were performed using PAUP 4b10 (Swofford 2001) and CONSEL (Shimodaira and Hasegawa 2001; Shimodaira 2002). The following hypotheses were tested: (1) all serpulids are monophyletic, with the exception of Spirorbinae (i.e., Spirorbinae represents a ‘family’ of its own; e.g. Pillai 1970); (2) all serpulids are monophyletic, with the exception of Filograninae (i.e., Filiograninae is basal; e.g. Kupriyanova 2003); and (3) *Pomatoceros* + Spirorbinae form a monophyletic clade (ten Hove 1984).

Results

After the exclusion of ambiguous sites, the final alignment contained 1579 positions: 928 were constant, 159 parsimony uninformative, and 492 parsimony informative.

The likelihood-mapping analysis indicates that the data support a dissolved dichotomous tree with 93.6% support, a star-shaped structure with 4.1%, and a polytomous tree structure with 2.3%. Thus the aligned data display highly informative phylogenetic signals and few contradictory phylogenetic signals. This result has to be taken with some reservation, because likelihood mapping is likely to produce false positives (Nieselt-Struwe and von Haeseler 2001; Struck et al. 2002).

Maximum likelihood and Bayesian analyses resolve trees with congruent topologies. This topology (Fig. 2) has a likelihood value of $-\ln L$ 10854.6592. Maximum parsimony results in one most parsimonious tree (Fig. 3) with a length of 1836 steps and a consistency index (CI) of 0.5784.

The monophyly of the Serpulidae is highly supported by all chosen inference methods, as evidenced by 100% likelihood bootstrap support (Lbs), 100% parsimony bootstrap support (Pbs), and 1.00 Bayesian posterior probability (Pp). Within the Serpulidae, four monophyletic clades are recovered; these are hereafter referred to as the *Protula*-group, *Serpula*-group, *Pomatoceros*-group, and Spirorbinae. The monophyly of each group is well supported by all methods (Lbs and Pbs from 82.7 to 100%, and Pp 1.00 for all clades). The *Protula*-group includes two species of *Protula*, *Vermiliopsis infundibulum* (Philippi, 1844), *Salmacina* sp., and *Filograna implexa* Berkeley, 1835. Monophyly of *Protula* receives high support (Lbs and Pbs >96%; Pp 1.00), as does a sister-group relationship of *Salmacina* + *Filograna*. *Vermiliopsis* is sister to the two *Protula* species, but this branching receives only low bootstrap support (Lbs 56.6%; Pbs 55.6%; Pp 0.97). Sister to the *Protula*-group is Spirorbinae (Lbs 66.3%; Pbs 52.8%; Pp 0.99), represented by two *Spirorbis* species and *Circeis armoricana* Saint-Joseph, 1894.

The *Serpula*-group and the *Pomatoceros*-group form a well-supported monophyletic clade in all our analyses (Lbs 92%; Pbs 94%; Pp 1.00). The *Serpula*-group consists of two species of *Serpula*, *Crucigera zygophora* (Johnson, 1901), and *Hydroides pseudoumcinatus* Zibrowius, 1968. Monophyly of *Serpula* is recovered; and *Crucigera* and *Hydroides* branch off successively. All clades within the *Serpula*-group are supported by Lbs and Pbs >70% and Pp >0.98.

The *Pomatoceros*-group comprises three clades: a monophyletic *Pomatoceros* spp. + *Spirobranchus* (Lbs and Pbs >93.6%, Pp 1.00), *Ditrupa* + *Pseudochitinopoma* (Lbs and Pbs >98.9%; Pp 1.00) and *Galeolaria* + *Ficopomatus* (low Lbs of 61% and no Pbs, but recovered by all methods). The ML and Bayesian analysis infer a sister-group relationship between *Galeolaria* + *Ficopomatus* and *Pomatoceros* + *Spirobranchus* (Fig. 2). However, the MP analysis (Fig. 3) infers the sister group of *Galeolaria* + *Ficopomatus* to be *Ditrupa* + *Pseudochitinopoma*. In both cases support values for these groupings are poor at best.

Hypothesis testing (Table 2) reveals that, based on the AU and NP tests, we cannot significantly reject the hypothesis that Spirorbinae represents the sister group of all other serpulids. The hypothesis that Filograninae are the basal-most serpulids is significantly rejected by the NP test, but not by the AU test. A possible monophyletic group consisting of *Pomatoceros* + Spirorbinae is significantly rejected by both tests.

Discussion

This study represents the first phylogenetic analysis of serpulimorph relationships based on molecular sequence data. It confirms previous hypotheses that the Serpulidae (inclusive of the Serpulinae, Filograninae and Spirorbinae) are a monophyletic group, a grouping that has been long substantiated by the possession of thoracic membranes and calcareous tubes (ten Hove 1984). Bartolomaeus and Quast (2005) added larval protonephridia with a multiciliated terminal cell as a further autapomorphy for the Serpulidae.

A long debate about serpulid ingroup relationships can be found in the literature of the last 100 years. Early classifications (e.g. Fauvel 1927; Fauchald 1977) divided Serpulidae into the subfamilies Serpulinae, Filograninae and Spirorbinae – a classification based on the structure of the operculum (namely the appearance of pinnulae on the stalk), and on the number of thoracic chaetigers. Some authors proposed family status for the Spirorbinae (“Spirorbidae”; Pillai 1970; Fauchald 1977; Knight-Jones 1978; Uchida 1978; Bianchi 1979) because of their unique morphological characteristics (e.g. spirally coiled tube, less than four thoracic chaetigers, and sometimes brood chambers under the opercular plate). Translated

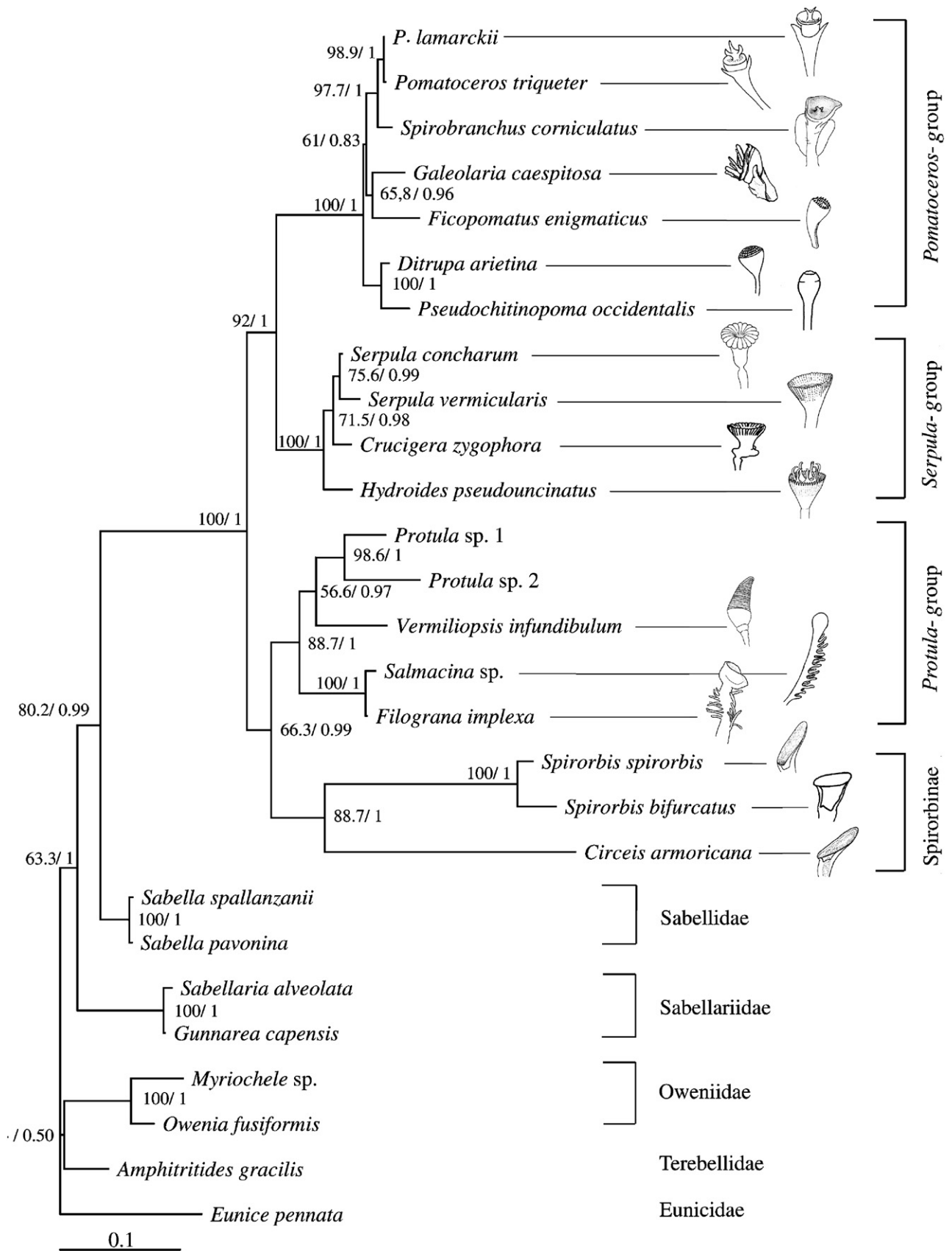


Fig. 2. Maximum-likelihood tree of the 18S rRNA gene dataset based on the TrNef+I+Γ model of sequence evolution (−lnL 10854.65928), with schematic representation of the operculum of each species. Values separated by slashes at nodes represent ML bootstrap support (at left) and Bayesian posterior probability, respectively.

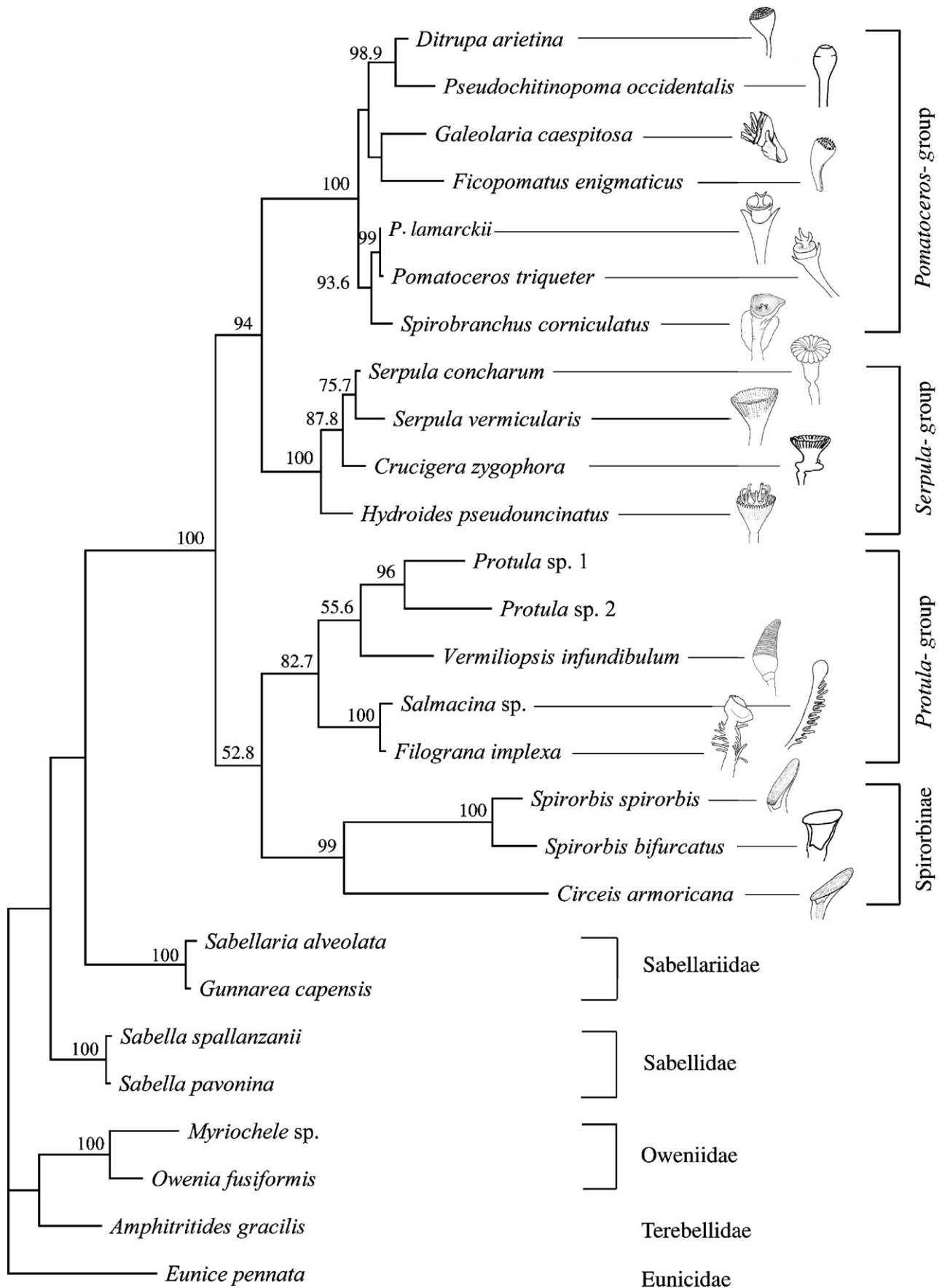


Fig. 3. Most parsimonious tree (tree length = 1836, CI = 0.5784) from maximum parsimony analysis of the 18S rRNA gene dataset, with schematic representation of the operculum of each species. Bootstrap frequencies shown above branches; nodes without values received bootstrap support < 50%.

Table 2. Results of approximately unbiased (AU) and non-scaled bootstrap probability (NP) tests

	Difference to best trees	AU	NP
Best tree	–	0.952	0.890
Serpulids excl. Spirorbinae monophyletic	6.8	0.094	0.063
Serpulids excl. Filograninae monophyletic	19.9	0.057	0.048*
Pomatoceros + Spirorbinae monophyletic	120.2	2×10^{-6} *	6×10^{-6} *

Significant differences ($p < 0.05$) indicated by asterisks.

into cladistic terms, this classification would render the spirorbins the sister taxon of the remaining serpulids.

Ten Hove (1984) used a Hennigian interpretation of transformation series of the branchial crown, as well as chaetal characters, to analyse serpulid relationships. Due to the lack of potential synapomorphies, Filograninae were regarded as paraphyletic. Within Serpulinae, monophyly of a *Serpula*-complex (*Serpula*, *Crucigera* and *Hydroides*) and a *Pomatoceros*-complex (*Pomatoceros*, *Spirobranchus*, *Ficopomatus*, and other genera) were assumed, but a possible Spirorbinae–*Pomatoceros* relationship was discussed. According to ten Hove (1984) *Vermiliopsis* branches off first within Serpulinae.

Two recent cladistic analyses using morphological and ontogenetic characters are available for serpulid taxa. Macdonald (2003) analysed the relationships of Spirorbinae and Kupriyanova (2003) those of serpulids. In congruence with ten Hove (1984), Filograninae were placed basally within the serpulid tree, and regarded as paraphyletic in the latter analyses. As ten Hove (1984) had predicted, non-operculate forms were found to resemble the serpulid ground pattern. Monophyletic Spirorbinae and Serpulinae were recovered as sister taxa by Kupriyanova (2003) but, interestingly, *Vermiliopsis* species were not included in this analysis. Spirorbins were monophyletic in all studies, but their position within Serpulidae remained unclear. Evidence was given for paraphyly of Filograninae, and the non-operculate genus *Protula* was seen as the sister taxon of all other serpulids.

Our molecular 18S rRNA gene sequence data corroborate the monophyly of Spirorbinae. ‘Filograninae’ and ‘Serpulinae’ are both recovered as paraphyletic in all analyses. In our study four major monophyletic clades can be found within Serpulidae: the Spirorbinae; a group including the filogranin taxa and *Vermiliopsis* that we refer to as the *Protula*-group; and two clades consisting of former members of the Serpulinae and named *Serpula*-group and *Pomatoceros*-group, which

both are in congruence with ten Hove’s (1984) complexes. The *Serpula*- and the *Pomatoceros*-group are sister taxa, as are the *Protula*-group and Spirorbinae.

Protula-group

Surprisingly, an operculate member of Serpulinae, *Vermiliopsis*, is found within this group consisting of filogranins. Moreover, the analysis gives some evidence for a sister-group relationship between *Vermiliopsis* and *Protula*. *Vermiliopsis* species have a conical, chitinized functional operculum with no pinnulae on the stalk in the adult stage; in contrast, *Protula* develops no operculum at all (all tentacles have pinnulae). Ten Hove (1984) and Kupriyanova (2003) assumed *Protula* as the most basal taxon within serpulids and that the Filograninae represent a paraphyletic grade basal to all other serpulids. This interpretation is not supported by our results and has been rejected in the NP test, though not in the AU test. The *Protula* + *Vermiliopsis* clade has a sister-group relationship to *Salmacina* + *Filograna*; both clades are supported. Members of *Salmacina* and *Filograna* are very small as adults, and show remarkable sequence similarity (98.3%), which pertains to the discussion of their possible synonymy (McIntosh 1919; Day 1955, 1967; Zibrowius 1968, 1973; Uchida 1978; Nogueira and ten Hove 2000). Traditionally, *Salmacina* and *Filograna* have been distinguished by the presence of a pair of opercula in *Filograna*, absent in *Salmacina*, although the radiolar tips in *Salmacina* may be swollen (e.g. Fauvel 1927). Some authors found operculate and non-operculate specimens within the same colony (McIntosh 1919; Faulkner 1929; Day 1955) and considered presence or absence of an operculum as ecological adaptation rather than a taxonomic character. However, ten Hove and Pantus (1985) and Nogueira and ten Hove (2000) regard the operculate forms as a separate taxon, though doubting whether it should be distinguished on the genus level.

Spirorbinae

The monophyly of Spirorbinae and the taxon’s status as a serpulid ingroup is supported both by recent morphological studies (ten Hove 1984; Fitzhugh 1989; Smith 1991; Kupriyanova 2003; Macdonald 2003) and our molecular study. Thus, spirorbins should be regarded as a derived taxon within serpulids that does not show plesiomorphic characters as suggested by Pillai (1970), Fauchald (1977), Uchida (1978) and Knight-Jones (1978), who postulated spirorbins as the sister-group to Serpulidae (i.e. consisting of “Serpulinae” and “Filograninae”). This classification was based on an idea of the spirorbin operculum as non-homologous to

that of serpulids (Pillai 1970). The spirorbin operculum is always modified from the second-from-dorsal radiole, whereas the operculum in serpulids was suggested to be derived from the first, dorsal-most radiole. However, ten Hove (1984) has shown that the operculum actually is a modified second dorsal radiole in most serpulids. It is only in the genera *Filograna* (“Filograninae”), *Ditrupa* (“Serpulinae”) and *Rhodopsis* (“Serpulinae”) that the insertion precedes the first normal radiole.

Based on cladistic analyses, ten Hove (1984) and Kupriyanova (2003) suggested that the Spirorbiniae are more closely related to “Serpulinae” (especially *Pomatoceros*) than to the “Filograninae”. This interpretation is not in line with our results, which show the exact opposite; the spirorbins are sister to the *Protula*-group in all analyses, though support for this is not significant.

Ten Hove (1984) considered the possibility that spirorbins are an offshoot of *Pomatoceros*-like genera, because both taxa develop their opercula directly (Segrove 1941 for *P. triqueter*; Nott 1973 for Spirorbiniae; Smith 1991 for *Spirobranchus*). In addition, *Pomatoceros* and *Spirobranchus* species show incidental moulting of the opercular plate (ten Hove 1970), which is reminiscent of the cyclic replacement of brood chambers below the opercular plate in spirorbins. Our molecular data do not support this hypothesis.

Even though we cannot rule out significantly that Spirorbiniae represent the sister group to all other serpulids, a close relationship to *Pomatoceros* is significantly rejected by the AU and NP tests.

Serpula-group

According to Kupriyanova (2003), *Hydroides*, *Crucigera* and *Serpula* form a monophyletic group within the Serpulinae, with *Hydroides* as the sister taxon to *Crucigera*+*Serpula*. This is in contrast to ten Hove (1984), who considered *Hydroides* and *Crucigera* as sister groups. According to ten Hove (1984), a monophyletic clade consisting of *Pomatoceros*-like genera and spirorbins is most closely related to the *Serpula*–*Crucigera*–*Hydroides* clade. Our molecular data place spirorbins widely apart from the *Pomatoceros*-group.

Kupriyanova (2003) did not include *Vermiliopsis* in her analysis; thus her “Serpulinae” are congruent with our clade consisting of the *Serpula* and *Pomatoceros* groups.

Pomatoceros-group

Within the *Pomatoceros*-group, the hypothesized sister-group relationship between *Spirobranchus* and *Pomatoceros* (based on a homologous organization and development of opercula and other morphological characters; H.A. ten Hove, unpublished data; Kupriya-

nova 2003) is confirmed and highly supported by our molecular data. Both taxa possess a distal calcareous opercular plate bearing a variable numbers of spines; the opercular stalk is winged and the operculum develops directly. Kupriyanova (2003) suggests a sister-group relationship between the *Pomatoceros*+*Spirobranchus* cluster and *Galeolaria*, because the latter also shows calcareous spines on the opercular plate and a winged peduncle. According to our molecular data, *Galeolaria* is sister to the brackish-water *Ficopomatus*, and in the likelihood analyses this cluster is sister to the *Pomatoceros*+*Spirobranchus* cluster. In the parsimony analysis, the cluster is more closely placed to the *Ditrupa*+*Pseudochitinopoma* cluster. However, none of these relationships achieve support. Nevertheless, the position of *Ficopomatus* within the *Pomatoceros*-group is remarkable. Admittedly, due to weak bootstrap support along the branches leading towards *Ficopomatus*, we cannot discard a possible sister-group relationship to the remaining taxa of the *Pomatoceros*-group. *Ficopomatus* shows direct opercular development as in *Spirobranchus* and *Pomatoceros*, whereas it does not possess a winged peduncle, nor a calcareous opercular plate, in contrast to *Galeolaria*, *Spirobranchus* and *Pomatoceros*. Nothing is known about the opercular development in *Galeolaria*, *Ditrupa* and *Pseudochitinopoma*. Kupriyanova (2003) showed an unresolved position for *Ficopomatus* within the Serpulinae.

Evolution of opercula

Ten Hove’s (1984) gradual evolutionary series starts with filogranin forms that do not develop opercula (*Protula*), followed by those with branchial radioles each endowed with swollen tips (*Salmacina*), and leads to forms that have two fronting thin, horny opercula on a pinnulated radiole (*Filograna*). The swollen radiolar tips of *Salmacina* and the small, smooth opercula of *Filograna* are reminiscent of early ontogenetic stages of other serpulids; thus they are regarded as ancestral as well. In addition, animals in these three taxa are bilaterally symmetrical, in contrast to asymmetry in the remaining genera. For functional reasons the asymmetric condition is thought to be the derived character state. This condition is probably found in *Apomatus* species; here a functional operculum as well as a small pseudoperculum (rudimentary operculum) are present on a normal pinnulated radiole. When the functional operculum is lost, reversal of symmetry occurs. The selective advantage of this arrangement may lie in the possibility that a new operculum can be formed in case of heavy damage, while the other is still in place.

According to ten Hove (1984) the next step in evolution may have been the acquisition of distal

reinforcements of the functional operculum by horny or calcareous structures. In many serpulids, this reinforcement of the opercular bulb occurs during late development. This pattern, first visible in his scheme in the genus *Josephella*, is combined with the loss of pseudopercula. Further steps would be a loss of pinnules on the stalk in adult age (indirect opercular development), the reappearance of pseudopercula, and modifications of the distal opercular plate (*Serpula*, *Crucigera*, *Hydroides*). At the least, there is a tendency of: (1) the peduncle moving out from the centre of the branchial crown, (2) direct opercular development, and (3) a loss of symmetry reversal (*Pomatoceros*, *Spirobranchus*). The functional opercula become highly modified and no pseudopercula develop (in the event of injury, a new operculum is regenerated from the same peduncle; ten Hove 1970 for *Spirobranchus*). A possible selective advantage of distal calcareous structures on the operculum, as found in *Pomatoceros* and *Spirobranchus* species, may be better protection against predators. Since spirorbins, too, possess calcareous plates on the distal surface of the operculum, which develops directly, Spirorbinae have been regarded as derived serpulids (Caullery and Mesnil 1897; Uchida 1978; ten Hove 1984). Based on this gradual series, as well as on ontogenetic studies (Müller 1864; Ludwig and Ludwig 1954; Ludwig 1957; Vuillemins 1965), ten Hove hypothesized a phylogeny within the Serpulidae consisting of 10 genera and Spirorbinae. In this classification, *Protula* branches off first, followed successively by *Salmacina*, *Filograna*, *Apomatus*, *Josephella*, and *Vermiliopsis* which is the closest relative to the *Serpula*–*Hydroides*–*Cruciger* cluster. The next cluster consists of the spirorbins and *Pomatoceros*. Our findings partly corroborate these relationships (*Serpula* cluster; *Salmacina*–*Filograna* sister-group relationship), but also show that the successive transformation series of opercula cannot be supported and the proposed *Pomatoceros* + spirorbin relationship is significantly rejected.

According to our molecular data *Protula* sp., *Salmacina* sp. and *F. implexa*, historically classified as primitive, form a monophyletic clade with *V. infundibulum*. In contrast to the filogranins, the latter taxon possesses a well-developed (conical) operculum in the adult stage, with no pinnulae on its stalk. *Salmacina* and *Filograna* species have pinnulae on their opercular stalks as adults; *Vermiliopsis* species develop pinnulae only in early ontogenetic stages (Ludwig, 1957; indirect opercular development, ten Hove 1984). This aggregation of filogranin members with a member of the Serpulinae, and the possible positions of Spirorbinae, make it impossible to retain the proposed polarity of ten Hove's (1984) transformation series. Instead, it is more parsimonious to assume that the opercula of *Protula* and *Salmacina* are reduced secondarily and that those of *Filograna* species are duplicated.

Reductions of opercula in serpulids have been described in the literature before, mainly from taxa with alternative defence mechanisms. For instance, *Spiraserpula* spp. only develop two pseudopercula and secrete sharp ridges and spines on the inner mouth of the tube as an alternative defence against being pulled out from their tubes by predators (Pillai and ten Hove 1994). In a population of *Hydroides spongicola* Benedict, 1887, 75–95% of the individuals possess two small pseudopercula instead of one functional and one rudimentary operculum (ten Hove and Jansen-Jacobs 1984). This species lives as a symbiont in a toxic sponge, *Neofibularia nolitangere* (Duchassaing de Fombressin and Michelotti 1864), significantly called “touch-me-not sponge”, which might be the alternative defense of *H. spongicola* (ten Hove and Jansen-Jacobs 1984). *Spirobranchus nigranucha* (Fischli, 1903), clearly a member of the *Spirobranchus giganteus* complex, living deep inside the branches of *Acropora* corals, shows no trace of an operculum as opposed to all other members of the genus (ten Hove 1989). Knight-Jones et al. (1997) described *Hyalopomatus cancerum*, a species that differs from others of the genus in lacking opercula, and proposed that in this case the condition might be an adaptation to low oxygen levels.

Our molecular study indicates that the absence of opercula in *Protula* and *Salmacina* is not a plesiomorphic character state as suggested by Uchida (1978), ten Hove (1984), Smith (1991), and Kupriyanova (2003). Judging from the small body sizes in *Salmacina* and *Filograna* species (2 mm length; up to 0.5 mm tube diameter) and the fact that the swollen tips in *Salmacina* species and the small membranous opercula in *Filograna* species are reminiscent of other operculate serpulids in their early ontogenetic stages, these two taxa might be progenetic.

Our molecular data do not support the present taxonomic classification of Serpulidae into the Filograninae, Serpulinae and Spirorbinae. The results suggest convergent evolution of direct opercula development, once in the stem of the *Pomatoceros*-group and once in the stem of the Spirorbinae clade.

Note added in proof

While this manuscript was in press, Kupriyanova et al. (2006) reported similar results using comparable datasets.

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References

- Bailey, J.H., 1969. Methods of brood protection as a basis for reclassification of the Spirorbinae (Serpulidae). *Zool. J. Linn. Soc.* 48, 387–407.
- Bartolomeus, T., Quast, B., 2005. Structure and development of nephridia in Annelida and related taxa. *Hydrobiologia* 535/536, 139–164.
- Bianchi, C.N., 1979. Serpuloidea (Annelida, Polychaeta) delle acque italiane: elenco delle specie e chiavi per la determinazione. *Ann. Mus. Civ. Sto. Nat. Genova* 82, 266–294.
- Bianchi, C.N., 1981. Policheti Serpuloidei. Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane AQ/1/96 5, Consiglio Nazionale delle Ricerche, Rome.
- Bleidorn, C., 2005. Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. *Zool. J. Linn. Soc.* 144, 59–73.
- Bleidorn, C., Vogt, L., Bartolomeus, T., 2005. Molecular phylogeny of lugworms (Annelida, Arenicolidae) inferred from three genes. *Mol. Phylogenet. Evol.* 34, 673–679.
- Borda, E., Siddall, M.E., 2004. Arhynchobdellida (Annelida: Oligochaeta: Hirundinida): Phylogenetic relationships and evolution. *Mol. Phylogenet. Evol.* 30, 213–225.
- Caulery, M., Mesnil, F., 1897. Études sur la morphologie comparée et la phylogénie des espèces chez les Spirorbés. *Bull. Sci. Fr. Belg.* 30, 185–233.
- Chamberlin, R.V., 1919. The Annelida Polychaeta (Rep. Sci. Res. Exp. “Albatross”). *Mem. Mus. Comp. Zool. Harvard* 48, 1–514.
- Day, J.H., 1955. The polychaeta of South Africa. 3. Sedentary species from Cape shores and estuaries. *Zool. J. Linn. Soc.* 42, 407–452.
- Day, J.H., 1967. A Monograph on the Polychaeta of Southern Africa. Part 2. Sedentaria. British Museum of Natural History, London, pp. 459–878.
- Duchassaing de Fombressin, P., Michelotti, G., 1864. Spongiaires de la mer Caraïbe. *Natuurk. Verh. Holl. Maatsch. Wetensch. Haarlem* 21 (2), 1–124.
- Fauchald, K., 1977. The polychaete worms. Definitions and keys to the orders, families and genera. *Nat. Hist. Mus. L. A. Co. Sci. Ser.* 28, 1–188.
- Faulkner, G.H., 1929. The anatomy and histology of bud-formation in the serpulid *Filograna implexa*, together with some cytological observations on the nuclei of the neoblasts. *Zool. J. Linn. Soc.* 37, 109–189.
- Fauvel, P., 1927. Polychètes sédentaires. Addenda aux Errantes, Archiannelides, Myzostomaires. *Faune Fr.* 5, 346–405.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Fitzhugh, K., 1989. A systematic revision of the Sabellidae–Caobangidae–Sabellongidae complex (Annelida: Polychaeta). *Bull. Am. Mus. Nat. Hist. NY* 192, 1–104.
- Hall, T.A. (Ed.), 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucl. Acids Symp. Ser.* 41, 95–98.
- Hartmann-Schröder, G., 1996. Annelida, Borstenwürmer, Polychaeta, vol. 58, 2nd ed. *Die Tierwelt Deutschlands*, pp. 560–574.
- Hayward, P., Ryland, J.S., 1996. Handbook of the Marine Fauna of North West Europe. Oxford University Press, Oxford, pp. 255–261.
- Hobson, K.D., Banse, K., 1981. Sedentary and archiannelid polychaetes of British Columbia and Washington. *Can. Bull. Fish. Aquat. Sci.* 209, 1–144.
- ten Hove, H.A., 1970. Serpulinae (Polychaeta) from the Caribbean: I. The genus *Spirobranchus*. *Stud. Fauna Curaçao Carib. Islds.* 32, 1–57.
- ten Hove, H.A., 1984. Towards a phylogeny in serpulids (Annelida, Polychaeta). *Proc. Int. Polychaete Conf. (Linn. Soc. N.S. Wales)* 1, 181–196.
- ten Hove, H.A., 1989. Serpulinae (Polychaeta) from the Caribbean: IV — *Pseudovermilia madracicola* sp. n., a symbiont of corals. *Studies in honour of Dr. Pieter Wagenaar Hummelinck. Found. Sci. Res. Surinam Neth. Antilles* 123, 135–144.
- ten Hove, H.A., Jansen-Jacobs, M.J., 1984. A revision of the genus *Crucigera* (Polychaeta, Serpulidae); a proposed methodical approach to serpulids, with special reference to variation in *Serpula* and *Hydroides*. *Proc. Int. Polychaete Conf. (Linn. Soc. N.S. Wales)* 1, 143–180.
- ten Hove, H.A., Pantus, F.J.A., 1985. Distinguishing the genera *Apomatus* Philippi, 1844 and *Protula* Risso, 1826 (Polychaeta: Serpulidae). A further plea for a methodical approach to serpulid taxonomy. *Zool. Med. Leiden* 59 (32), 419–437.
- Huelsenbeck, J.P., Ronquist, F., 2001. MrBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Knight-Jones, E.W., Knight-Jones, P., Oliver, P.G., Mackie, S.Y., 1997. A new species of *Hyalopomatus* (Serpulidae: Polychaeta) which lacks an operculum: is this an adaptation to low oxygen? In: Naumov, A.D., Hummel, H., Sukhotin, A.A., Ryland, J.S. (Eds.), *Interactions and Adaptation Strategies of Marine Organisms. Hydrobiologia* 355, 141–151 (4 figures).
- Knight-Jones, P., 1978. New Spirorbidae (Polychaeta, Sedentaria) from the east Pacific, Atlantic, Indian and southern oceans. *Zool. J. Linn. Soc.* 64, 201–240.
- Kupriyanova, E.K., 2003. Life history evolution in serpulid polychaetes: a phylogenetic analysis. *Hydrobiologia* 496, 105–114.
- Kupriyanova, E.K., Jirkov, J.A., 1997. Serpulidae (Annelida, Polychaeta) of the Arctic Ocean. *Sarsia* 82, 203–236.
- Kupriyanova, E.K., Macdonald, T.A., Rouse, G.W., 2006. Phylogenetic relationships within Serpulidae (Sabellida, Annelida) inferred from molecular and morphological data. *Zool. Scr.* 35, 421–439.
- Ludwig, H.W., 1957. Morphologische Untersuchungen zur Regeneration der Operkula der Serpuliden. *Verh. Dt. Zool. Ges.* 1956, 272–277.
- Ludwig, W., Ludwig, H.W., 1954. Untersuchungen zur kompensatorischen Regeneration an *Hydroides norvegica*. *Roux’ Arch. Entwickl.-mech.* 147, 259–287.

- Macdonald, T.A., 2003. Phylogenetic relations among spirorbid subgenera and the evolution of opercular brooding. *Hydrobiologia* 496, 125–143.
- McIntosh, W.C., 1919. Notes from the Gatty Marine Laboratory, St. Andrews. No XLII. 1. Preliminary studies on *Filograna*: a, historical; b, faunistic; c, structural; d, general 2. On *Harmothoe watsoni*, M'I., and var. *H. marphysae*, M'I. *Ann. Mag. Nat. Hist. Lond.* 9th Ser. 3, 125–538.
- Müller, F., 1864. Für Darwin. Wilhelm Engelmann, Leipzig.
- Nieselt-Struwe, K., von Haeseler, A., 2001. Quartet-mapping, a generalization of the likelihood-mapping procedure. *Mol. Biol. Evol.* 18, 1204–1219.
- Nogueira, J.M., ten Hove, H.A., 2000. On a new species of *Salmacina* Claparède, 1870 (Polychaeta: Serpulidae) from São Paulo State, Brazil. *Beaufortia* 50, 151–153.
- Nott, J.A., 1973. Settlement of the larvae of *Spirorbis spirorbis* L. *J. Mar. Biol. Assoc. UK* 53, 437–453.
- Nygren, A., Sundberg, P., 2003. Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida). *Mol. Phylogenet. Evol.* 29, 235–249.
- Orrhage, L., 1980. On the structure and homologues of the anterior end of the polychaete families Sabellidae and Serpulidae. *Zoomorphology* 96, 113–168.
- Pillai, T.G., 1970. Studies on a collection of spirorbids from Ceylon, together with a critical review and revision of spirorbid systematics and an account of their phylogeny and zoogeography. *Ceylon J. Sci. Biol. Sci.* 8, 100–172.
- Pillai, T.G., ten Hove, H.A., 1994. On recent species of *Spiraserpula* Regenhardt, 1961, a serpulid polychaete genus hitherto known only from Cretaceous and Tertiary fossils. *Bull. Nat. Hist. Mus. (Zool. Ser.)* 60, 39–104.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 917–918.
- Posada, D., Crandall, K.A., 2001. Selecting the best-fit model of nucleotide substitution. *Syst. Biol.* 50, 580–601.
- Rioja, E., 1923. Estudio sistemático de las especies Ibéricas del suborden Sabelliformia. *Trab. Mus. Nac. Cienc. Nat. Ser. Zool.* 48, 1–144.
- Rouse, G.W., Fauchald, K., 1997. Cladistics and polychaetes. *Zool. Scr.* 26, 139–204.
- Rouse, G.W., Pleijel, F., 2001. Polychaetes. Oxford University Press, London.
- Segrove, F., 1941. The development of the serpulid *Pomatoceros triqueter* L. *J. Microsc. Sci.* 82, 467–540.
- Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* 51, 492–508.
- Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17, 1246–1247.
- Smith, R.S., 1991. Relationships within the order Sabellida (Polychaeta). In: Petersen, M.E., Kirkegaard, J.B. (Eds.), *Proceedings of the International Polychaete Conference 2*, *Ophelia Supplement*, vol. 5, pp. 249–260.
- Strimmer, K., von Haeseler, A., 1997. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. *Proc. Acad. Nat. Sci. Philadelphia* 94, 6815–6819.
- Struck, T., Hessling, R., Purschke, G., 2002. The phylogenetic position of the Aeolosomatidae and Parergodrilidae, two enigmatic oligochaete-like taxa of the “Polychaeta”. *J. Zool. Syst. Evol. Res.* 40, 155–163.
- Swofford, D.L., 2001. PAUP*. Phylogenetic Analysis Using Parsimony, version 4.0b8. Sinauer, Sunderland, MA.
- Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512–526.
- Thomas, J.G., 1940. *Pomatoceros*, *Sabella* and *Amphitrite*. In: Daniel, R.J. (Ed.), *On Typical British Marine Plants and Animals*. University Press, Liverpool, pp. 1–88.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22, 4673–4680.
- Uchida, H., 1978. Serpulid tube worms (Polychaeta, Sedentaria) from Japan with a systematic review of the group. *Bull. Mar. Park Res. Stat.* 2, 1–98.
- Vuillemin, S., 1965. Contribution à l'étude écologique du lac de Tunis. *Biologie de Mercierella enigmatica* Fauvel. Thèse d'Etat, Université Paris VI.
- Zeleny, C., 1905. Compensatory regulation. *J. Exp. Zool.* 2, 1–102.
- Zibrowius, H., 1968. Étude morphologique, systématique et écologique des Serpulidae de la région de Marseille. *Rec. Trav. Stat. Mar. d'Endoume Bull.* 43, 81–252.
- Zibrowius, H., 1973. Revision of some Serpulidae (Annelida, Polychaeta) from abyssal depths in the Atlantic and Pacific, collected by the “Challenger” and Prince of Monaco expeditions. *Bull. Brit. Mus. (Nat. Hist.) Zool.* 24, 427–439.