

Article

The Role of Aragonite in Producing the Microstructural Diversity of Serpulid Skeletons

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Abstract: Aragonite plays an important role in the biomineralization of serpulid polychaetes. Aragonitic structures are present in a wide range of serpulid species, but they mostly belong to one clade. Aragonitic structures are present in a wide range of marine environments, including the deep ocean. Aragonitic tube microstructures were studied using a scanning electron microscope. X-ray powder diffraction was used to identify the aragonite. Aragonite is used to build five different types of microstructures in serpulid tubes. The most common aragonitic irregularly oriented prismatic structure (AIOP) is also, evolutionarily, the most primitive. Some aragonitic microstructures, such as the spherulitic prismatic (SPHP) structure, have likely evolved from the AIOP structure. Aragonitic microstructures in serpulids are far less numerous than calcitic microstructures, and they lack the complexity of advanced calcitic microstructures. The reason why aragonitic microstructures have remained less evolvable than calcitic microstructures is currently unknown, considering their fit with the current aragonite sea conditions (Paleogene–recent).

Keywords: aragonite; microstructure; ultrastructure; tubeworms; evolution



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1. Introduction

In temperate regions of the ocean, serpulids can have an important role in the carbonate sink [1]. Serpulids start to build calcareous tubes in their post-larval stage [2]. Serpulid worms are not attached to their tubes and can move freely within them. Serpulid tubes lack an external organic cover, such as periostracum of molluscs and barchiopods; however, their tube interior is covered by a thick organic sheet [3]. The serpulid tubes are composed of aragonite, calcite or a mixture of both polymorphs [4,5]. The mineralogical composition of serpulid tubes depends on their phylogenetic position (Figure 1), so that dominantly calcitic species form one clade, whereas dominantly aragonitic species form the other clade (i.e., *Chitinopoma*, *Filograna*, *Metavermilium*, *Protis*, *Protula*, *Salmacina*, and *Vermiliopsis*) [5]. Serpulid tube microstructures are relatively well known [4], with fifteen distinct types of microstructures, hitherto described. Vinn et al. [4,6] interpreted the occurrence of the complex oriented biomineral structures in *Spirobranchus americanus*, and many other serpulids, as an indicator of organic matrix-controlled crystallization, analogous to that of the biomineralization of mollusc shells. Some serpulids have biomineralized opercula, which can contain up to two layers with distinct microstructures [7]. The calcareous serpulid opercula are also secreted by the epithelium, via organic matrix-controlled crystallization [7]. On the other hand, much less is known about the mineralogical composition of serpulid tube microstructures. The aim of this paper is to identify aragonitic structures among the known serpulid microstructures. Serpulids possess several unique tube microstructures, and at least one of them is aragonitic [8]. Aragonitic structures are present in a wide range of marine environments, including the hadal zone [9]. Chan et al. [2] found that juvenile tubes of *H. elegans* were predominantly aragonitic and contained more amorphous calcium carbonate, whereas adult tubes were always bimineralic and contained much more calcite. Similar mineralogical changes are likely to occur during the ontogeny of other bimineral serpulid taxa. Two-layered and multilayered serpulid tubes often have an outer layer composed of denser mineral structures than the inner layers [10].

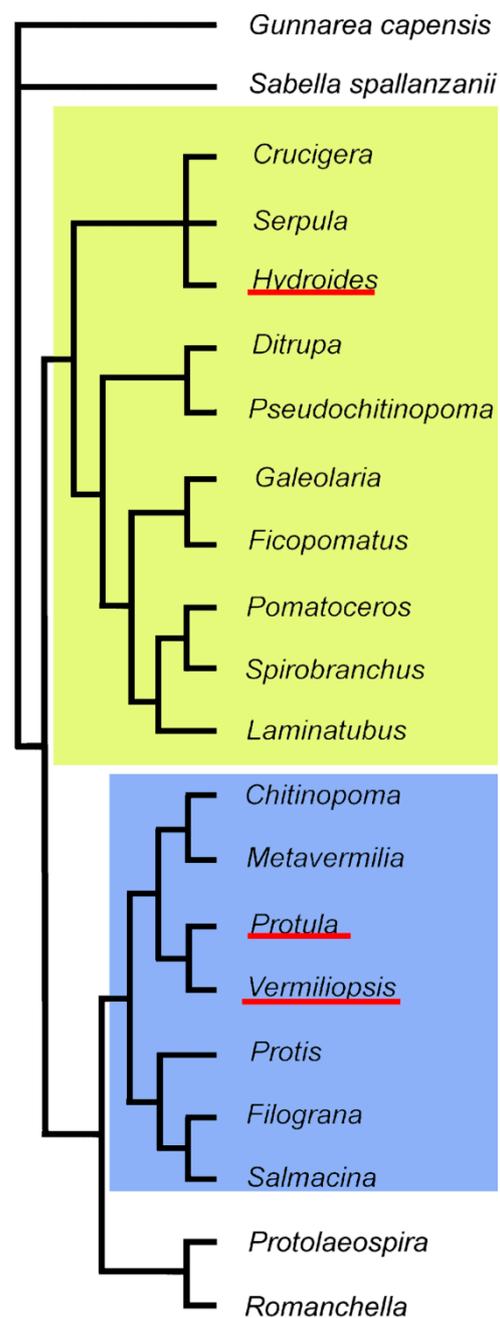


Figure 1. Phylogeny of serpulids. Studied genera are underlined. Dominantly calcitic serpulids are marked with yellow and dominantly aragonitic serpulids with blue modified after Vinn [11].

The terminology of serpulid tube microstructures follows Vinn et al. [4]. The present paper addresses the following questions: (1) are aragonitic serpulid structures as complex and diverse as calcitic serpulid structures? (2) How did the aragonitic microstructures evolve? (3) To which invertebrates is the complexity and diversity of aragonitic serpulid structures most similar.

2. Materials and Methods

Serpulid tubes were collected intertidally or by diving, dredging or trawling. The collected serpulids were fixed in 4% (buffered) formalin and were later transferred to 70% ethanol. All specimens originate from the Zoological Museum of Amsterdam, Amsterdam, the Netherlands. Present study (Table 1) covers most known species of aragonitic serpulids

(for list of known aragonitic serpulids see Smith et al. [5]). All tubes were cut using small electrical saw, oriented, and mounted in Canada balsam for machine grinding. Five- to ten-millimeter-long longitudinal sections, and one to three cross sections of each serpulid species were prepared. Sectioned tubes were polished with polishing machine and etched in a 1% solution of acetic acid for 1 min. All samples were gold sputtered prior to SEM study with a Hitachi S-4300 SEM at the Swedish Museum of Natural History. During the SEM study, beam was operated at 5–10 kV and 1 nA. The mineral composition of serpulid microstructures was identified using X-ray diffraction (XRD) in powdered samples on a Dron-3M diffractometer, at the Department of Geology, Institute of Ecology and Earth Sciences, University of Tartu. The mineral composition of tubes was studied on single tube or tube fragment.

Table 1. The locality information and data on the tube microstructures.

Species	Locality	Structures
<i>Apomatus globifer</i> Théel, 1878	Kara Sea, 71° N, 64° E	AIOP
<i>Filigranella elatensis</i> (Ben-Eliahu and Dafni, 1979)	Japan, Sesoko Island, Okinawa	AIOP, ASPHP
<i>Hydroides spongicola</i> Benedict, 1887	Netherlands Antilles, Curaçao, Sta.2048Ba	ASIOP
<i>Josephella marenzelleri</i> Langerhans, 1884	France, Marseille	AIOP
<i>Pomatostegus stellatus</i> (Abildgaard, 1798)	Netherlands Antilles, Curaçao, Sta.2093	ARHC
<i>Protula diomedea</i> Benedict, 1887	USA, Florida, 27° N, 84° W	ASOIOP
<i>Pseudovermilia occidentalis</i> (McIntosh, 1885)	Cape Verde Islands, Sta.Luzia, Sta.6.D07	AIOP
<i>Spiraserpula caribensis</i> Pillai and ten Hove, 1994	Netherlands Antilles, Curaçao, Sta.2061A	ASPHP, ASIOP
<i>Vermiliopsis infundibulum</i> (Philippi, 1844)	Canary Islands, Lanzarote, Sta.4.071	ASIOP

3. Results

Aragonitic structures (confirmed by XRD) are present in the tube wall of the following nine serpulid species (Table 1): *Apomatus globifer*, *Filigranella elatensis*, *Hydroides spongicola*, *Josephella marenzelleri*, *Pomatostegus stellatus*, *Protula diomedea*, *Pseudovermilia occidentalis*, *Spiraserpula caribensis*, and *Vermiliopsis infundibulum*. Aragonitic tubes are mostly single layered, but in *Spiraserpula caribensis*, the tube wall contains two distinct microstructural layers. In serpulids, the aragonite is used to build five different microstructures (Table 1). Both isotropic and anisotropic aragonitic structures are present in serpulids.

The aragonitic irregularly oriented prismatic (AIOP) structure is most common in serpulids, and it occurs in *Apomatus globifer* (Figure 2), *Filigranella elatensis*, *Josephella marenzelleri*, and *Pseudovermilia occidentalis*. This isotropic structure is composed of irregularly oriented, elongated (1 to 2 µm in diameter and 2 to 4 µm long) aragonite crystallites. The substructure is composed of parallel elongate rods, about 0.2 µm in diameter, and about 1 to 1.5 µm long. The AIOP structure is somewhat porous, and the elongate crystallites are not intergrown.

The second most common aragonitic structure in serpulids is the aragonitic spherulitic, irregularly oriented prismatic structure (ASIOP), and it is present in *Hydroides spongicola*, *Spiraserpula caribensis* (Figure 3), and *Vermiliopsis infundibulum*. The ASIOP is an isotropic structure and is composed of irregularly oriented intergrown spherulitic sectors, with a maximum diameter of about 2.5 µm and about 3 µm long. The substructure is composed of slender aragonitic rods (0.09 µm in diameter and up to 0.4 µm long) that are oriented subparallel, to parallel, to each other within a spherulitic sector.

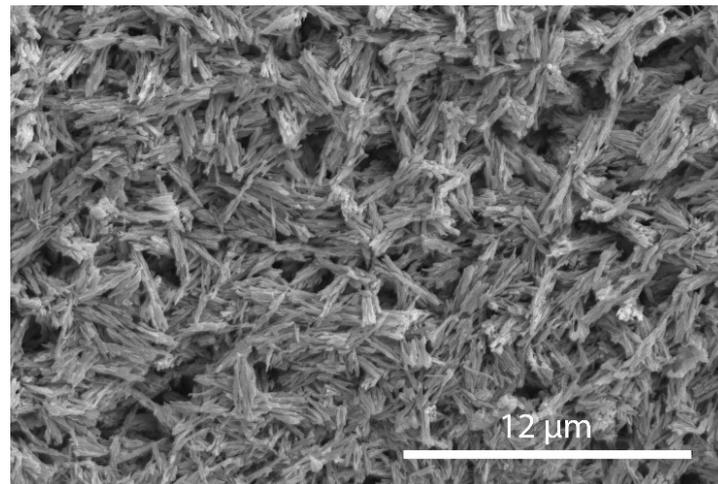


Figure 2. The transverse section of aragonitic irregularly oriented prismatic (AIOP) structure in *Apomatus globifer*.

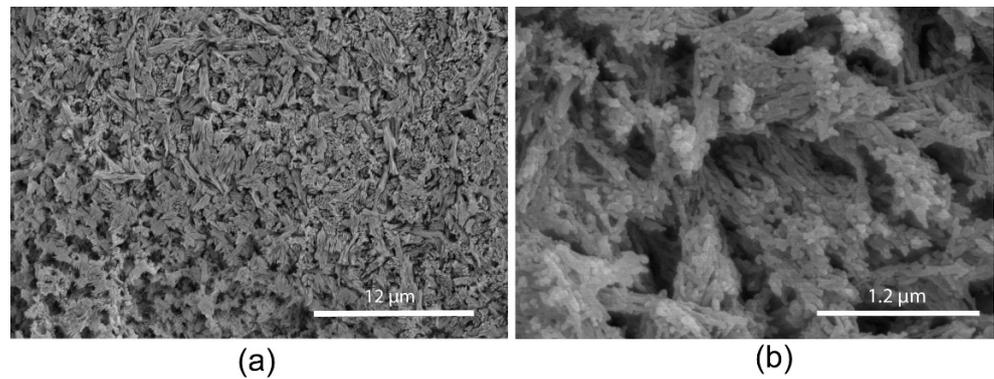


Figure 3. The ASIOP structure in the inner layer of *Spiraserpula caribensis*. (a) Longitudinal section, general view; (b) longitudinal section, showing substructure composed of elongated rods.

The aragonitic spherulitic prismatic (ASPHP) structure is present as a distinct layer in only *Spiraserpula caribensis* (Figure 4), but it can also be found in the form of lenses in the tube wall of *Filogranella elatensis* and *Pseudovermilia occidentalis*. The ASPHP is an anisotropic structure, where prismatic crystallites (0.9–1.0 μm in diameter and up to 6 μm long) of slightly spherulitic appearance are oriented perpendicular to the tube wall of the serpulid. The substructure is composed of slender elongated aragonitic rods that are subparallel, to parallel, to each other (0.09 μm in diameter and up to 0.4 μm long).

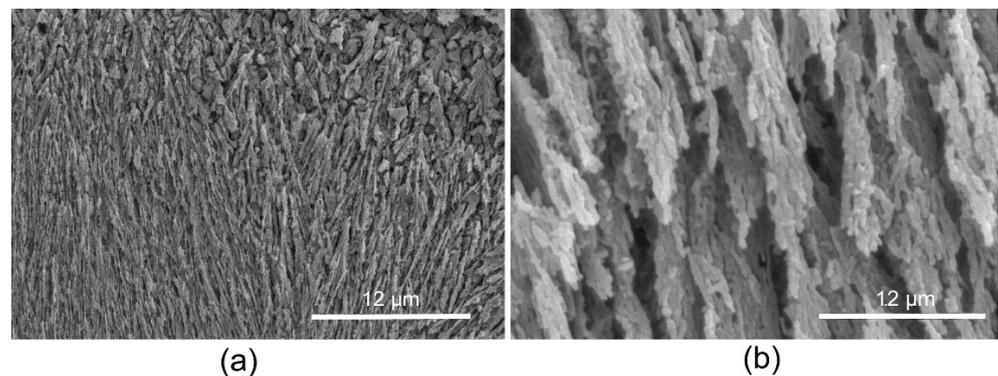


Figure 4. The ASPHP structure in the inner layer of *Spiraserpula caribensis*. (a) Longitudinal section, general view; (b) longitudinal section, showing substructure composed of elongated rods.

The aragonitic rounded homogeneous crystal (ARHC) structure is only present in *Pomatostegus stellatus* (Figure 5). The ARHC structure is an isotropic structure that is composed of rounded aragonitic granules (2 to 3 μm in diameter) of generally similar sizes.

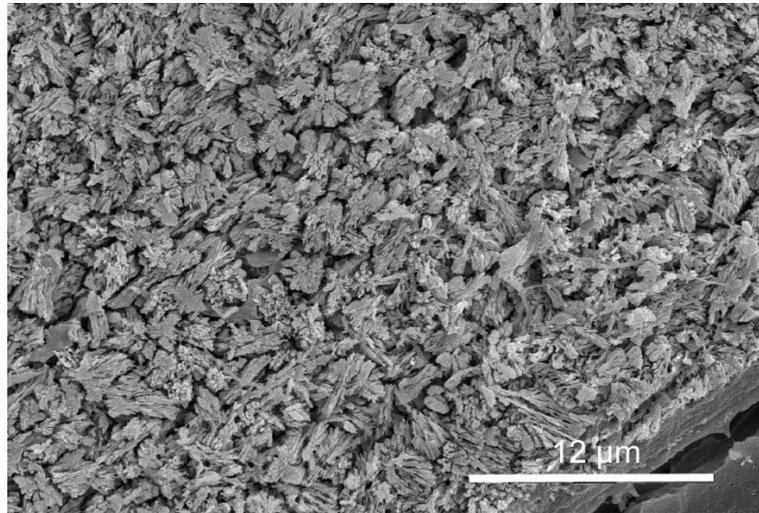


Figure 5. The longitudinal section of ARHC structure in *Pomatostegus stellatus*.

The aragonitic, semi-oriented, irregularly oriented prismatic (ASOIOP) structure is only present in *Protula diomedea* (Figure 6). The ASOIOP structure is an anisotropic structure composed of elongated crystallites (about 1.2 μm in diameter and 4 to 6 μm long), which are somewhat irregularly oriented within each growth increment, but, at the same time, their longitudinal axis is more often oriented parallel with the tube surface.

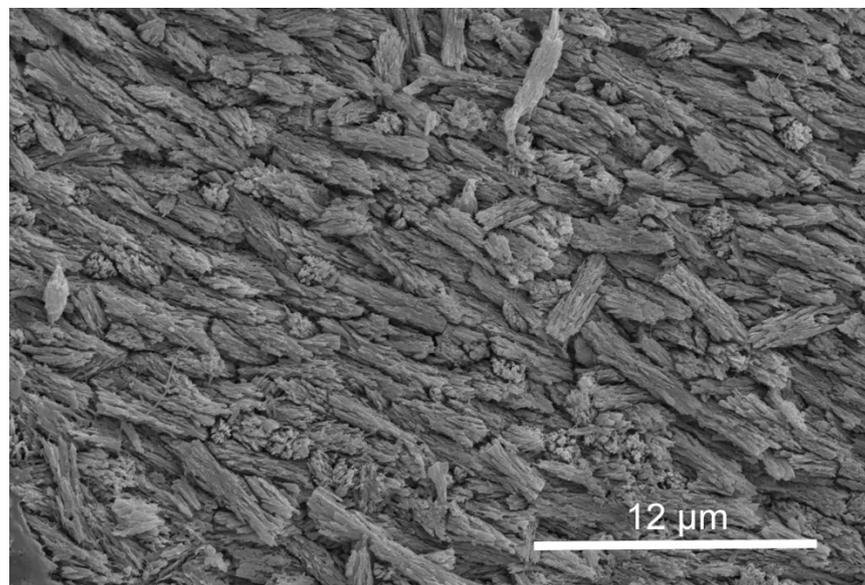


Figure 6. The transverse section of ASOIOP structure in *Protula diomedea*.

There are no obvious organic sheets visible in aragonitic structures in serpulid tubes. The thick organic sheets are likely lacking in aragonitic structures because the etching of the polished tube structures with diluted (1%) acetic acid for one minute would not have destroyed them, as organic sheets have been discovered in calcitic serpulid structures using the same method [12]. Nevertheless, etching of the polished surface dissolves the minerals, and, probably, at least a part of the organic components.

The substructures of anisotropic and isotropic aragonitic structures in *Spiraserpula* are similar, and they are both anisotropic. The substructures of all other aragonitic structures are also anisotropic. The boundaries between the isotropic ASIOP structure and the anisotropic ASPHP structure are somewhat transitional in *Spiraserpula*.

4. Discussion

4.1. Isotropic versus Anisotropic Aragonitic Structures and Evolutionary Implications

Recently, Buckman and Harries [13] have tried to explain the orientation mechanism of calcitic anisotropic structures. They argued that the difference in orientation observed between unordered and ordered fabrics can be explained by the application (or lack of) of a rotational force by the serpulid collar. However, Vinn [11] has demonstrated that the same growth increment occurs across different microstructures in serpulids, and it is impossible that, with the same rotational movement of the collar, the worm would create multiple types of microstructures and a multitude of crystallite orientations. According to Vinn [11], serpulids most likely use matrix-controlled crystallization for the biomineralization of oriented microstructures. Alternatively, serpulid biomineralization could be a combination of both the organic matrix-mediated process and the rotational movements of the collar.

The transitional nature of the boundaries between anisotropic and isotropic aragonitic structures within the same growth increment suggests that the change in crystal orientation was likely achieved by the organic matrix-mediated process. It is likely that the serpulid altered the chemical composition of the organic matrix, and, in the contact zone of two different aragonitic structures, the composition of the organic matrix has a mixed composition.

All aragonitic serpulid structures have two levels of organization. At the substructural level, such structures are anisotropic, but at the microstructural level, they can either be isotropic or anisotropic. This two-leveled organization can help us to understand the evolution of oriented microstructures in serpulids. For example, one could ask whether the orientation of biominerals is controlled by a similar mechanism at the substructure and microstructure levels. If the plesiomorphic aragonitic microstructures were all isotropic [14], then the anisotropic microstructures may have evolved from isotropic ones by continuing substructural orientation at the microstructural level. Similarly, the evolution of isotropic structures from anisotropic structures, via a loss of control over the crystallite orientation at the microstructural level, seems to be feasible. Among isotropic aragonitic structures, the ASIOP structure has likely evolved from a precursory AIOP structure, as the former is mechanically stronger, due to intergrown crystallites. The latter scenario is likely because evolution towards a mechanically stronger structure seems to be more likely than evolution towards a weaker tube structure.

4.2. Aragonitic versus Calcitic Microstructures

In serpulids, all complex oriented structures, such as the lamello-fibrillar (LF) structure, for example, are made of calcite. It should be noted that Buckman and Harries [13] suggest the use of ordered fabrics as a replacement for the lamello-fibrillar structure, based on their more complex nanostructure, and, in the case of ordered vs. lamello-fibrillar fabrics, the latter should be restricted to usage amongst the Mollusca, as originally described; both are likely to have had divergent origins/modes of formation. The most similar aragonitic analog to the calcitic, complex oriented structures is the aragonitic, semi-oriented, irregularly oriented prismatic (ASOIOP) structure, which is somewhat similar to the ordered fibrillar (OF) structure of *Spirobranchus*, but exhibits less control over the orientation of crystallites. Thus, the most complex aragonitic structures are less complex than the most complex calcitic structures in serpulids. The properties of aragonite as the biomineral are in no way inferior to the calcite and aragonite used in most complex structures of molluscs, such as, for example, the nacreous structures [15]. The reason why aragonitic structures are less complex than calcitic structures in serpulids likely has evolutionary roots. The serpulid appeared in the Permian [16]. However, the

major diversification of serpulids took place during the Jurassic and Cretaceous calcitic seas [17], when there were favorable conditions for the evolution of calcitic structures. Still, it is unclear why aragonitic microstructures have remained less evolved than calcitic microstructures, considering their fit with the current aragonite sea conditions, where the precipitation of aragonite is chemically favored (Paleogene–recent). The other likely reasons why aragonite is not favored for producing complex microstructures in serpulids involve the characteristics of serpulid biomineralization. In molluscs, for example, complex microstructures are favorably made from aragonite, but in bryozoans, on the contrary, calcite is the preferred mineral for building the complex microstructures [15]. Thus, the preferred biominerals for complex structures vary among different groups of invertebrates. The biomineralization of serpulids resembles bryozoans more than molluscs in the latter aspect. The biomineralization of complex microstructures in corals is also not that different from serpulids, as their most complex structure is calcitic.

Aragonite undoubtedly contributes to the diversity of skeletal microstructures in serpulids, as five out of fifteen serpulid structures are made from aragonite. However, only the aragonitic, spherulitic, irregularly oriented prismatic (ASIOP) structure and the aragonitic, rounded, homogeneous crystal (ARHC) structure do not have a calcitic analogue, and the number of calcitic microstructures that do not have an aragonitic analogue is two times larger. Thus, the contribution of aragonite to the diversity of the skeletal microstructures of serpulids is smaller than the contribution of calcite. In molluscs, a major part of the diversity of skeletal microstructures is created by aragonite, whereas, in bryozoans, the calcitic microstructures are more numerous than the aragonitic microstructures [15]. In this respect, serpulid biomineralization is also more similar to bryozoans than to molluscs.

The lack of organic sheets in aragonitic serpulid microstructures is noteworthy because such sheets are common in many calcitic serpulid structures, such as the lamello-fibrillar structure, for example [10]. Nevertheless, this is not necessarily caused by differences in the biomineralization of serpulid aragonite and calcite. The calcitic serpulids form one clade, whereas the aragonitic serpulids belong to another clade [5,18]. It is possible that the organic sheets appeared as an apomorphy in the calcitic clade, and they have not yet appeared in the aragonitic clade, just by chance. If the latter is true, it could be interpreted as an indication of the low adaptive value of the organic sheets in the serpulid microstructures.

5. Conclusions

The transitional nature of the boundaries between the anisotropic and isotropic aragonitic structures within the same growth increment suggests that the change in crystal orientation was achieved by chemical means. All aragonitic serpulid structures have two levels of organization, where, at the substructure level, aragonitic serpulid structures are always anisotropic, but at the microstructural level, they can either be isotropic or anisotropic. In serpulids, the complexity of aragonitic structures is less than in calcitic structures. The contribution of aragonite to the diversity of the skeletal microstructures of serpulids is smaller than the contribution of calcite.

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Data Availability Statement: All data generated are included in the present paper.

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Conflicts of Interest: The author declares no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. ten Hove, H.A.; van den Hurk, P. A review of Recent and fossil serpulid ‘reefs’; actuopalaeontology and the ‘Upper Malm’ serpulid limestones in NW Germany. *Geol. Mijnb.* **1993**, *72*, 23–67.
2. Chan, V.; Vinn, O.; Li, C.; Lu, X.; Kudryavtsev, A.B.; Schopf, J.W.; Shih, K.; Zhang, T.; Thiyagarajan, V. Evidence of compositional and ultrastructural shifts during the development of calcareous tubes in the biofouling tubeworm, *Hydroides elegans*. *J. Struct. Biol.* **2015**, *189*, 230–237. [[CrossRef](#)] [[PubMed](#)]
3. Vinn, O. The role of an internal organic tube lining in the biomineralization of serpulid tubes. *Carnets Géol.* **2011**, *11*, 13–16. [[CrossRef](#)]
4. Vinn, O.; ten Hove, H.A.; Mutvei, H.; Kirsimäe, K. Ultrastructure and mineral composition of serpulid tubes (Polychaeta, Annelida). *Zool. J. Linn. Soc.* **2008**, *154*, 633–650. [[CrossRef](#)]
5. Smith, A.M.; Riedi, M.A.; Winter, D.J. Temperate reefs in a changing ocean: Skeletal carbonate mineralogy of serpulids. *Mar. Biol.* **2013**, *160*, 2281–2294. [[CrossRef](#)]
6. Vinn, O.; Kirsimäe, K.; ten Hove, H.A. Tube ultrastructure of *Pomatoceros americanus* (Polychaeta, Serpulidae): Implications for the tube formation of serpulids. *Est. J. Earth Sci.* **2009**, *58*, 148–152. [[CrossRef](#)]
7. Vinn, O.; ten Hove, H.A. Microstructure and formation of the calcareous operculum in *Pyrgopolon ctenactis* and *Spirobranchus giganteus* (Annelida, Serpulidae). *Zoomorphology* **2011**, *130*, 181–188. [[CrossRef](#)]
8. Vinn, O. On the unique isotropic aragonitic tube microstructure of some serpulids (Polychaeta, Annelida). *J. Morph.* **2013**, *274*, 478–482. [[CrossRef](#)] [[PubMed](#)]
9. Kupriyanova, E.K.; Vinn, O.; Taylor, P.D.; Schopf, J.W.; Kudryavtsev, A.; Bailey-Brock, J. Serpulids living deep: Calcareous tubeworms beyond the abyss. *Deep Sea Res.* **2014**, *90*, 91–104. [[CrossRef](#)]
10. Vinn, O.; Kupriyanova, E.K. Evolution of a dense outer protective tube layer in serpulids (Polychaeta, Annelida). *Carnets Géol.* **2011**, *11*, CG2011_L05.
11. Vinn, O. Biomineralization in Polychaete Annelids: A Review. *Minerals* **2021**, *11*, 1151. [[CrossRef](#)]
12. Vinn, O. Occurrence, formation and function of organic sheets in the mineral tube structures of Serpulidae (Polychaeta, Annelida). *PLoS ONE* **2013**, *8*, e75330. [[CrossRef](#)] [[PubMed](#)]
13. Buckman, J.O.; Harries, D.B. Reef forming *Serpula vermicularis* from Scotland and Ireland: Tube structure, composition and implications. *Zool. Anz.* **2020**, *288*, 53–65. [[CrossRef](#)]
14. Vinn, O.; Jäger, M.; Kirsimäe, K. Microscopic evidence of serpulid affinities of the problematic fossil tube “*Serpula*” etalensis from the Lower Jurassic of Germany. *Lethaia* **2008**, *41*, 417–421. [[CrossRef](#)]
15. Carter, J.G.; Bandel, K.; de Buren, V.; Carlson, S.J.; Castanet, J.; Crenshaw, M.A.; Dalingwater, J.E.; Francillion-Vieillot, H.; Geradie, J.; Meunier, F.J.; et al. Glossary of skeletal biomineralization. In *Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends*; Carter, J.G., Ed.; Wiley: Hoboken, NJ, USA, 1990; pp. 609–671.
16. Sanfilippo, R.; Rosso, A.; Reitano, A.; Insacco, G. First record of sabellid and serpulid polychaetes from the Permian of Sicily. *Acta Palaeontol. Pol.* **2017**, *62*, 25–38. [[CrossRef](#)]
17. Ippolitov, A.P.; Vinn, O.; Kupriyanova, E.K.; Jäger, M. Written in stone: History of serpulid polychaetes through time. *Mem. Mus. Vict.* **2014**, *71*, 123–159. [[CrossRef](#)]
18. Kupriyanova, E.K.; Macdonald, T.; Rouse, G.W. Phylogenetic relationships within Serpulidae (Sabellida, Annelida) inferred from molecular and morphological data. *Zool. Scr.* **2006**, *35*, 421–439. [[CrossRef](#)]