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

Janina Lehrkea,*, Harry A. ten Hoveb, Tara A. Macdonaldb, Thomas Bartolomaeusa, Christoph Bleidorna

a Institute for Zoology, Animal Systematics and Evolution, Freie Universitaet Berlin, Koenigin-Luise-Street 1-3, 14195 Berlin, Germany
b Zoological Museum, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands
c Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada, V0R 1B0

Received 19 December 2005; accepted 2 June 2006

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.

Keywords: Serpulidae; Phylogeny; Operculum; 18S rRNA gene; Annelida; Polychaeta

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 Currently, there are approximately 343 species which are assigned to 74 genera (H.A. ten Hove, unpublished data).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, Filogranae, 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 Filogranae...
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 filogranin taxa (Apomatus, Josephella, Filograna), and of some Serpulinae (Vermiliopsis, Serpula, Hydroides, Crucigera). Here the operculum develops on a pinnulated peduncle. In the Filograninae, 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 filogranin serpulids) and leads to highly modified operculate genera (Serpulinae and Spirorbinae).

While division of Serpulidae into Spirorbinae, Filograninae and Serpulinae is a widely used classification scheme (Fauvel 1927; Bianchi 1981; Hobson and Banse 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 Filogranae and Serpulinae are regarded as paraplethytic 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; Macdonald 2003). Therefore, two sabellid species are included as outgroup taxa, together with representatives of the Sabellariidae, the Oweniidae and the Terebellidae. All outgroup taxa, together with representatives of the Serpulidae and Sabellidae, 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; Smith 1991; Kupriyanova 2003; Macdonald 2003).

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 equal base frequencies, invariant sites and gamma distribution (TrNef+I+Γ), is the optimal model.

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 Filogranae and Serpulinae are regarded as paraplethytic 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 Sabellariidae, the Oweniidae and the Terebellidae. All outgroup taxa, together with representatives of the Serpulidae and Sabellidae, 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; Smith 1991; Kupriyanova 2003; Macdonald 2003).

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 equal base frequencies, invariant sites and gamma distribution (TrNef+I+Γ), is the optimal model.
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.

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

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

*aVouchers are deposited in the Zoological Museum, Amsterdam or in the South Australian Museum, Adelaide.*
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 Filogranae (i.e., Filogranae 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 polytomy of three species 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 $-\ln 10854.6592$. 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 impexa 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 zygomorpha (Johnson, 1901), and Hydrodias pseudouncinatus Zibrowius, 1968. Monophyly of Serpula is recovered; and Crucigera and Hydrodias 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. + Spirorbranchus (Lbs and Pbs > 93.6%, Pp 1.00), Ditrupa + Pseudochitino- poma (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 + Spirorbranchus (Fig. 2). However, the MP analysis (Fig. 3) infers the sister group of Galeolaria + Ficopomatus to be Ditrupa + Pseudochitino- poma. 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 Filogranae 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, Filogranae 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, Filogranae 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 Spirorbidae (“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%.
Our molecular 18S rRNA gene sequence data corroborate the monophyly of Spirorbinae. Filogranae and ‘Serpulinae’ are both recovered as paraplythic 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; a clade comprising 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), Fauvel (1977), Uchida (1978) and Knight-Jones (1978), who postulated spirorbins as the sister-group to Serpulidae (i.e. consisting of “Serpulinae” and “Filogranae”). This classification was based on an idea of the spirorbin operculum as non-homologous to other serpulid opercula.

### 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.

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

<table>
<thead>
<tr>
<th>Difference to best trees</th>
<th>AU</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best tree</td>
<td>0.952</td>
<td>0.890</td>
</tr>
<tr>
<td>Serpulids excl. Spirorbinae monophyletic</td>
<td>6.8</td>
<td>0.094</td>
</tr>
<tr>
<td>Serpulids excl. Filograninae monophyletic</td>
<td>19.9</td>
<td>0.057</td>
</tr>
<tr>
<td>Pomatoceros + Spirorbinae monophyletic</td>
<td>120.2</td>
<td>$2 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

Significant differences ($p<0.05$) indicated by asterisks.
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 (“Filigraninaceae”), Ditrupa (“Serpulinaceae”) and Rhodopsis (“Serpulinaceae”) that the insertion precedes the first normal radiole.

Based on cladistic analyses, ten Hove (1984) and Kupriyanova (2003) suggested that the Spirorbinae are more closely related to “Serpulinae” (especially Pomatoceros) than to the “Filigraninaceae”. 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 Spirorbinae; 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 Spirorbinae 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, Cruciger and Serpula form a monophyletic group within the Serpuliniae, with Hydroides as the sister taxon to Cruciger + Serpula. This is in contrast to ten Hove (1984), who considered Hydroides and Cruciger 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–Cruciger–Hydroides clade. Our molecular data place spirorbins widely apart from the Pomatoceros-group.

Kupriyanova (2003) did not include Vermiliopsis in her analysis; thus her “Serpuliniae” 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; Kupriyanova 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 Serpuliniae.

**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, horned 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 *Josephelloa*, 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, Cruciger, 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 operculum 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, *Verrilliopsis* 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, Josephelloa*, and *Verrilliopsis* 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 pinnules on its stalk. *Salmacina* and *Filograna* species have pinnules on their opercular stalks as adults; *Verrilliopsis* species develop pinnules 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* *Benedit 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* (*Duchassaying de Fonbressin 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 membraneous 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 Filogranae, 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.

**Acknowledgements**

The authors are grateful to Torkild Bakken (Norwegian University of Science and Technology, Trondheim,
Norway) who collected *Filograna implexa* in Norway for this study.

We thank two anonymous reviewers for their comments.

References


