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Ultrastructure and mineral composition of serpulid tubes (Polychaeta, Annelida)

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We have studied the tube ultrastructure of 44 recent species from 36 serpulid genera. Twelve distinct ultrastructures are identified. Serpulids possess very diverse tube ultrastructures, in contrast with the traditional point of view. Most species show single-layered tubes, but 34% of these species have between two and four ultrastructurally different layers. Tubes are mostly bimineralic, and are composed of aragonite and calcite; however, one of the polymorphs is always dominant. All the studied single-layered tubes with a lamello fibrillar tube ultrastructure are exclusively calcitic; prismatic structures, both in regular or irregular orientation, are either calcitic or aragonitic in composition. There is no correlation between tube mineralogy, and ultrastructure, and marine, brackish, and freshwater environments. We find that 47% of the serpulid species studied possess a unique combination of tube structure characters. © 2008 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2008, 154, 633–650.

ADDITIONAL KEYWORDS: biomineralization – Serpulidae – skeletal ultrastructure.

INTRODUCTION

Serpulids build a calcareous tube that, in its embryonic stage, of the recently settled trochophore larva, is open at both ends. Later, the open beginning of the tube is closed off, leaving only the anterior mouth open, from which the branchiae may protrude. The tubes usually have a smooth interior. Several serpulid species can form tabulae, perpendicular to the tube lumen (Hedley, 1958; ten Hove, 1973; ten Hove & Smith, 1990), and, rarely, may also form internal longitudinal keels (*Spiraserpula*; Pillai & ten Hove, 1994). The asexually reproducing *Salmacina* builds escape hatches in its tube wall for its clonal offspring (Pernet, 2001). The calcareous tubes of serpulids are internally covered by a thin organic layer (Nishi, 1993), but they lack a thick external organic layer, such as the periostracum of molluscs and brachio-

pods. Serpulid tubes can show faint growth striations or variably developed sculptures, such as transverse and longitudinal ridges and peristomes (ten Hove, 1973, 1975; Bastida-Zavala & ten Hove, 2002; Aliani, Bianchi & Meloni, 1995). Growth lamellae of serpulids are narrow, and are commonly chevron shaped in longitudinal section (Weedon, 1994) or, more rarely, are straight bands (Sanfilippo, 1996). At the contact between the lateral tube wall and the substrate, the tube may contain cavities (e.g. Hedley, 1958).

The mineral composition of serpulid tubes has been reported to be either calcitic, aragonitic, or a combination of both (Lowenstam, 1954; Bornhold & Milliman, 1973; Simkiss & Wilbur, 1989; Vovelle, Grasset & Truchet, 1991). Locally, serpulids may have an important role in the ocean carbonate sink (Mas-trangelo & Passeri, 1975; ten Hove & Van den Hurk, 1993; Medernach *et al.*, 2000). Serpulid tube ultrastructure has never been extensively studied, although some structures and properties of the tubes

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have been described previously (Pillai & ten Hove, 1994; Zibrowius & ten Hove, 1987; ten Hove & Zibrowius, 1986; ten Hove & Smith, 1990; Nishi, 1993; Weedon, 1994; Aliani *et al.*, 1995; Sanfilippo, 1996, 1998; Sanfilippo & Mollica, 2000; Vinn, 2007; Vinn, ten Hove & Mutvei, 2008). Because of the high variability in the size of the elongate prismatic crystals forming the hitherto studied serpulid tubes, Nishi (1993) ascribed low taxonomical value to this character. According to Carter *et al.* (1990), annelids possess the smallest number of known shell ultrastructures, when compared with brachiopods, bryozoans, or molluscs. The aim of this paper is to describe the overall diversity of tube structure in the serpulid polychaetes found in a representative large number of taxa. The main questions to be addressed are: do serpulids possess more diverse skeletal ultrastructures than have previously been reported; can their tube ultrastructures be used taxonomically; are there any evolutionary trends in biomineralization and tube ultrastructure; is there a straightforward relationship between ultrastructure and transparency of the tube; are calcitic and aragonitic tube ultrastructures different; and are tube ultrastructure and composition different between marine and fresh-water species?

MATERIAL AND METHODS

Material was collected intertidally or by diving, trawling, or dredging; it was fixed in 4% (buffered) formalin and was later transferred to 70% ethanol for museum storage. Serpulid species were selected for the study in order to cover most of the accepted genera.

Serpulid tubes (Table 1) were cut using either a small electrical saw or a razor blade. Pieces of tubes were then oriented and mounted in Canada balsam for machine grinding. Sections of tubes were polished and etched in a 1% solution of acetic acid for 1 min. All preparations were gold sputtered prior to SEM investigation. SEM studies were performed on a Hitachi S-4300 SEM, equipped with an Inca EDX system, at the Swedish Museum of Natural History, Stockholm. The beam was operated at 5–10 kV and 1 nA. In our study, we used 5–15-mm-long longitudinal sections, and between one and three cross sections of each serpulid species. Some samples were repolished and treated with a 1 : 1 mixture of 25% glutaraldehyde and 1% acetic acid, to which alcian blue was added (GA solution) before performing the SEM study (Schöne *et al.*, 2005). After this treatment, the organic-rich parts of the tubes have a more intensive blue color. In addition, some samples were repolished and bleached with NaHCl before the SEM study. The overall mineral composition of serpulid tubes was studied by means of X-ray diffraction (XRD) in pow-

dered samples on a Dron-3M diffractometer, at the Institute of Geology, University of Tartu. Tube mineralogy was studied on single tube or tube fragment of each species. The studied material is stored at the Zoological Museum, University of Amsterdam.

RESULTS

THE ULTRASTRUCTURE OF SERPULID TUBES

We have distinguished 12 different types of tube ultrastructure in the 44 serpulid species studied (Tables 1, 2), subdivided here into four categories on the basis of crystal orientation.

Here, the basic units of ultrastructure are termed crystals, and substructural units of crystals are termed crystallites.

UNORIENTED STRUCTURES (CRYSTALLIZATION AXIS LACKS UNIFORM ORIENTATION)

The irregularly oriented prismatic (IOP) structure

This structure is formed by elongate crystals of prismatic shape, which are irregularly oriented within each growth increment (Figs 1A–F, 9A). In all of the species studied with an IOP structure, the crystals have a substructure of long rod-like crystallites oriented parallel with the longitudinal axis of the crystals. They are 0.2–0.3- μm thick and 0.8–1.5- μm long (Fig. 1A, B). The boundaries between substructure crystallites are not sharp. This structure forms the entire tubes of *Apomatus globifer* Théel, 1878 (Fig. 1A, B), *Chitinopoma serrula* (Stimpson, 1853), *Crucigera websteri* (Benedict, 1887), *Ficopomatus enigmaticus* (Fauvel, 1923), *Ficopomatus uschakovi* (Pillai, 1960), *Filograna implexa* (Berkeley, 1835), *Filogranella elatensis* (Ben-Eliahu & Dafni, 1979) (excluding spherulitic prismatic inner tube lenses; see Fig. 9 and Table 2), *Hyalopomatus marenzelleri* Langerhans, 1884, *Janita fimbriata* Delle Chiaje, 1828, *Josephella marenzelleri* Caullery & Mesnil, 1896, *Marifugia cavatica* Absolon & Hrabec, 1930 (Fig. 1C–E), *Metavermilia multicristata* (Philippi, 1844), *Paraprotis pulchra* (Imajima, 1979), *Protis arctica* (Hansen, 1878), *Pseudovermilia occidentalis* (McIntosh, 1885), *Rhodopsis pusilla* (Bush, 1905), *Salmacina incrustans* (Claparède, 1870), and *Semivermilia crenata* (Costa, 1861) (Fig. 1F); is found in the outer tube layer of *Floriprotis sabiuraensis* Uchida, 1978; is found in the second tube layer from the outside of *Filogranula gracilis* Langerhans, 1884, *Crucigera zygophora* (Johnson, 1901), *Hydroides dianthus* (Verrill, 1873), and *Pomatoleios kraussii* (Baird, 1865); and is found in the inner tube layer of *Hydroides norvegicus* Gunnerus, 1768, *Laminatubus alvini* ten Hove & Zibrowius, 1986, *Neovermilia falcigera* (Roule, 1898), and *Serpula crenata* (Ehlers, 1908) (Table 2).

Table 1. List of serpulid taxa studied and locality information

Species	Locality
1. <i>Apomatus globifer</i>	V.Pol.0105.01 Kara Sea, 71 °N, 64 °E
2. <i>Bathyvermilia langerhansi</i>	V.Pol.4041 Madeira archipelago, Porto Santo, Sta.4.180
3. <i>Chitinopoma serrula</i>	V.Pol.5034 Iceland, Snaefellsnes Peninsula
4. <i>Chitinopomoides wilsoni</i>	V.Pol.3166 Antarctica, USNM Acq.224443
5. <i>Crucigera websteri</i>	V.Pol.3589 Shelf of Surinam
6. <i>Crucigera zygophora</i>	V.Pol.3287 Canoe Bay, Alaska, USA
7. <i>Ficopomatus enigmaticus</i>	V.Pol.3779 Tunis, lake of Tunis
8. <i>Ficopomatus uschakovi</i>	V.Pol.3647 Thailand, Klong Prao, legit Mortensen
9. <i>Filograna implexa</i>	V.Pol.3767 United Kingdom, Orkney Island, mainland
10. <i>Filogranella elatensis</i>	V.Pol.3911 Japan, Sesoko Island, Okinawa
11. <i>Filogranula gracilis</i>	V.Pol.4047 Cape Verde Islands, Boa Vista, Sta.6.071
12. <i>Floriprotis sabiuraensis</i>	V.Pol.3929 Shimoshima Isl., Amakusa, Japan
13. <i>Galeolaria hystrix</i>	V.Pol.3576 New Zealand, Queen Charlotte Sound
14. <i>Hyalopomatus marenzelleri</i>	V.Pol.4522 Canary Islands, Lanzarote, Sta.4.066
15. <i>Hydroides dianthus</i>	V.Pol.3661 USA, Florida, Anna Maria Island
16. <i>Hydroides norvegicus</i>	V.Pol.0463. Norway, Skjerstad (Saltenfjord)
17. <i>Hydroides spongicola</i>	V.Pol.3584 Netherlands Antilles, Curaçao, Sta.2048Ba
18. <i>Janita fimbriata</i>	V.Pol.4072 Canary Islands, Lanzarote, Sta.4.091
19. <i>Josephella marenzelleri</i>	V.Pol.3030 France, Marseille
20. <i>Laminatubus alvini</i>	V.Pol.3512 East Pacific Rise, 09 °N, 104 °W
21. <i>Marifugia cavatica</i>	V.Pol.3102 Hercegovina, Popovopolje
22. <i>Metavermilia multicristata</i>	V.Pol.4300 Seychelles, N. of d'Arros Island, Sta.752
23. <i>Neovermilia falcigera</i>	V.Pol.3501b Italy, Calabria, Pleistocene
24. <i>Neovermilia sphaeropomatus</i>	V.Pol.3274 New Zealand, Cape Saunders
25. <i>Paraprotis pulchra</i>	V.Pol.3379 off Kushimoto, Japan, Sta. 11
26. <i>Placostegus tridentatus</i>	V.Pol.1105 Norway, Bergensfjord
27. <i>Pomatoceros triquetter</i>	Sweden, Tjärnö
28. <i>Pomatoleios kraussii</i>	V.Pol.4748 Indonesia, E. of Komodo
29. <i>Pomatostegus stellatus</i>	V.Pol.5170 Netherlands Antilles, Curaçao, Sta.2093
30. <i>Protis arctica</i>	V.Pol.3833 NE off Iceland, 'Ingolf' Sta.104
31. <i>Protula diomedea</i>	V.Pol.4271 USA, Florida, 27 °N, 84 °W
32. <i>Pseudovermilia madracicola</i>	V.Pol.3751 Netherlands Antilles, Curaçao, Salinja Fuik
33. <i>Pseudovermilia occidentalis</i>	V.Pol.4090 Cape Verde Islands, Sta.Luzia, Sta.6.D07
34. <i>Pyrgopolon ctenactis</i>	V.Pol.4969 Netherlands Antilles, Bonaire, Sta.2117
35. <i>Rhodopsis pusilla</i>	V.Pol.3623 Reunion, Indian Ocean, Sta. PL53
36. <i>Salmacina incrustans</i>	V.Pol.3814 Costa Brava, Spain
37. <i>Semivermilia crenata</i>	V.Pol.3045 Marseille, France
38. <i>Serpula crenata</i>	V.Pol.1739 Indonesia, Siboga Sta.159
39. <i>Serpula israelitica</i>	Cape Verde Islands, São Vicente, Sta.7.160
40. <i>Serpula vermicularis</i>	V.Pol.3780 Ireland, Ardbear Lough
41. <i>Spiraserpula caribensis</i>	Netherlands Antilles, Curaçao, Sta.2061A
42. <i>Spirobranchus giganteus</i>	Netherlands Antilles, Curaçao, Sta.2073Aa
43. <i>Vermiliopsis infundibulum</i>	V.Pol.4036 Canary Islands, Lanzarote, Sta.4.071
44. <i>Vitreotubus digeronimoi</i>	V.Pol.4308 Seychelles, Platte Island, Sta.795

The spherulitic irregularly oriented prismatic (SIOP) structure

This structure is formed by sectors of spherulites that are irregularly oriented within each growth increment (Figs 2A–F, 9B). The substructure is composed of elongate slender crystallites subparallel with the longitudinal axis of crystals forming the SIOP structure. The structure occurs in the whole tube of *Chiti-*

nopomoides wilsoni Benham, 1927, *Hydroides spongicola* Benedict, 1887, *Pseudovermilia madracicola* ten Hove, 1989 (Fig. 2A, B), and *Vermiliopsis infundibulum* (Philippi, 1844); in the second tube layer from the outside in *Spiraserpula caribensis* Pillai & ten Hove, 1994 (Fig. 2F); in the inner tube layer of *C. zygophora* (Fig. 2C–E), *Galeolaria hystrix* Mörch, 1863, *P. kraussii*, and *Spirobranchus*

Table 2. Tube ultrastructure of serpulid polychaetes

Species	IOP	SIOP	IOPL	HAC	RHC	SOIOP	SOSIOP	SPHP	SP	LF	SLF	OF	Spher.	Hcement
1. <i>Apomatus globifer</i>	1													1a
2. <i>Bathyvermilia langerhansi</i>				1.2										
3. <i>Chitinopoma serrula</i>	1													1a
4. <i>Chitinopomoides wilsoni</i>		1												
5. <i>Crucigera websteri</i>	2							1.4		3				
6. <i>Crucigera zygophora</i>	2	3						1, [4]						2a
7. <i>Ficopomatus enigmaticus</i>	1												1	1a
8. <i>Ficopomatus uschakovi</i>	1													
9. <i>Filograna implexa</i>	1													1a
10. <i>Filigranella elatensis</i>	1							[2]						1a
11. <i>Filigranula gracilis</i>	1													1a
12. <i>Floriprotis sabiuraensis</i>	1	[4]						[4]		2	3			1a
13. <i>Galeolaria hystrix</i>		2								1				
14. <i>Hyalopomatus marenzelleri</i>	1													1a
15. <i>Hydroides dianthus</i>	2	[4]						1		3				
16. <i>Hydroides norvegicus</i>	2							1						
17. <i>Hydroides spongicola</i>		1												
18. <i>Janita fimbriata</i>	1													1a
19. <i>Josephella marenzelleri</i>	1													1b
20. <i>Laminatubus alvini</i>	2			1										2a
21. <i>Marifugia cavatica</i>	1.2													1a, 2b
22. <i>Metavermilia multicristata</i>	1													1b
23. <i>Neovermilia falcigera</i>	2		1					cav.						2a
24. <i>Neovermilia sphaeropomatus</i>										1				1a
25. <i>Paraprotis pulchra</i>	1													1a
26. <i>Placostegus tridentatus</i>									1				1	
27. <i>Pomatoceros triqueter</i>										1				[1]
28. <i>Pomatoleios kraussii</i>	2	4						1		3				2a
29. <i>Pomatostegus stellatus</i>					1									
30. <i>Protis arctica</i>	1													
31. <i>Protula diomedea</i>						1								
32. <i>Pseudovermilia madracicola</i>		1												
33. <i>Pseudovermilia occidentalis</i>	1							[1]						1a
34. <i>Pyrgopolon ctenactis</i>							1	2						
35. <i>Rhodopsis pusilla</i>	1													1b
36. <i>Salmacina incrustans</i>	1													1b
37. <i>Semivermilia crenata</i>	1													1b
38. <i>Serpula crenata</i>	2								1					2a
39. <i>Serpula israelitica</i>										1				
40. <i>Serpula vermicularis</i>										1				
41. <i>Spiraserpula caribensis</i>		2						1.3						
42. <i>Spirobranchus giganteus</i>		2										1		
43. <i>Vermiliopsis infundibulum</i>		1												
44. <i>Vitreotubus digeronimoi</i>									1					

Unoriented structures: HAC, homogeneous angular crystal structure; IOP, irregularly oriented prismatic structure; IOPL, irregularly oriented platy structure; RHC, rounded homogeneous crystal structure; SIOP, spherulitic irregularly oriented prismatic structure.

Semi-oriented structures: SOIOP, semi-ordered irregularly oriented prismatic structure; SOSIOP, semi-ordered spherulitic oriented prismatic structure.

Oriented prismatic structures: SP, simple prismatic structure; SPHP, spherulitic prismatic structure.

Oriented complex structures: LF, lamello-fibrillar structure; OF, ordered fibrillar structure; SLF, spherulitic lamello-fibrillar structure.

Other abbreviations: [], denotes discontinuous layer or lenses; a, denotes the occurrence of some carbonate cement; b, denotes the occurrence of much carbonate cement; Cav., denotes that structure occurs in the tube wall, surrounding the cavities at the contact between the tube and the substrate; Hcement, homogeneous carbonate cement; Spher, spherulite.

The location of the tube layers is indicated by numbers (1–4) counted from outside.

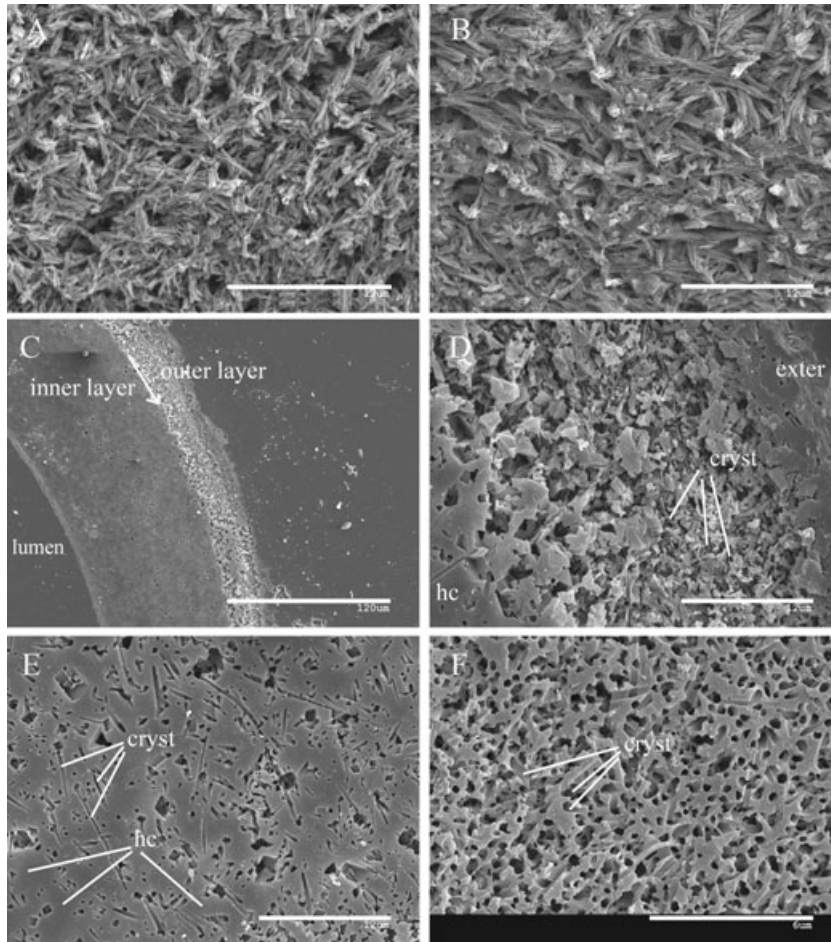


Figure 1. A–B, *Apomatus globifer*, irregularly oriented prismatic structure, polished and treated with GA (see Material & Methods) solution for 5 min. A, cross section. B, longitudinal section. C–E, *Marifugia cavatica*, polished, and treated with 1% acetic acid for 2 min. C, cross section, two-layered tube with irregularly oriented prismatic structure. D, longitudinal section, outer tube layer, with a low content of homogeneous carbonate cement. E, longitudinal section, inner tube layer, with a high content of homogeneous carbonate cement. F, *Semivermilia crenata*, irregularly oriented prismatic structure with a high content of homogeneous carbonate cement, polished cross section, treated with GA solution for 5 min. Abbreviations: cryst, crystals; exter, exterior; hc, homogeneous carbonate cement.

giganteus (Pallas, 1766); in the inner tube lenses in *Floriprotis sabiuraensis* Uchida, 1978 and *Hydroides dianthus* (Verrill, 1873) (Table 2).

The irregularly oriented platy (IOPL) structure

This structure is formed by slightly elongate crystal sheets that are irregularly oriented within each growth increment (Figs 3A, B, 9C). The substructure is composed of elongate slender crystallites, oriented subparallel with the longitudinal axis of crystals forming the IOPL structure. The structure occurs in the outer tube layer of *N. falcigera* (Figs 3A, B, Table 2).

The homogeneous angular crystal (HAC) structure

This structure is composed of angular crystals of irregular shape and varying size (1–30 µm) (Figs 3C,

D, 9D). The substructure of the crystals is composed of elongate crystallites with variable orientation. This structure forms the entire tube of *Bathyvermilia langerhansi* (Fauvel, 1909) (Fig. 3C, D), and the outer tube layer of *L. alvini* (ten Hove & Zibrowius, 1986) (Fig. 7F, Table 2).

The rounded homogeneous crystal (RHC) structure

This structure is composed of crystals of slightly rounded shape and relatively uniform size (1.0–4.0 µm) (Figs 3E, F, 9E). The crystals have an orientated substructure of parallel fibre-like crystallites that are 0.8–2.0-µm long and 0.1–0.3-µm thick (Fig. 3E, F, Table 2). This structure occurs in the entire tube of *Pomatostegus stellatus* (Abildgaard, 1798) (Table 2).

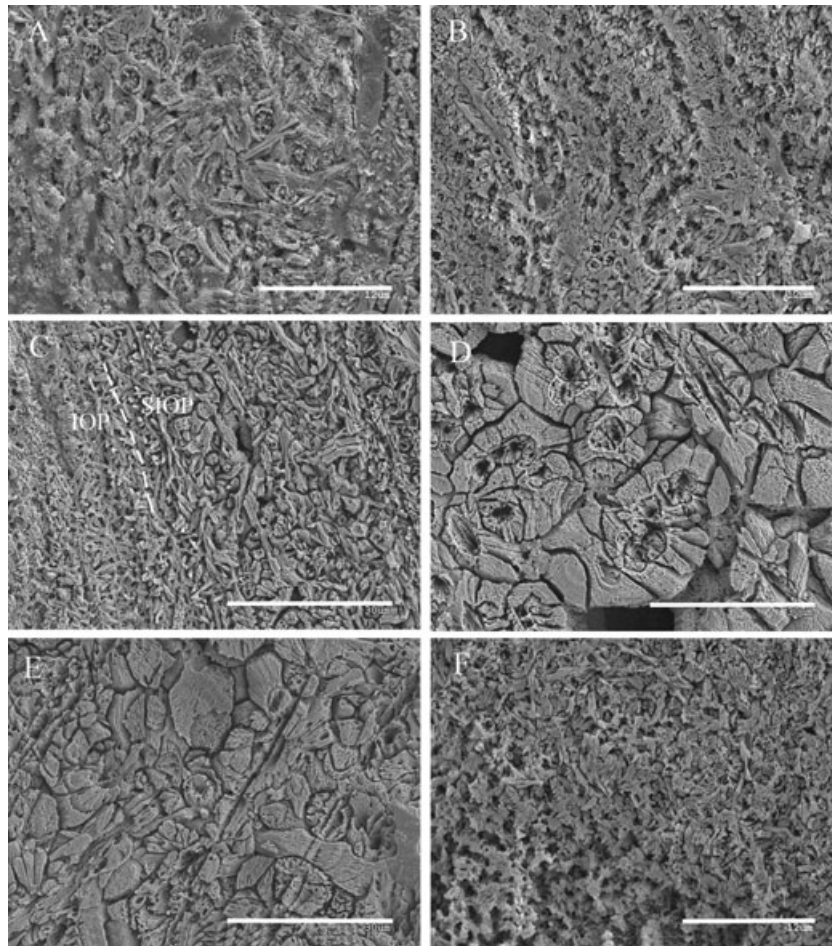


Figure 2. A–B, *Pseudovermia madracicola*, spherulitic irregularly oriented prismatic structure. A, cross section. B, longitudinal section. C–E, *Crucigera zygophora*. C, transition between irregularly oriented prismatic and spherulitic irregularly oriented prismatic structure in cross section. D, spherulitic irregularly oriented prismatic structure, cross section. E, spherulitic irregularly oriented prismatic structure, longitudinal section. F, *Spiraserpula caribensis*, spherulitic irregularly oriented prismatic structure. All sections were polished and treated with 1% acetic acid for 2 min. Abbreviations: IOP, irregularly oriented prismatic structure; SIOP, spherulitic irregularly oriented prismatic structure.

SEMI-ORIENTED STRUCTURES (THE CRYSTALLIZATION
AXIS HAS A SEMI-UNIFORM ORIENTATION)

*The semi-ordered irregularly oriented prismatic
(SOIOP) structure*

This structure is composed of prismatic crystals, which are in general irregularly oriented within each growth increment, but still have a slight tendency to have their longitudinal axis more often oriented subparallel or parallel with the tube surface (Figs 4A, B, 9F). The substructure is composed of elongate slender crystallites parallel with the longitudinal axis of SOIOP crystals. The structure forms the entire tube of *Protula diomedea* Benedict, 1887 (Fig. 4A, B, Table 2).

*The semi-ordered spherulitic irregularly oriented
prismatic (SOSIOP) structure*

This structure is composed of sectors of spherulites that are in general irregularly oriented within each

growth increment, but still have a slight tendency to have their longitudinal axis more often oriented subparallel or parallel with the tube surface (Figs 4C, D, 9G). The substructure is composed of elongate slender crystallites subparallel with the longitudinal axis of SOSIOP crystals. The structure occurs in the outer tube layer of *Pyrgopolon ctenactis* (Mörch, 1863) (Fig. 4C, D, Table 2).

ORIENTED PRISMATIC STRUCTURES (THE
CRYSTALLIZATION AXIS HAS A UNIFORM ORIENTATION,
AND IS CONTINUOUS THROUGH SUCCESSIVE
GROWTH INCREMENTS)

The spherulitic prismatic (SPHP) structure

This structure is formed by prismatic crystals of slightly spherulitic arrangement, oriented perpen-

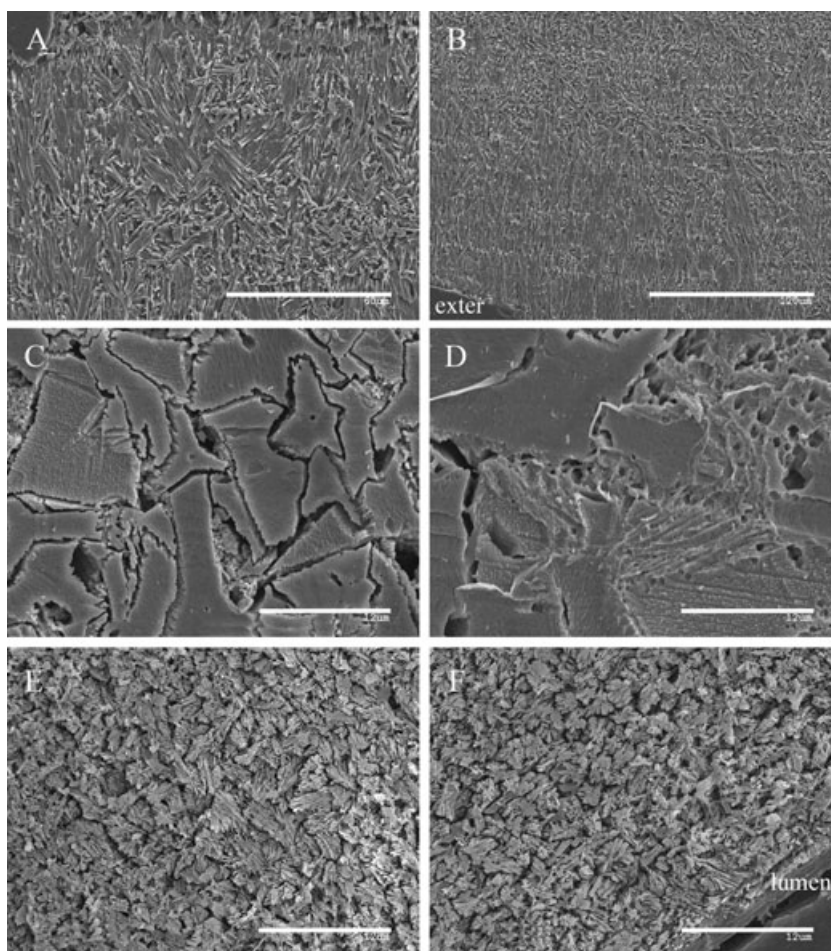


Figure 3. A–B, *Neovermilia falcigera*, irregularly oriented platy structure, outer tube layer. A, cross section. B, longitudinal section. C–D, *Bathyvermilia langerhansi*, homogeneous angular crystal structure, outer tube layer. C, cross section. D, longitudinal section. E–F, *Pomatostegus stellatus*, homogeneous rounded crystal structure. E, cross section. F, longitudinal section. All samples were polished and treated with 1% acetic acid for 2 min. Abbreviations: exter, exterior.

dicularly to the tube wall (Figs 4E, F, 9H). In distinct tube layers, the irregular spherulitic prismatic structure has an incremental zonation (e.g. *S. caribensis*). The structure lacks incremental zonation in the walls of tube cavities, such as in *F. elatensis* and *N. falcigera*. The structure occurs in the outer and inner tube layers of *C. websteri* (Fig. 4E, F) and *S. caribensis*; in the outer tube layer and inner tube lenses of *C. zygophora*; in the outer tube layer of *H. dianthus*, *H. norvegicus*, and *P. kraussii*; in the inner tube layer of *P. ctenactis*; in the inner tube lenses of *F. elatensis* and *F. sabiuraensis*; in the walls of tube cavities of *N. falcigera*; as dispersed spherulitic sectors in the IOP structure of the tube wall of *P. occidentalis* (Table 2).

The simple prismatic (SP) structure

This structure is formed by parallel prismatic crystals oriented perpendicularly to the tube wall (Figs 5A, B,

9I). All prismatic crystals show incremental zonation. This structure forms the entire tube of *Placostegus tridentatus* (Fabricius, 1779) and *Vitreotubus digeronimoi* Zibrowius, 1979 (Fig. 5A, B), and constitutes the outer tube layer of *S. crenata* (Table 2). It slightly resembles the SP structure in molluscs, as described by Carter *et al.* (1990: 610).

ORIENTED COMPLEX STRUCTURES (THE CRYSTALLIZATION AXIS OF THE CRYSTALS HAS A UNIFORM ORIENTATION THAT IS NOT CONTINUOUS THROUGH SUCCESSIVE GROWTH INCREMENTS)

The lamello-fibrillar (LF) structure

This structure is formed by elongate crystals that have a uniform orientation within each growth increment, and a different orientation in adjacent growth increments (Figs 4E, F, 5C, D, 9J). The substructure

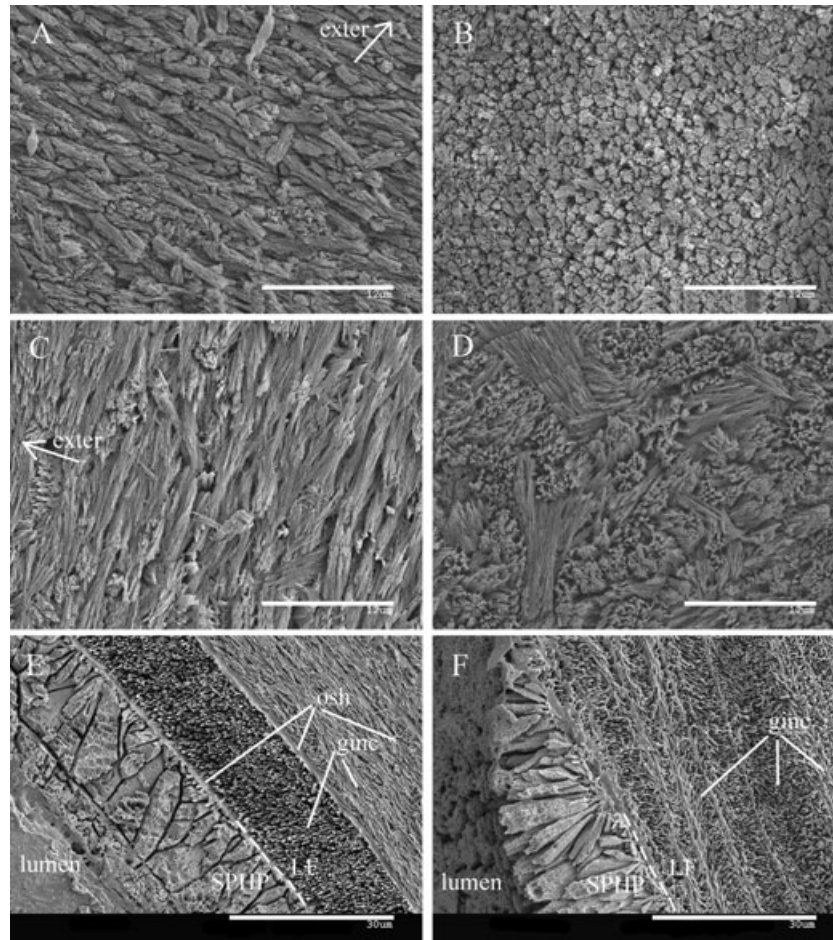


Figure 4. A–B, *Protula diomedeeae*, semi-ordered irregularly oriented prismatic structure. A, cross section. B, longitudinal section. C–D, *Pyrgopolon ctenactis*, semi-ordered spherulitic irregularly oriented prismatic structure, outer tube layer. C, cross section. D, longitudinal section. E–F, *Crucigera websteri*, contact of inner tube layer with spherulitic prismatic structure, and third tube layer from outside with lamello-fibrillar structure. E, cross section. F, longitudinal section. All samples were polished and treated with 1% acetic acid for 2 min. Abbreviations: exter, exterior; ginc, growth increments; LF, lamello-fibrillar structure; osh, organic sheet; SPHP, spherulitic prismatic structure.

is composed of elongate slender crystallites that are parallel with the longitudinal axis of the crystals forming the LF structure. The structure forms the entire tube of *Neovermilia sphaeropomatus* (Benham, 1927), *Pomatoceros triqueter* (Linnaeus, 1758) (Fig. 5C, D), *Serpula israelitica* Amoureux, 1977, and *Serpula vermicularis* Linnaeus, 1767; the outer tube layer of *G. hystrix*; the second tube layer from the outside of *F. sabiuraensis*; the third tube layer from the outside of *C. websteri* (Fig. 4E, F) and *P. kraussii*; and the inner tube layer of *H. dianthus* (Table 2).

The spherulitic lamello-fibrillar (SLF) structure

This structure is formed by sectors of spherulites that have a uniform orientation within each growth increment, and a different orientation in adjacent growth

increments (Figs 5E, F, 9K). The substructure is composed of elongate slender crystallites, oriented subparallel with the longitudinal axis of the crystals forming the SLF structure. The structure occurs in the inner tube layer of *F. sabiuraensis* (Fig. 5E, F, Table 2).

The ordered fibrillar (OF) structure

This structure is formed by elongate crystals that have a uniform orientation within each growth increment and the same orientation in adjacent growth increments (Figs 6A, B, 9L). The longitudinal axis of the crystals is oriented at an angle of about 90° to the radius of the tube, in the plane of cross section of the tube. The substructure is composed of elongate slender crystallites oriented parallel with the longitudinal axis of crystals

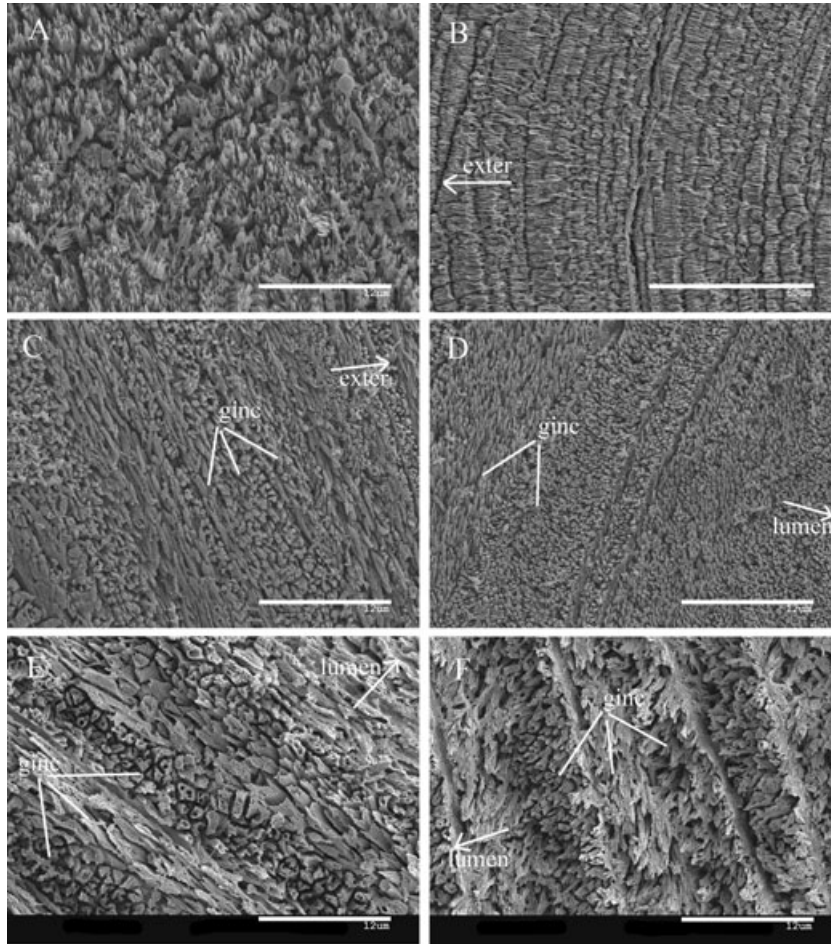


Figure 5. A–B, *Vitreotubus digeronimoi*, simple prismatic structure, polished, and treated with GA solution for 5 min. A, cross section. B, longitudinal section. C–D, *Pomatoceros triqueter*, lamello-fibrillar structure. C, cross section. D, longitudinal section. E–F, *Floriprotis sabiuraensis*, spherulitic lamello-fibrillar structure, inner tube layer. E, cross section. F, longitudinal section. Samples (C–F) were treated with 1% acetic acid for 2 min. Abbreviations: exter, exterior; ginc, growth increments.

forming the OF structure. The structure is found in the outer tube layer of *S. giganteus* (Fig. 6A, B, Table 2).

The most common tube ultrastructure is the IOP, occurring in 61% of the 44 species studied. The SPHP and the SIOP structures occur in 25% of species, and the LF structure occurs in 20% of species. The rest of the tube ultrastructures appear to play minor roles in serpulid tube construction.

HOMOGENEOUS CARBONATE CEMENT

Unevenly distributed homogeneous carbonate cement usually occurs between the crystals of the IOP structure of *C. serrula*, *F. enigmaticus*, *F. implexa*, *F. gracilis*, *H. marenzelleri*, *J. marenzelleri*, *M. cavatica*

(Fig. 1C–E), and *S. crenata* (Fig. 1F). This cement may be completely invisible in some parts of the tube wall, whereas it may be abundant in other parts. In some species, the IOP crystals may be completely surrounded by homogeneous carbonate cement, as in *M. cavatica* (Fig. 1E), *J. marenzelleri*, and *S. crenata* (Fig. 1F). To a lesser extent it may also occur in the most external portion of the tube wall in *P. triqueter*.

SPHERULITIC INCLUSIONS

Spherulitic inclusions may occur in the granular structures, as in *F. enigmaticus* (Fig. 6), and rarely occurs in the prismatic structures of *P. tridentatus* (Fig. 6F), inside the narrow split between the tooth and the tube wall (Table 2).

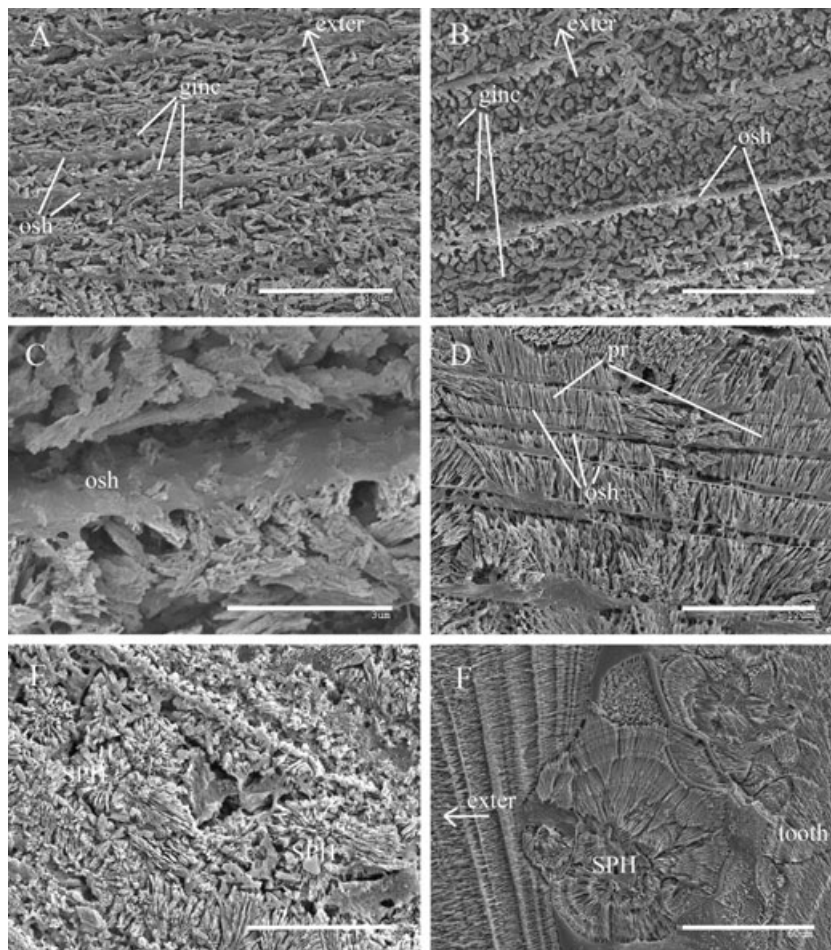


Figure 6. A–D, *Spirobranchus giganteus*, polished, and treated with GA solution for 5 min. A, outer tube layer with oriented fibrillar structure, cross section. B, outer tube layer, oriented fibrillar structure, longitudinal section. C, detail of (B), showing the close-up of the organic sheet. D, outer layer with spherulitic irregularly oriented prismatic structure; note the epitaxial growth of prisms through several organic sheets. E, *Ficopomatus enigmaticus*, polished longitudinal section, spherulites in irregularly oriented prismatic structure, treated with 1% acetic acid for 2 min. F, *Placostegus tridentatus*, calcitic spherulite, longitudinal section of the tube with simple prismatic structure. Abbreviations: exter, exterior; ginc, growth increments; osh, organic sheet; pr, prismatic crystals; SPH, spherulite.

ORGANIC SHEETS IN THE MINERAL TUBE STRUCTURES

Thin organic sheets parallel with the accretion surface of the tube are common inside the calcareous serpulid tubes (Figs 4E, 6A–D). They occur at semi-regular intervals in *S. giganteus* (treated with GA solution for 5 min), both in the inner SIOP structure and in the outer OF structure (Fig. 6A–D). The sheets are about 0.1–0.2- μm thick and their intervals are 0.5–6.0 μm . The spherulitic sectors of the inner SIOP structure grow epitaxially through several sheets in *S. giganteus*. The crystals of the outer ordered fibrillar layer have not been observed to grow through the sheets. In *C. websteri*, organic sheets may or may not occur at the boundaries of different growth incre-

ments, or inside a single growth increment in the LF layer.

ORGANIC MATRIX IN THE INTERSPACES BETWEEN THE CRYSTALS IN THE IOP STRUCTURE

The crystals are usually loosely packed in the IOP structure, and their interspaces can be filled with organic matrix, homogeneous carbonate cement, or with both (see above). In *S. crenata*, crystals of the IOP structure are completely embedded in homogeneous organic matrix (Fig. 7A). The organic matrix is coloured blue after treatment with GA solution, and is partially decomposed (Fig. 7B). The structure shows

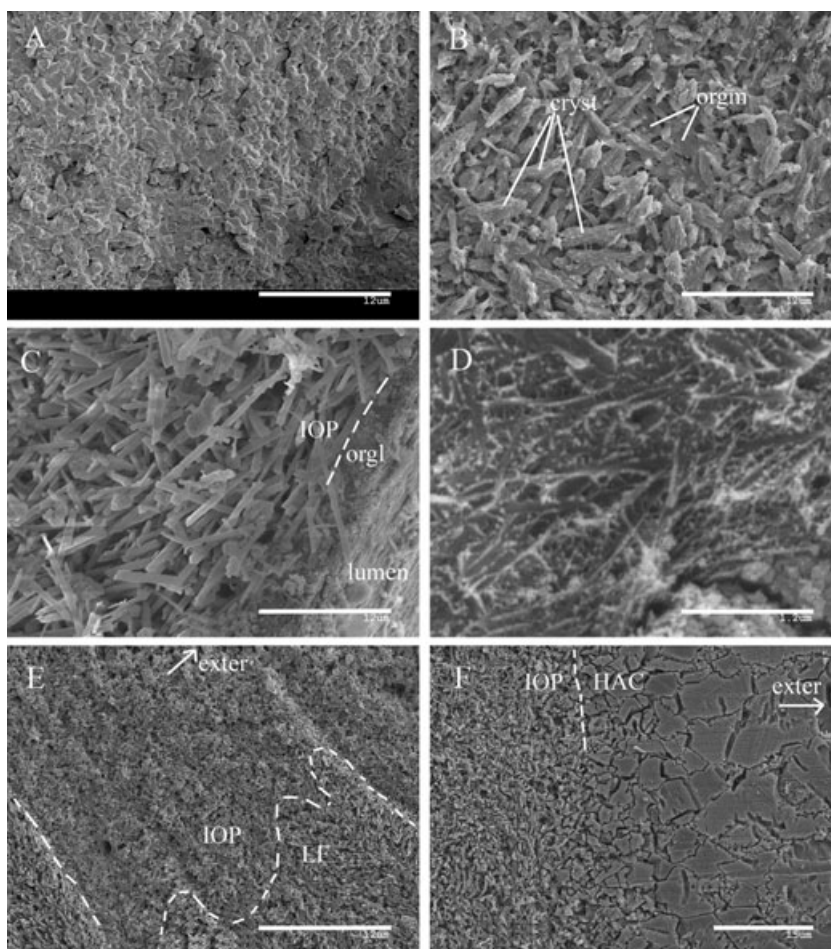


Figure 7. A–B, *Serpula crenata*, inner tube layer. A, cross section, fresh fracture surface, showing the crystals of irregularly oriented prismatic structure completely embedded into the organic matrix. B, polished cross section, treated with GA solution for 5 min; note that the organic matrix is partially decomposed. C, *Marifugia cavatica*, longitudinal section, fresh fracture surface, inner mineral irregularly oriented prismatic structure, and inner organic tube layer. D, *Spirobranchus giganteus*, tube lumen, inner organic layer; note the mesh-like composition of the layer. E, *Hydroides dianthus*, polished longitudinal section, treated with 1% acetic acid for 2 min, showing transition between irregularly oriented prismatic and lamello-fibrillar structure. F, *Laminatubus alvini*, polished cross section, treated with 1% acetic acid for 2 min, showing transition between inner irregularly oriented prismatic and outer homogeneous angular crystal structure. Abbreviations: cryst, crystals; exter, exterior; HAC, homogeneous angular crystal structure; IOP, irregularly oriented prismatic structure; LF, lamello-fibrillar structure; orgm, organic matrix; orgl, inner organic layer of the tube.

loose contact between the aragonitic crystals in the irregularly oriented prismatic structure in *F. elatensis*.

INNER ORGANIC TUBE LAYER

The presence and structure of the thin inner organic tube layer described by Nishi (1993) is beyond the scope of the present study. However, we found a well-developed inner organic layer in *M. cavatica* (Fig. 7C), which is about 3.0- μm thick, and in *S. giganteus* (Fig. 7D).

MINERAL LAYERS OF SERPULID TUBES

Ultrastructurally, serpulid tubes can be either single layered or multilayered. Tube layers are recognised as separate layers, if, in adjacent layers, (1) the orientation, morphology, and size of crystals is different, or (2) the quantity of homogeneous carbonate cement between the IOP crystals is different.

Single-layered tubes

A total of 66% of the 44 serpulid species studied here are single layered. Most of the single-layered tubes have an IOP structure, but they can also be of LF (e.g.

S. vermicularis, *S. israelitica*, and *P. triqueter*) or SP structures (e.g. *P. tridentatus* and *V. digeronimoi*) (Table 2).

Multilayered tubes

We found that 34% of the 44 serpulid species studied have more than one distinct tube layer (Table 2). In general, such tubes have two layers (e.g. *B. langerhansi*, *G. hystrix*, *H. norvegicus*, *L. alvini*, *M. cavatica*, *N. falcigera*, *P. ctenactis*, *Serpula crenata*, and *S. giganteus*), with the exception of *C. zygophora*, *F. sabiuraensis*, *H. dianthus*, and *S. caribensis*, which have three-layered tubes, and *C. websteri* and *P. kraussii*, which have four-layered tubes (Table 2). In tubes with a multilayered structure, each layer is almost always composed of a different type of ultrastructure, with the exception of a few species, such as *B. langerhansi*, where the crystals of the outer and inner layers differ only in size, and *M. cavatica*, where the inner IOP layer is distinguished from the outer IOP layer by an increased quantity of homogeneous carbonate cement (Fig. 1C–E). If present, the oriented prismatic structures (e.g. the SPHP, SP, and regularly ridged prismatic structures) always form the outer or the inner tube layer, whereas additional layers have other types of ultrastructures (Table 2).

Tube layers are mostly continuous in the cross and longitudinal sections studied. However, in several species (e.g. *C. zygophora*, *F. elatensis*, *F. sabiuraensis*, *H. dianthus*, and *P. occidentalis*) the tube wall contains lenses of a distinct ultrastructure, which usually form the innermost part of the tube wall (Table 2).

Boundaries between the tube layers, and transitions between the ultrastructures

The boundary is distinct between the inner SPHP and internal LF layers in *C. websteri* (Fig. 4E, F). However, boundaries between the tube layers are often somewhat transitional in other species studied. A gradual structural transition occurs: (1) in *C. zygophora* between the outer SPHP layer, the middle IOP layer (Fig. 2C), and the inner SIOP layer (Fig. 2C, D); (2) in *H. dianthus* between the inner LF and the middle IOP layers (Fig. 7E); (3) in *F. sabiuraensis* between the outer IOP layer, middle LF, and inner SLF layers; (4) in *L. alvini* between the outer coarse angular crystal homogeneous layer and the inner IOP layer (Fig. 7F); and (5) in *B. langerhansi* between the outer coarse angular crystal homogeneous layer and the inner fine angular crystal homogeneous layer. In *S. giganteus* there are a few growth increments with slightly different crystal orientation within the outer ordered fibrillar structure, making it somewhat similar to the LF structure.

MINERAL COMPOSITION OF SERPULID TUBES

Most of the species studied here are of aragonitic–calcitic composition (Table 3). Two-layered species can contain both polymorphs, as in *H. dianthus*, or can be completely calcitic, as in *M. cavatica*. However, among the aragonitic–calcitic species, only the two-layered *P. ctenactis* has a tube containing both polymorphs in equal quantities. Single-layered species are of mixed composition and are rarely monomineral (Tables 2, 3). However, the single-layered *P. tridentatus* and *V. digeronimoi* are purely calcitic. In single-layered species of mixed composition, one component always dominates, contributing at least 75% of the material to the structure (Table 3).

TRANSPARENCY OF TUBE LAYERS

Macroscopically, serpulid tubes are either opaque or hyaline, or are composed of hyaline and opaque layers. *Placostegus tridentatus* and *V. digeronimoi* show completely hyaline tubes: the whole tube has an SP ultrastructure. A hyaline external cover or layer has been observed in *A. globifer* (the whole tube has an IOP structure), *B. langerhansi* (the outer tube layer has an HAC structure), *C. zygophora* (the outer tube layer has an SPHP structure), *G. hystrix* (the outer tube layer has an LF structure), *H. dianthus* (the outer tube layer has an SPHP structure),

Table 3. Mineral composition of serpulid tubes (quoted as the percentage weight of carbonate phases)

Species	Calcite	Aragonite
<i>Marifugia cavatica</i>	100.0	
<i>Placostegus tridentatus</i>	100.0	
<i>Vitreotubus digeronimoi</i>	100.0	
<i>Hydroides norvegicus</i>	98.4	1.6
<i>Pomatoceros triqueter</i>	98.4	1.6
<i>Filigrana implexa</i>	96.8	3.2
<i>Serpula vermicularis</i>	93.6	6.4
<i>Galeolaria hystrix</i>	91.2	8.8
<i>Ficopomatus enigmaticus</i>	90.7	9.3
<i>Serpula israelitica</i>	88.3	11.7
<i>Laminatubulus alvini</i>	86.9	13.1
<i>Neovermilia sphaeropomatus</i>	84.2	15.8
<i>Pyrgopolon ctenactis</i>	50.9	49.1
<i>Apomatus globifer</i>	24.2	75.8
<i>Filigranella elatensis</i>	17.2	82.8
<i>Pseudovermilia occidentalis</i>	15.6	84.4
<i>Vermiliopsis infundibulum</i>	13.8	86.2
<i>Spiraserpula caribensis</i>	7.2	92.8
<i>Protula diomedea</i>	5.8	94.2
<i>Hydroides spongicola</i>	5.0	95.0
<i>Pomatostegus stellatus</i>	3.1	96.9
<i>Josephella marenzelleri</i>	2.2	97.8

H. norvegicus (the outer tube layer has an SPHP structure), *L. alvini* (the outer tube layer has an HAC structure), *M. cavatica* (the outer tube layer has an IOP structure), *N. falcigera* (the outer tube wall has an IOPL structure), *N. sphaeropomatus* (the whole tube wall has an LF structure), *P. ctenactis* (the outer tube layer has an SOIOP structure), *S. crenata* (the outer tube layer has an SP structure), *S. vermicularis* (the whole tube wall has an LF structure), and *S. caribensis* (the outer tube layer has an SPHP structure) (Table 2). A hyaline inner layer with an SPHP structure was observed in *C. websteri* and *F. elatensis*, with the latter containing SPHP lenses. A hyaline inner tube layer with an SIOP structure was also observed in *N. sphaeropomatus*. In *P. triqueter*, with an inner hyaline layer, the whole tube wall has an LF structure. A hyaline inner layer occurs in *P. ctenactis* and *S. caribensis*, in which the inner tube layer has an SPHP structure (Table 2). See the Discussion.

THE ULTRASTRUCTURE OF TABULAE

Tabulae were found in the tube of *P. triqueter*. They are about 30- μm thick and are composed of irregularly oriented short prismatic-to-cubic crystals in a rich organic matrix. The structure is termed here as the fine angular crystal homogeneous structure (Fig. 8). The crystals forming the structure are about 0.15–0.4 μm in size, and do not show any substructure.

DISCUSSION

TUBE ULTRASTRUCTURE AND TRANSPARENCY

The transparency of serpulid tubes has been interpreted to reflect the oriented prismatic ultrastructure

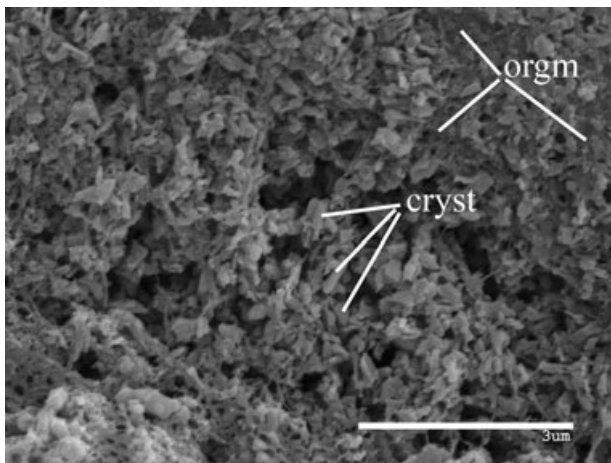


Figure 8. *Pomatoceros triqueter*, polished section of the tabula, fine crystal homogeneous structure, treated with 1% acetic acid for 2 min. Abbreviations: cryst, crystals; orgm, organic matrix.

of the tube (Zibrowius & ten Hove, 1987). Our SEM observations confirm that all tubes composed of the SP structure (e.g. *P. tridentatus*, *V. digeronimoi*, and *S. crenata*) or of the SPHP structure (e.g. *C. websteri*, *C. zygophora*, *F. elatensis*, *H. dianthus*, *H. norvegicus*, *P. ctenactis*, and *S. caribensis*) are always optically hyaline. In addition to oriented prismatic structures, the coarse crystals of the HAC structure (e.g. *B. langerhansi* and *L. alvini*), the IOPL structure (e.g. *N. falcigera*), and the SOSIOP structures are also optically hyaline. In most of the species, an IOP structure is optically opaque. However, in some species (e.g. *M. cavatica*) the outer tube layer of a similar IOP structure is also hyaline. Thus, most hyaline tube layers have oriented prismatic structures, but irregularly oriented structures can sometimes also look hyaline.

MINERALOGY AND ULTRASTRUCTURE

Serpulids of mixed mineralogy are very common (Table 3), in agreement with previous studies (Lowenstam, 1954; Bornhold & Milliman, 1973). In species of mixed mineralogy, aragonite and calcite were hitherto thought to be present in separate layers, as is commonly the case with most bimineralic molluscs (Simkiss & Wilbur, 1989). Although we have not been able to study the layers of tubes separately, we provide some deductive observations below. Two-layered bimineralic species (e.g. *P. ctenactis*) may prove to have a different polymorph in each layer. However, the two-layered tubes of *M. cavatica* are of a single polymorph, calcite (see below). Moreover, most single-layered serpulids are also bimineralic, with the exception of completely calcitic prismatic *P. tridentatus* and *V. digeronimoi*. This raises the question of whether the crystals of single-layered species such as *S. israelitica*, *F. enigmaticus*, and *A. globifer* are of mixed composition, or if the presence of the minor component should be attributed to the very fine crystals of homogeneous carbonate cement between the large crystals. We believe that the crystals of aragonite and calcite are still separated similarly to the mixed argonitic–calcitic layer in the archaeogastropod *Haliotis* (Mutvei, Dauphin & Cuif, 1985; Mutvei, 1989). On the other hand, in the completely calcitic *F. implexa* and *M. cavatica*, the homogeneous carbonate cement can only be of the same mineral composition as the granules forming the IOP structure.

The species studied here with FL ultrastructures have calcitic (*S. vermicularis*) or dominantly calcitic tubes (*S. israelitica* and *P. triqueter*). Species with tubes that are composed only of IOP structure are predominantly aragonitic (e.g. *A. globifer*, *F. elatensis*, and *V. infundibulum*), exclusively calcitic (e.g. *M. cavatica*), or predominantly calcitic (e.g.

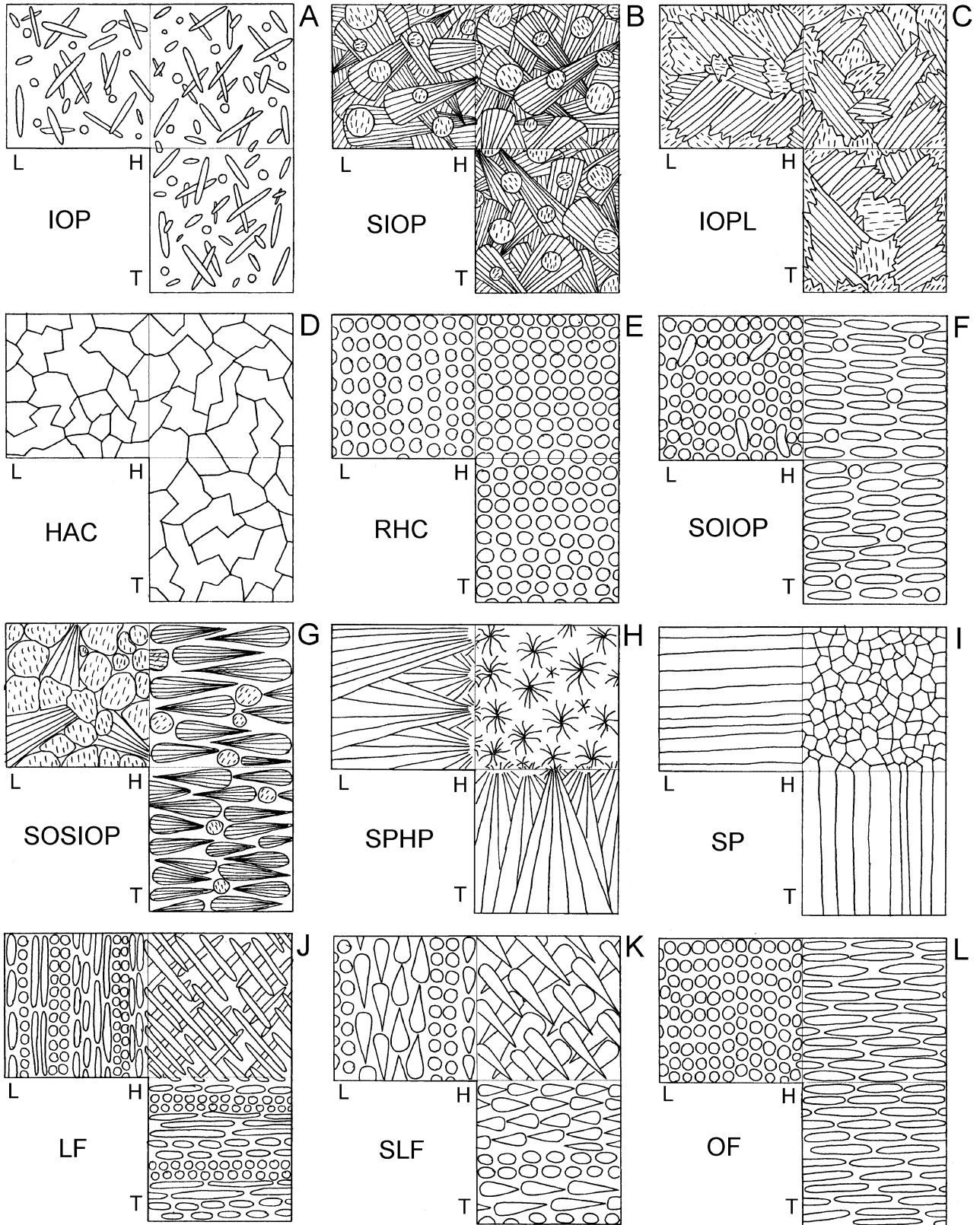


Figure 9. Schematic line drawings of serpulid tube ultrastructures. A, irregularly oriented prismatic (IOP) structure. B, spherulitic irregularly oriented prismatic (SIOP) structure. C, irregularly oriented platy (IOPL) structure. D, homogeneous angular crystal (HAC) structure. E, rounded homogeneous crystal (RHC) structure. F, semi-ordered irregularly oriented prismatic (SOIOP) structure. G, semi-ordered spherulitic irregularly oriented prismatic (SOSIOP) structure. H, spherulitic prismatic (SPHP) structure. I, simple prismatic (SP) structure. J, lamello-fibrillar (LF) structure. K, spherulitic lamello-fibrillar (SLF) structure. L, ordered fibrillar (OF) structure. Abbreviations: H, horizontal section; L, longitudinal section; T, transverse section.

F. enigmaticus and *F. implexa*). Bornhold & Milliman (1973) reported *F. implexa* as an aragonitic species, which is contradictory to our results. However, *F. implexa* had a confused taxonomy in 1973, and even included non-operculate forms (see Nogueira & ten Hove, 2000). Without access to Bornhold & Milliman's material, the identity of their material must be deemed questionable; it may very well belong to a different species from our material. An alternative explanation would necessitate an extremely high flexibility of this particular taxon concerning the mineral composition of its tube. Further studies are needed to solve this problem. Tubes of the two-layered *M. cavatica* are completely calcitic. Thus, both polymorphs can be used by the worm to form an IOP structure.

ECOLOGICAL IMPLICATIONS

The calcium for the tubes of serpulid worms is supplied at least in part from the surrounding water. Therefore, differences have been expected to occur between marine, brackish, and freshwater species (Oglesby, 1978). Our observations do not support this notion: neither in mineralogy nor in ultrastructure (see Tables 2, 3). The purely calcitic freshwater species *M. cavatica* has an IOP tube ultrastructure that is similar to calcitic marine species, such as the 90% calcitic *F. implexa* (Tables 2, 3). There are also several purely calcitic marine species, such as *P. tridentatus* and *V. digeronimoi*. The brackish *F. enigmaticus* has the most common serpulid tube ultrastructure (e.g. an IOP structure), as well as a mixed mineralogy, as is found in many marine species (Tables 2, 3). Thus, the most common tube ultrastructure of marine species (i.e. the IOP structure) also occurs in brackish and freshwater species.

BIOMINERALIZATION CONTROL OVER CRYSTAL ORIENTATION

With respect to biomineralization control over crystal orientation, serpulid tube ultrastructures can be divided into four major categories: (1) structures lacking orientation (homogeneous structures and irregularly oriented structures); (2) semi-oriented structures; (3) a simple type of oriented structure

(prismatic), where the orientation of crystals can involve epitaxy; and (4) a complex type of oriented structure, where the crystals cannot be oriented by epitaxy (e.g. LF, SLF, and OF structures).

In the case of oriented prismatic structures, the orientation of the crystals can be achieved by epitaxial continuation of the crystal along its longitudinal axis. In contrast, in LF and SLF structures the orientation of crystals is different in adjacent growth increments. In the OF structure, the crystals have a uniform orientation in adjacent growth increments, but their axes are not continuous through the growth increments. The orientation of crystals in the latter structures is thus presumably completely controlled by the organic matrix. Most of the 44 serpulid species studied (52%) are characterized by tubes lacking an oriented ultrastructure, or by tubes with a semi-oriented ultrastructure. We found that 23% of the species are capable of producing oriented tube ultrastructures of a simple type, where epitaxy can be involved in control over the crystal orientation. However, 25% of the species can mineralize oriented structures, where the crystal orientation must result from a complex control mechanism. This presumably implies a mechanism capable of detecting or 'memorizing' the orientation of crystals in the previously deposited growth increment.

Complex oriented serpulid tube structures, similar to LF structures in molluscs, presumably have a tube-strengthening function. These complex structures may have material properties that are mechanically superior, compared with structures lacking orientation, and compared with simple types of oriented structures. The latter structures lacking orientation might therefore be plesiomorphic. Simple types of oriented structures (an SPHP structure) also occur in *Glomerula* (Sabellidae) and cirratulids (Vinn *et al.*, 2008). Complex oriented structures may have evolved in serpulids to combine fast tube growth with mechanical strength.

Traditionally, serpulids have been described as having a small number of simple skeletal ultrastructures, and as lacking the complex ultrastructures that are characteristic of molluscs, bryozoans, and brachiopods. Our study shows that biomineralization mecha-

nisms of serpulid polychaetes lead to far more complex tube ultrastructures than have been recorded in the literature.

EVOLUTION OF TUBE ULTRASTRUCTURE

The phylogeny of Serpulidae has been discussed by several authors on the basis of morphological characters (Uchida, 1978; ten Hove, 1984; Fitzhugh, 1989; Kupriyanova, 2003). Traditionally, Serpulidae are subdivided into three subfamilies: Filograninae Rioja, 1923, Serpulinae Macleay, 1840, and Spirorbinae Chamberlin, 1919. A recent study (Kupriyanova, Macdonald & Rouse, 2006) of serpulid phylogeny, based on combined molecular and morphological characters of 29 species, supports two major clades within Serpulidae (Fig. 10). The first major clade includes Spirorbinae as a sister group to a monophyletic group comprising both 'filogranin' taxa (*Salmacina*, *Filograna*, *Protis*, and *Protula*) and 'serpulid' taxa, such as *Chitinopoma* and *Vermiliopsis*. The second major clade includes the following 'serpulid' genera: *Crucigera*, *Hydroides*, *Serpula*, *Ditrupa*, *Pseudochitinopoma*, *Ficopomatus*, *Galeolaria*, *Pomatoceros*, and *Laminatubus* (see Kupriyanova *et al.*, 2006: 432, Fig. 7).

Comparison of the tube ultrastructure data (Table 2) with the phylogeny of Serpulidae described by Kupriyanova *et al.* (2006) reveals several patterns. Most striking is the difference in the complexity of tube ultrastructures between the two major clades. The complex oriented structures, such as LF, SLF, and OF structures, occur only in genera of the second clade. Similarly, the oriented prismatic structures also only occur in genera of the second clade (Fig. 10). However, a SOIOP structure occurs in *Protula*, representing the most advanced tube ultrastructure for the first clade (Fig. 10). Thus, one could hypothesize that if the oriented tube ultrastructures of the second clade are more derived than the unoriented structures of the first clade, then the latter could represent the plesiomorphic condition for Serpulidae. However, the occurrence of the LF tube structure has high intrageneric variability (Table 2), indicating that it may have evolved several times independently within the second clade. Probably the superior mechanical properties of the LF structure, as compared with the unoriented or simple oriented structures, would make this a useful adaptation, thereby favouring a multiple appearance of the character over a multiple disappearance of the character.

Among annelids, serpulids possess the highest number of different skeletal ultrastructures. Two skeletal ultrastructures are known in sabellids

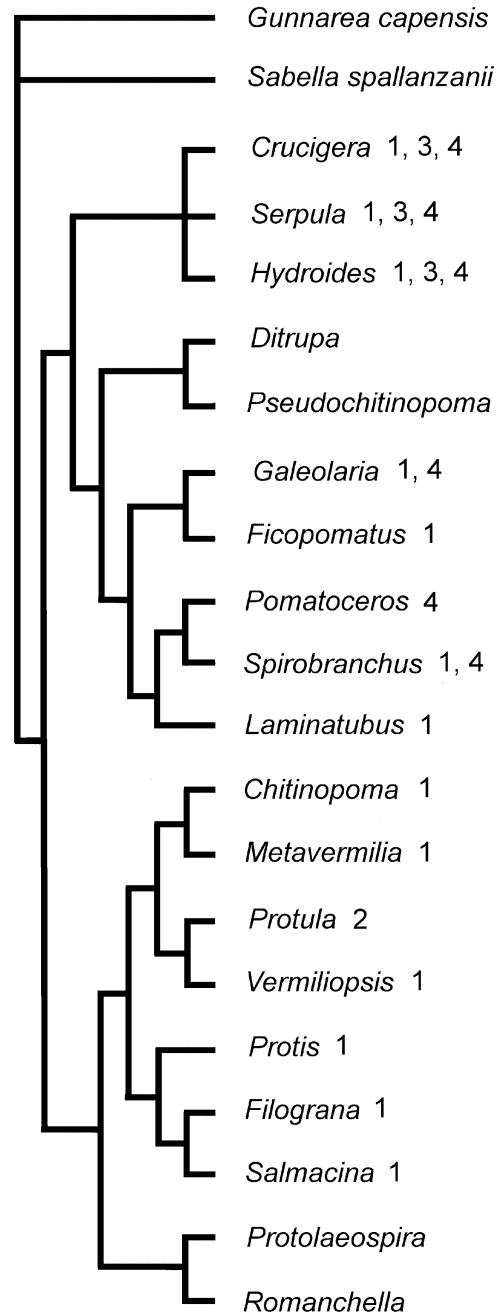


Figure 10. Evolution of serpulid tube ultrastructures. Phylogenetic relationships of serpulid genera derived from Kupriyanova *et al.* (2006), Bayesian majority consensus phylogram of the combined molecular and morphological data set. Numbers denote the presence of the ultrastructures in a particular genus: 1, unoriented structures; 2, semi-oriented structures; 3, oriented prismatic structures; 4, oriented complex structures.

(spherulitic and spherulitic prismatic, in *Glomerula*) and one (spherulitic prismatic) in cirratulids (Vinn *et al.*, 2008). However, the spherulitic structure of the external tube layer of *Glomerula* is not known in

serpulids. There were seven different skeletal ultrastructures previously known in annelids, fewer than in arthropods (11), corals (15), brachiopods (16), bryozoans (19), and molluscs (42), and slightly more than in echinoderms (5), according to Carter *et al.* (1990). Our new data on serpulids show that the diversity of annelid skeletal ultrastructures is comparable with that of arthropods and corals, and is higher than has been previously reported.

TAXONOMIC IMPLICATIONS

Serpulid tube structure has been regarded as being too variable, and at the same time too similar, to warrant taxonomic importance in the 12 species studied by Nishi (1993). Our preliminary observations, based on 44 species in 36 genera (of a total of about 350 species in 50 genera), contradict this opinion, and show that diverse and discrete structures occur in serpulids, and are possibly taxon specific. According to our observations, 47% of our 44 species possess a unique combination of tube structure characters. Because of the high intrageneric variability (see Table 2), serpulid tube structure seems more likely to be a species-level rather than a generic-level character. However, certain more complex ultrastructures, such as LF, SLF, and OF structures, could have some importance at a higher taxonomic level (Fig. 10).

CONCLUSIONS

1. Serpulids possess more diverse skeletal ultrastructures than have been previously reported. We have distinguished 12 different types of tube ultrastructure.
2. Serpulid tube ultrastructure can be used taxonomically.
3. There are evolutionary trends in tube ultrastructure (Fig. 10). The complex oriented structures, such as LF, SLF, and OF structures, occur only in genera of the *Crucigera*, *Hydroides*, *Serpula*, *Ditrupa*, *Pseudochitinopoma*, *Ficopomatus*, *Galeolaria*, *Pomatoceros*, and *Laminatubus* clade.
4. There is a relationship between ultrastructure and transparency of the tube. Oriented prismatic ultrastructures are always transparent. However, other structures can also be transparent.
5. Calcitic and aragonitic tube ultrastructures are not always different. Species with FL ultrastructures have calcitic (*S. vermicularis*) or dominantly calcitic tubes (*S. israelitica* and *P. triqueter*).
6. The tube ultrastructure and composition is not different between marine, brackish, and freshwater species.

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