Journal of *Marine Science and Engineering*

MDPI

Article

Bio-Engineers of Marine Animal Forests: Serpulidae (Annelida) of the Biostalactite Fields in the Submarine Cave "lu Lampiùne" (Mediterranean Sea, Italy)

Margherita Licciano and Genuario Belmonte *

Laboratory of Zoogeography and Fauna, Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100 Lecce, Italy; margherita.licciano@unisalento.it
* Correspondence: genuario.belmonte@unisalento.it

Abstract: Marine caves are complex habitats characterized by intense environmental gradients from the entrance towards the innermost dark sectors. The submarine caves at the Cape of Otranto (Mediterranean, SE Italy) host skeletonized invertebrates able to build 3D structures by intermingling their hard body parts with microbial carbonates, thus acting as bio-constructors of true marine animal forests. Complex bio-constructions named "biostalactites" (BSTs) with a core of calcareous tubes of *Protula* sp. (Serpulidae, Annelida) have been recently found in the dark sector of the "lu Lampiùne" submarine cave, one of the most complex and largest in the area. In the present study, we examined the outer surface of a BST from "lu Lampiùne" in order to evaluate species richness, abundance and distribution of Serpulidae at proximal, intermediate, and distal positions along the BST and on the two opposite sides of the BST with different textures (coarse vs. smooth). The BST surface hosted 1252 specimens belonging to 9 Serpulidae species differently distributed along the BST and on differently textured surfaces. As expected, sciaphilic Serpulidae dominated in terms of number of species and individuals. Remarkably, the large *Protula* tubes of the BST core that allowed it to grow from 6000 years ago have been largely replaced by small-sized Serpulidae species. The present study contributes to increase the knowledge of the metazoans associated with biostalactite fields from "lu Lampiùne" cave and allows for a comparison with findings from other Mediterranean BSTs.

Keywords: bioconstructions; biostalactites; marine animal forests; Mediterranean Sea; Salento Peninsula; Serpulidae; submarine caves

check for updates

Academic Editor: Taewon Kim

Received: 19 February 2025
Revised: 12 March 2025
Accepted: 18 March 2025
Published: 23 March 2025

Citation: Licciano, M.; Belmonte, G. Bio-Engineers of Marine Animal Forests: Serpulidae (Annelida) of the Biostalactite Fields in the Submarine Cave "lu Lampiùne" (Mediterranean Sea, Italy). *J. Mar. Sci. Eng.* **2025**, *13*, 639. https://doi.org/10.3390/jmse13040639

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Marine caves are complex habitats characterized by light, hydrodynamics and trophic input variations from the entrance toward the darkest inner sectors where total darkness occurs, water movement is negligible, and microbial communities and sessile active filter-feeders dominate [1–4]. In these confined systems, sessile skeletonized invertebrates may act as bio-constructors of 3D biogenic frames by intertwining their tubes or skeletons in association with carbonates whose precipitation is induced by microbial activity [5–7]. In submarine dark caves, the continuous overlapping of colonizers, whose skeletons are in turn colonized by the next generations of encrusting organisms intermingled with microbial carbonate, give rise to bio-constructions which may reach considerable sizes and complexity, components of the so-called "Marine Animal Forests".

Marine cave-dwelling taxa able to modify their physical environment and defined as ecosystem engineers, can be assigned to different categories according to their activity [8].

Contrary to the 'bio-constructors', which build structures by their own mineral skeletons that remain also after their death, and the 'binders', able to unit and expand the habitat framework by agglomerating its components, the 'borers' are those organisms that actively penetrate the substrate with their erosive activity [4,6,9]. Bio-construction features vary markedly depending on the local prevalence of builders and consumers. In the submarine dark caves, the composition of the builder's guild is affected by the distance from the cave entrance, the depth, and connections to the open sea. At the entrance of the caves, the suitable light intensity and hydrodynamic energy allow coralligenic-type bio-structures similar to those of coralligenic concretions occurring on rocky bottoms outside caves and mainly depending on algae, with calcareous algae, large-sized erect Bryozoa species and stony corals acting as the main constructors, opposed to Bivalvia and Porifera promoting and enhancing erosive processes [10–14]. Conversely, algae disappear as the distance from the entrance increases and the animal component, represented mainly by Serpulidae (Annelida), Bryozoa and Porifera, dominates in the innermost sectors of the cave.

Recently, peculiar structures with an unusual cylindrical/conical shape, up to 2 m in length, have been discovered in the innermost dark sector of the submarine cave "lu Lampiùne", one of the most complex and largest caves of the Salento Peninsula (Mediterranean, SE Italy) [15,16]. These bio-constructions, named "Bio-stalactites" (BSTs) due to their shape resembling true stalactites, are completely organogenic and based on calcareous tubes of the serpulid *Protula* sp., which constitute the core of the structure. Similar bio-constructions have already been described by Macintyre and Videtich [17] who named "pseudostalactites" club-shaped aggregations of Serpulidae tubes belonging to the genus *Vermiliopsis* projecting from the ceiling of a submarine cave in the Belize barrier-reef platform. The BSTs from the "lu Lampiùne" cave represent the first record for the whole Mediterranean basin; however, their presence was later also reported in other Mediterranean marine caves in the Ionian Sea, Levantine Sea, and Aegean Sea [5,18–23].

Geobiological studies have revealed that Metazoa and bacteria are the main building engineers of the BSTs, with tubes of the genus *Protula* (Annelida, Serpulidae) as the structural components of the original inner core, which has been dated to approximately 6000 years [16]. A complex texture of microbialite and Metazoa skeletons enveloping the central core contributes to the general framework [7].

The role of Serpulidae as primary builders has been acknowledged not only by investigations on bio-structures in present-day environments, but also widely in studies concerning geological past [24–30]. In fact, the oldest evidence of fossil Serpulidae dates back to the middle Triassic (about 244 Mya), and from the late Jurassic (about 146 Mya), Serpulidae colonized hydrocarbon seep environments and possibly even the deep sea, where they developed even encrusting preexisting speleothemes [26].

These marine worms are sedentary filter-feeders living in a self-constructed tube cemented to the substrate and made of crystalline calcium carbonate in a mucopolysaccharide matrix, which provides protection from predators and adverse environmental conditions [31–34]. Serpulidae are among the few selected taxa living in the innermost dark sector of marine caves able to cope with the extreme environmental conditions hostile to most marine invertebrates [2,3,35,36]. In these confined sectors of the marine caves, they usually grow as isolated individuals or in clusters, forming crusts of variable thickness on the hard substrate [2,3,20–22,35–37].

The only available data regarding a BST from the "lu Lampiùne" cave come from the study of Rosso et al. [38], who provided the basis and highlighted the need for further research on this topic. These authors gave, for the first time, information on present-day BST-associated communities, which seems to differ from those of individual BSTs from other Mediterranean submarine caves. The data from this previous study, however, refer

only to one of the two halves into which the BST had been longitudinally sectioned, and available for analysis.

The present study is a contribution to increase the knowledge of the metazoans associated with BSTs which are the result of at least 6000 years of biological activities within the "lu Lampiùne" cave. An entire BST from the innermost dark sector of the cave was examined in order to detect and identify the encrusting Serpulidae on the whole outer surface. The coverage of Serpulidae hard skeletons on the two opposite sides of the BST, recognized on the basis of different textures of the surface (coarse vs. smooth), was evaluated. For each side, species richness, abundance and distribution were estimated along the longitudinal axis of the BST at proximal, intermediate, and distal positions in relation to the point of detachment from the ceiling of the cave.

A clear preferential distribution of Serpulidae at different positions along the length and on the differently textured sides of the BST was highlighted, with sciaphilic Serpulidae dominating in terms of the number of species and individuals. Remarkably, the large tubes of *Protula* individuals, which by 6000 years ago had built the core of the BST and provided biogenic substrates for the next bioconstructors, have been largely replaced by small-sized species.

2. Materials and Methods

The investigated BST was sampled in the submarine cave "lu Lampiùne" (Figure 1), one of the most complex and largest submerged caves of the Salento coast (Cape of Otranto, Apulian Peninsula, SE Italy, 40.0052778° N–18.5072222° E).

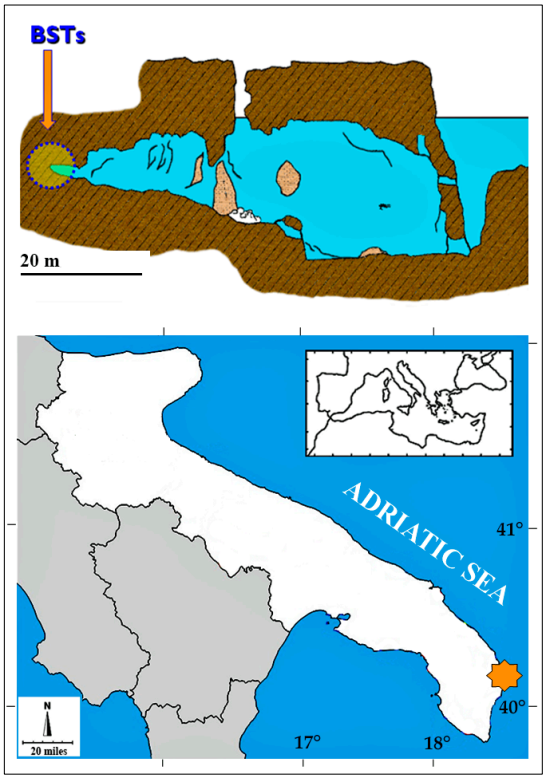
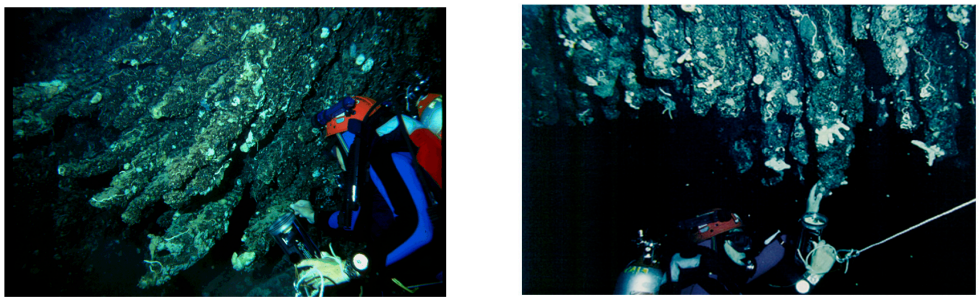


Figure 1. Map (section) of the "lu Lampiùne" cave with indication of the BST field position within the innermost chamber (dotted circle) and sampling site at Cape of Otranto (orange asterisk).

After the cave was discovered and mapped in 1989 [39], a new and more detailed topographic survey [15] was provided with the description of a new passage not described in the survey previously carried out. The cave consists of two chambers: a larger one communicating with the open sea at several points, and a smaller Y-shaped one, which is

internal (Figure 1). The two chambers are connected to each other by two narrow passages at 17 and 20 m depth below the sea surface which do not impede the passage of scuba speleo-divers. The ceiling of the shallower passage has an opening that allows light to penetrate inside, hence the name of the cave itself (lu Lampiùne = light spot). The innermost dark sector of the smaller chamber where the BST field is located (40 m from the entrance at the level/section of about 8–10 m below the sea surface and over the deepest point of the floor) is crowded with hundreds of BSTs hanging vertically from the ceiling or obliquely protruding from the walls of the cave towards the center of the chamber (Figure 2).



(**a**) (**b**)

Figure 2. Innermost dark sector of the "lu Lampiùne" cave (8 m depth, 40 m from the entrance) crowded with BSTs protruding from the walls and extending obliquely towards the center of the chamber (**a**) or vertically hanging from the ceiling (**b**).

In the present study, we examined a single BST (Figure 3) collected in 2003 in the innermost dark sector of the lu Lampiùne cave by detaching it from the ceiling covered by a field of tightly packed BSTs of considerable dimensions, with various lengths. In order to preserve this natural heritage, still novel for marine biology, we avoided a new sample collection, and we used for the study the BST collected in 2003 and conserved in the Marine Biology Museum "Pietro Parenzan" with the catalogue number MBMPPbst3. This BST is the only complete specimen of a group of three, the other two consisting only of longitudinal halves (the lacking halves being fragmented in preceding analyses). The size of the collected BSTs was chosen among structures of intermediate value (whose dimensions fell into the most common size group of about 50 cm in length) to avoid the removal of extra-sized structures, still unique to the science, from their natural site.

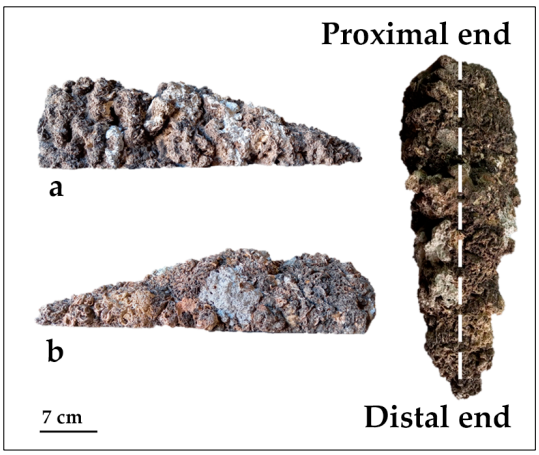


Figure 3. The BST collected from the "lu Lampiùne" cave oriented according to its vertical growth with the proximal part corresponding to the point of attachment to the ceiling. (**a**) Coarse side view of one of the two halves into which the BST was cut longitudinally, (**b**) Smooth side view of one of the two halves into which the BST was cut longitudinally.

The BST is roughly conical in shape, approximately 42 cm long, with a diameter of 16 cm measured at the point of detachment from the ceiling of the cave chamber.

We examined the outer surface of the BST in order to detect the calcareous tubes of the encrusting Serpulidae, which were identified at the species level. By examining the BST surface, however, many areas including skeletons appeared blackened by oxidation due to the exposure after sampling and/or mineralization processes, thus making it difficult to identify taxa underlying the uppermost biogenic crust or in some cases even those on the most external layer. In this case, Serpulidae were identified at the lowest possible taxonomic level.

Species richness, abundance and distribution were evaluated:

- on the two opposite sides of the BST, recognized on the basis of different textures (coarse vs. smooth) of the outer surface.
- for each side, at proximal, intermediate, and distal positions along the longitudinal axis of the BST, in relation to the point of detachment from the ceiling.

The BST was sectioned longitudinally in order to examine it for radiocarbon dating analyses, and micromorphological observations, UV-epifluorescence and micro-Raman spectroscopy analyses in order to investigate the internal structures and growth pattern. Therefore, the two different external surfaces were not considered in the cutting, and both a smooth and a wrinkled surface were present in each half (Figure 3a,b).

The identification of Serpulidae species was performed with a Zeiss magnifying glass (Zeiss, Oberkochen, Germany) and the images were acquired using the OM System Tough TG-7 (Olympus, Processor TruePic™ VIII, https://support.jp.omsystem.com/en/support/imsg/digicamera/download/notice/notice.html, accessed on 17 March 2025).

3. Results

The present study allowed the detection of 1252 serpulid tubes on the whole surface of the BST. A total of nine taxa belonging to the subfamilies Filograninae (7) and Serpulinae (2) were identified, with a different distribution of specimens along the BST longitudinal axis (Table 1). The total number of individuals detected along the longitudinal axis of the BST was lower at the intermediate position, where only 18% of the total Serpulidae were recorded, and higher at distal and proximal positions (about 41% of the total Serpulidae). The only exception to this pattern of distribution was the species *Josephella marenzelleri* showing the lowest abundance value recorded at the proximal position with only 13% of the total specimens belonging to this species, whereas higher values equal to 53% and 34% of the remaining individuals were found at the distal and intermediate positions, respectively (Figure 4).

Table 1. Abundance of Serpulidae tubules on the external surface of the investigated BST from the "lu Lampiùne" cave. The total specimens found along the BST longitudinal axis on the two opposite sides, coarse and smooth, are reported. Each side is divided into three positions: proximal, intermediate, and distal, according to their distance from the substrate/base.

Positions	-BST- Whole Surface (n. Specimens)	-BST- Coarse Surface (n. Specimens)	-BST- Smooth Surface (n. Specimens)
ThProximal	520	184	336
Intermediate	223	94	129
Distal	509	183	326
Total specimens	1252	461	791

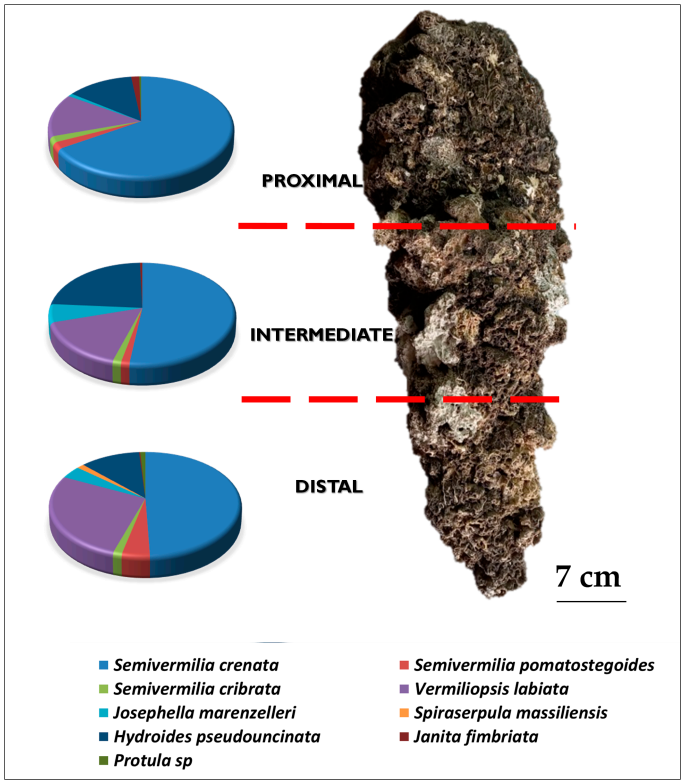


Figure 4. Species richness and distribution of Serpulidae on the outer surface of the BST from the "lu Lampiùne" cave at proximal, intermediate and distal positions along its longitudinal axis.

Semivermilia crenata was the most abundant and broadly distributed species on the whole BST, accounting for 57% of the total serpulid coverage, followed by *Vermiliopsis labiata* (20%) and *Hydroides pseudouncinata* (14%) (Figure 4). *Semivermilia crenata* and *H. pseudouncinata* were more abundant at the proximal position of the BST with 344 and 68 individuals, respectively, while the abundance of *V. labiata* was higher at the distal position where 138 individuals were identified (Table 1). The other two species, belonging to the genus *Semivermilia*, *Semivermilia pomatostegoides* and *Semivermilia cribrata*, showed lower abundances than *S. crenata*.

In addition to *S. pomatostegoides* and *S. cribrata*, the remaining taxa including *Spiraserpula massiliensis*, *Janita fimbriata*, *J. marenzelleri*, and *Protula* sp. also occurred with a low number of individuals on BST. Among these subordinated taxa, *J. fimbriata* and *S. cribrata* were more abundant at the proximal position, although with no more than 10 individuals, while *S. pomatostegoides* and *J. marenzelleri* showed higher abundances with greater values at the distal position than those reported for the other positions along the BST axis. *Spiraserpula massiliensis* and *Protula* sp. were present each with a total of only seven specimens, which were the lowest abundances recorded on the BST. Furthermore, the few specimens of *S. massiliensis* were detected only at the distal end while *Protula* sp. was absent at the intermediate position, occurring at distal and proximal ends as isolated and/or coiled intermingled tubes (Figure 5). Although detectable on the most external layer of the BST, *Protula* specimens were unrecognizable as belonging to a given species due to their heavy degradation caused by the mineralization process and the dark oxidation, hindering the identification.

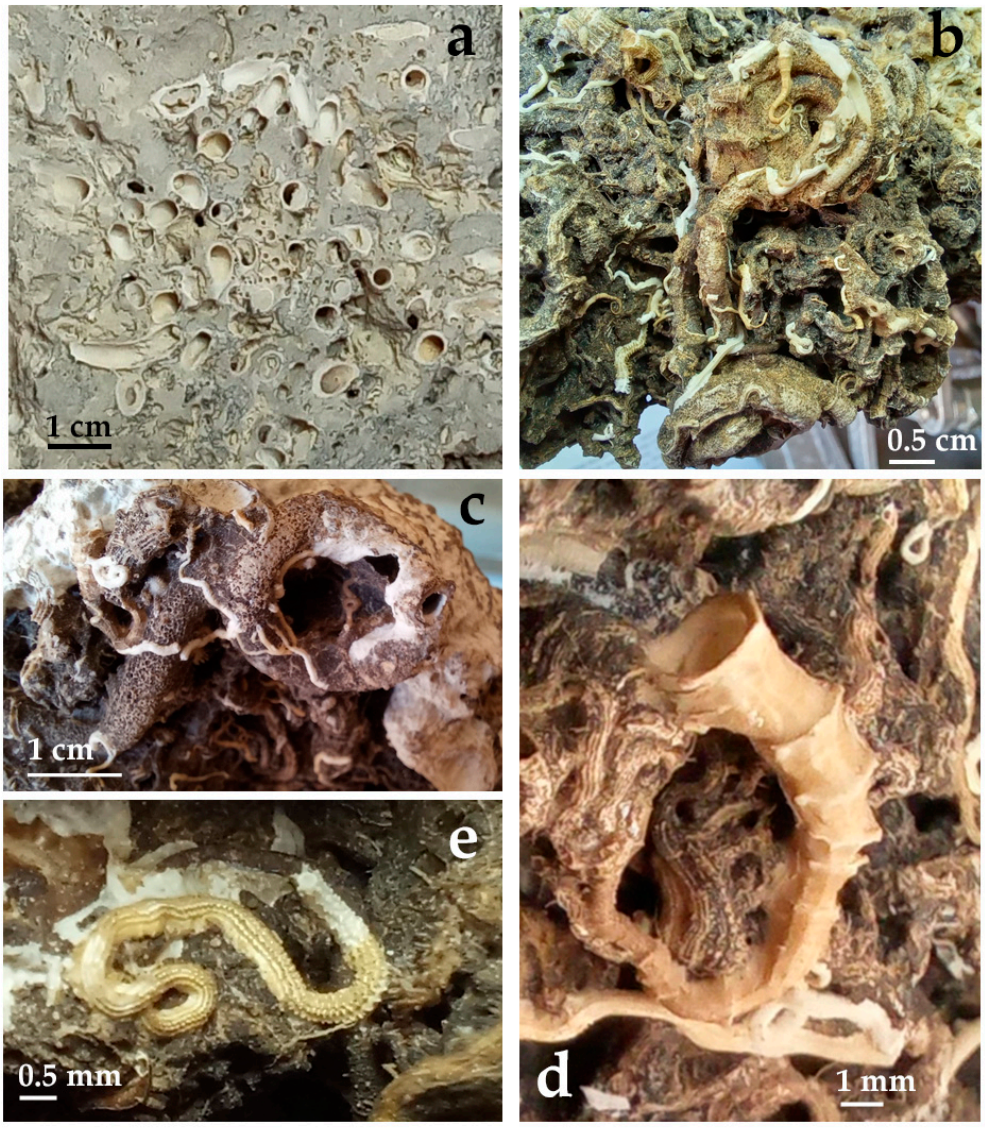


Figure 5. Serpulidae of the BST from the "lu Lampiùne" cave. (**a**) Detail of the BST transversal section of the investigated BST showing the core mainly constituting large Protula tubes; (**b**,**c**) aggregates of irregularly coiled Protula tubes colonized by other superimposed serpulids on outer BST surface; (**d**) Vermiliopsis labiata; (**e**) Semivermilia crenata.

The examination of the two BST sides, with different textures, highlighted differences in Serpulidae abundance (Table 1) and species distribution (Figure 6).

The coarse surface of the BST hosted 37% of the total Serpulidae; however, only 7% were positioned at the intermediate position. Although higher abundance values were found on the smooth surface, the number of individuals at the intermediate position was still lower on this side (10% of the total Serpulidae) compared to the proximal and distal ends.

The lowest values in terms of the number of taxa were also reported for the coarse side of the BST and at the intermediate position along its longitudinal axis. In particular, *S. massiliensis* and *Protula* sp. were not found on the coarse surface, being exclusive of the opposite smooth side, where were detected only at distal and proximal positions. On the contrary, *J. marenzellerii* was recorded only on the coarse surface where specimens colonized crevices and microcavities forming intricate networks with their long and thin tubes. On the smooth surface, *S. crenata* and *V. labiata* were present with double and triple the number of individuals, respectively, than on the rough surface.

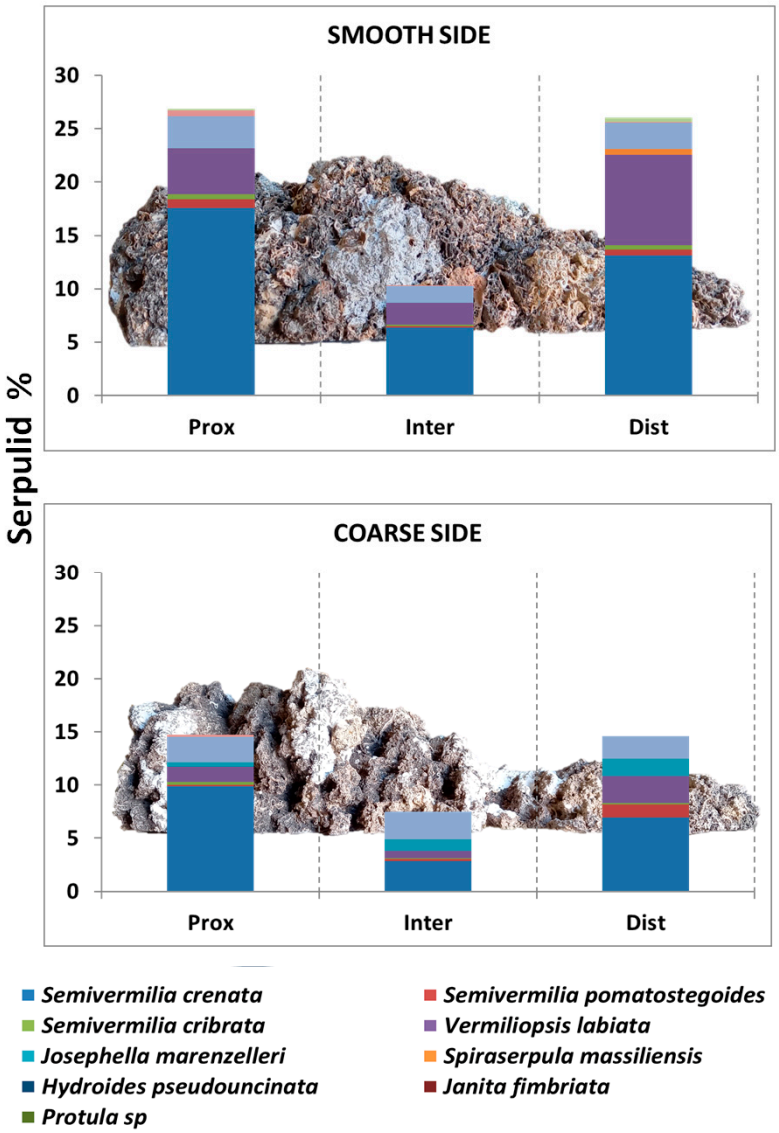


Figure 6. Species richness and distribution of Serpulidae on the outer surface of the BST from the "lu Lampiùne" cave on the two opposite sides with different textures (coarse vs. smooth).

4. Discussion

Through their mineral skeletons, Serpulidae are known to be able to form several types of assemblages based on a single or few species, depending on the environment and the species involved [26,37,40–43]. The occurrence of conspicuous Serpulidae aggregations, even overgrowing on preexisting speleothemes, has been reported from the geological past as well as the present-day [24–28,30,37,44–46]. In particular, the formation of BSTs in the Mediterranean submarine caves starts from a single or few Serpulidae species and proceeds after the outer surface of the assemblage is encrusted by other invertebrates, with Serpulidae still dominating, followed by Bryozoa, Porifera and Foraminifera [16,18,19,38].

Within marine animal forests, the BSTs recently described from the submarine cave "lu Lampiùne" are peculiar bio-constructions built by Serpulidae belonging to the genus *Protula* [15,16,18–20,38]. The BST core exclusively consists of *Protula* tubes while other superimposed species, also including other smaller Serpulidae, contribute to the building of the general framework. Our findings concerning the abundance and distribution of the encrusting Serpulidae on the outer surface of the BST from the "lu Lampiùne" submarine cave are in agreement with previous studies on Mediterranean marine caves that reported increased abundances of sciaphilic, deep water and cave Serpulidae inwards according

to the environmental gradients [20–22]. All the species detected in the present study are reported in the inventory of the Serpulidae fauna from the marine caves along the Italian coasts [3], which includes more than two-thirds of the whole Serpulidae living in the Mediterranean Sea, and the Salento coasts [47]. In particular, sciaphilic species largely dominated in terms of both the number of taxa and abundances, similar to what was reported by Rosso et al. [38] who first examined the external surface of one-half of a BST from "lu Lampiùne" cave. This is not surprising considering that submarine dark caves have been suggested as representing mesocosms of deep-sea conditions in the shallow littoral zone, providing simplified models for the study of less easily accessible marine ecosystems, such as ocean depths [2,48–51]. The innermost sector of a littoral submarine cave has a darkness that increases in a manner that is comparable to the reduction in light intensity at depths of 50 to 400 m in a deep-sea environment [52]. This lowlight level, combined with water confinement and oligotrophy, results in a submarine cave ecosystem that is analogous to deep-sea ecosystems and leads to faunal affinities [4,20,22,42,48,50,51]. Accordingly, *S. crenata* and *V. labiata*, which in the present study were the most abundant and broadly distributed species on the whole BST surface, fall within the ecological group "Sciaphilic and/or coralligenic taxa" according to the classification scheme of the Serpulidae by Rosso et al. [53] and Sanfilippo et al. [22]. Both these species, however, are also considered characteristic of the dark caves, although not exclusive to these environments [3,22,54,55]. Furthermore, *S. crenata* and *V. labiata* strongly increased the surface roughness of the BST due to the large number of individuals, thus providing diversification of microhabitats for further colonization by smaller organisms [19,22,38,56]. Compared to the previous data on the half BST investigated by Rosso et al. [38], the present study of a complete BST from the same cave did not detect the *Filogranula* species reported as subordinate species in the previous study. In addition, *Spiraserpula massiliensis*, and *Janita fimbriata* (both among the most typical species of the cave habitat), already known as fossils from the Pleistocene and recorded in Aegean caves and on Sicilian BSTs [20,22], occurred with very few specimens on the here-investigated BST. The deep-water species *S. pomatostegoides* and *S. cribrata* were also subordinate taxa, different from the congeneric *S. crenata***.** Conversely, *Hydroides pseudouncinata*, which is actually a very common species of the littoral hard substrates, is frequently found abundant in the caves, which likely constitutes intrusions from the outside [54]. The same considerations also apply to the microserpulid *Josephella marenzelleri.* Differently from *H. pseudouncinata*, however, this species occurred at low abundance and only on the coarse side of the examined BST, where the fragile thin tubes less than 0.2 mm in diameter and even longer than 20 mm formed intricate nets filling small cavities.

In the present study, differences in the abundance and distribution of Serpulidae were highlighted on the two opposite sides of the BST and along its longitudinal axis, with the coarse side and the intermediate position showing the lowest value in terms of both individuals and taxa. Differences in the distribution pattern related to the surface texture have been already described by Guido et al. [21] for the BSTs from Kakoskali submarine cave in Cyprus. Contrary to our findings, however, these authors reported a lower diversity and a negligible number of individuals on the smooth surface, whilst a higher species richness, albeit with a relatively low number of individuals, was noted on the rough surface. Similar results were also reported by Rosso et al. [38] for the "lu Lampiùne" cave. These authors observed a colonization degree higher in the distal part of the BST where the outer surface was rougher than in the proximal one. However, as only one-half of the BST was available for examination in that previous study, the different pattern was actually observed along the same BST side, with smoother areas only at the proximal end and an overall rougher distal end with cavities and crevices colonized by *J. marenzelleri*. Different BST features mainly related to the growth direction and spatial orientation could provide an explanation

for the differences between our results and previous data concerning the other known Mediterranean BSTs. Available information on the bioconstructions of the Mediterranean area recorded after Onorato et al. [15] and Belmonte et al. [16] first described BSTs from the "lu Lampiùne" cave allows the recognition of comparable features and highlight differences among those described for the submerged caves of another Italian locality at Plemmirio Marine Protected Area (SE Sicily, Ionian Sea) [18–20], Cyprus (Levantine Sea) [21,23] and Lesvos Island (N Aegean Sea) [22]. The BSTs from "lu Lampiùne" cave have a conical shape ranging in length from a few dm up to 2 m exceptionally and protrude from the walls of the cave inclined towards the center of the chamber or hang vertically from the ceiling. In particular, these bio-structures are the result of a complex intermingling of skeletons and micrite with two different building engineers, invertebrates and bacteria, differently acting from the internal core towards the outermost encrusting layers [7,16,57]. According to Guido et al. [7], in the BST core, the tubes of Serpulidae belonging to the *Protula* genus give rise to a self-supporting frame reinforced by the skeletons of other smaller organisms, including other Serpulidae, while microbialite plays a subordinate role representing only 8% of the BST main components. Conversely, the outer crust layers enveloping the core consist of 60% micrite and 85% mixing of micrite and skeletal remains on the downward- and upward-facing sides, respectively. Differently from BSTs occurring within "lu Lampiùne" cave, the bio-constructions recorded from two submerged caves in Sicily can have different morphologies although roughly cylindrical/conical, and be mushroom-, dome- or fan-shaped and also have a digitate surface [19,20]. In addition to the morphological diversity, also the dimensional range, which is in the order of cm to dm, differentiates the Sicilian bio-constructions from the former. However, comparable features to "lu Lampiùne" cave can be highlighted with regards to the growth direction of the Sicilian BSTs, which similarly can protrude obliquely from the walls or perpendicularly from the ceiling. In contrast to the investigated BSTs from the Apulian and Sicilian caves and the other known from the eastern Mediterranean areas, the BST from the Kakoskali cave (Cyprus) have been described as exclusively protruding from the walls and extending obliquely to nearly horizontally toward the center of the cave chamber [21]. Although circumscribed to a single BST, the present study suggests a succession of Serpulidae species either in time or in space. The core is dominated by *Protula* sp. tubes, but the species is almost absent on the surface. This could be explained by a core development (intertwined tubes) preceding that of the enveloping crust. On the surface, on the contrary, we can recognize a spatial succession of species from the basis to the free extremity of the BST. Notwithstanding the larger availability of surface at the basis (the BST is about "conical"), we note that only four species are more abundant than elsewhere: *S. crenata*, *S. cribrata*, *H. pseudouncinata*, and *J. fimbriata*. Four other species (*V. labiata*, *S. potamostegoides*, *J. marenzelleri*, *S. massiliensis*), on the other hand, are sensibly abundant on the free extremity, notwithstanding the smallest space available. Based on these observations, a spatial preference (and a different environment) can be supposed for the above-described situation, while a more general change in environmental conditions should have affected the structural change of builders from *Protula* sp. in the inner core to the encrusting community well described by Rosso et al. [38] and here limited to the serpulid component. However, an interaction of several small-scale environmental factors, including surface roughness, orientation and exposition to different flow regimes within the cave, has been suggested accounting for the differences between proximal and distal parts, as well as the opposite sides of the BSTs from "lu Lampiùne" examined by Rosso et al. [38]. In particular, the reason for the development of the BST towards the center of the cave and the orientation towards the main chamber of the cave could be dated back to the early phase of colonization of the *Protula* nucleus, with an inclination and downward growth direction of the specimens functional to intercept the main inflow carrying oxygen

and food [16,21,38,58]. Similarly, the oblique orientation of the BSTs within caves from Cyprus has been indicated as the result of a growth in predominantly incoming water circulation. In addition, the two opposite sides of the BSTs showing an oblique orientation are also differently affected by sediment deposition [21]. Therefore, although the light availability and hydro dynamics are the most obvious abiotic factors influencing the pattern of distribution, abundance and diversity of species-dwelling submarine caves, as well as recruitment and organism interactions [2,36,42,59–63] the settlement of fine sediment on the upper surface, which according to Harmelin [64] likely prevent larval attachment, should be also considered as to explain the distribution pattern inside caves as described for the submarine caves in the Plemmirio peninsula [20] and Cyprus [20,21].

Among the subordinate species found on the outer surface of the here-investigated BST, *Protula* sp. was detected to a lesser extent and with the same low number of individuals as *S. massiliensis*. Large-sized tubes of this taxon occurred isolated or forming intermingled aggregates of irregularly coiled tubes. Few isolated specimens belonging to the *Protula* genus have been already found in other Mediterranean marine caves [3,15,16,26,45,55,65], including others in the Salento peninsula neighboring "lu Lampiùne" [47] and often referred to as *P. tubularia*. As stressed by ten Hove and Kupryianova [66] in their review of the taxonomy of the Serpulidae genera, however, the genus *Protula* is the most problematic serpulid taxon. However, several authors [29,55,66] have advised against giving specific names to specimens of the genus *Protula*, given the need for a worldwide revision of the genus needed to clarify the taxonomic position of the Mediterranean specimens. In the present study, therefore, individuals identified as belonging to *Protula* genus have not been assigned a species name and have been reported as *Protula* sp.

According to Ten Hove and Van den Hurk [26], the gregarious behavior of *Protula* and other Serpulidae is controlled by environmental factors and is triggered in extreme or unstable marine habitats especially to avoid predation and/or competition. It is more likely that the relatively high food supply during the early phase of the cave colonization could have favored gregariousness leading pioneer *Protula* specimens to act as primary buildings promoting the formation of the BST [14,20]. The following growth of the structure started about 6000 years ago when during the last postglacial period the sea submerged the cave and the early nucleus of aggregated Serpulidae became substrate for succeeding colonizer generations superimposed to each other [14,16,57,58,67].

Our findings support the previous observation by Rosso et al. [38] that large *Protula* tubes that built the core of BSTs within "lu Lampiùne" cave are to date largely replaced by smaller-sized Serpulidae. In particular, on account of a large number of individuals, *S. crenata* and *V. labiata* strongly contributed to the increase in the roughness of the here-examined BST, providing crevices and microcavities for subsequent colonization by smaller Serpulidae. In agreement with previous data, there is evidence that among the superimposed species colonizing the outer BST surface, *S. crenata* act as secondary constructors, while others, coating the surface of the underlying tubes and filling crevices such as *J. marenzelleri*, act as binders [19,22,38,56,68]. The accretion of *Protula* tubes, however, started to slow down about 3000 years ago, as 14C dating suggests a shift in bioconstructors likely due to environmental changes [16,57,58,67]. In this scenario, it is likely that a low metabolic requirement linked to the small size of specimens as well as some environmental factors such as negligible water dynamics and the absence of light-dependent competitors may have facilitated the success of the detected small-sized constructors in the dark sector of the "lu Lampiùne" cave. Changes in environmental conditions allowing a shift in community composition and BST accretion rate have also been suggested for the BSTs from the Plemmirio caves in Sicily [19,20], showing a comparable pattern with a *Protula* core enveloped by smaller skeletonized organisms.

5. Conclusions

- Sciaphilic Serpulidae dominated in terms of number of taxa and abundances on the studied BST from "lu Lampiùne" submarine cave;
- *Semivermilia crenata* and *Vermiliopsis labiata*, characteristic of the dark cave, although not exclusive, largely contribute to the increase in the roughness of the BST surface providing crevices and microcavities;
- In an early phase of the cave colonization, the relatively high food supply likely led larger species (*Protula*) with a gregarious behavior to promote the formation and growth of the BST;
- Remarkably, the large *Protula* tubes that built the core of BTS and allowed it to grow 6000 years ago have now been replaced by smaller-sized Serpulidae
- As indicated by ^{14}C dating, the accretion of *Protula* tubes started to slow down about 3000 years ago, with a shift in Serpulidae bioconstructors, likely due to environmental changes;
- Among the superimposed species, *S. crenata* and *V. labiata* currently act as secondary builders, while others, including smaller specimens such as *Josephella marenzelleri,* act as binders coating the surface of the underlying invertebrate skeletons and filling crevices of the BST surface;
- Negligible water dynamics, the absence of light-dependent competitors, as well as a low metabolic requirement linked to the small size may have facilitated the success of small-sized constructors in the dark sector of the cave.

Author Contributions: Conceptualization, G.B. and M.L.; methodology, M.L.; formal analysis, M.L.; investigation, G.B.; resources, G.B.; writing—original draft preparation, G.B. and M.L.; writing—review and editing, G.B. and M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was realized in the frame of the project Ocean Citizen (Horizon Europe, CUP f83c23000020006)—scientific responsible, Sergio Rossi.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request.

Acknowledgments: Exploration of submarine caves at Cape of Otranto, and collection of BST, were realized with the members of the association of spelaeo-divers Apogon, and in detail with Andrea Costantini, Marco Poto, Raffaele Onorato, and Federico Fiorentino.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Riedl, R. *Biologie der Meereshöhlen*; Paul Parey: Hamburg, Germany, 1966.
2. Harmelin, J.G.; Vacelet, J.; Vasseur, P. Les grottes sous-marines obscures: Un milieu extrême et un remarquable biotope refuge. *Téthys* **1985**, *11*, 214–229.
3. Bianchi, C.N.; Sanfilippo, R. Policheti serpuloidei. In *Grotte Marine. Cinquant'anni di Ricerche in Italia*; Cicogna, F., Bianchi, C.N., Ferrari, G., Forti, P., Eds.; Ministero dell'Ambiente e della Tutela del Territorio: Roma, Italy, 2003; pp. 175–185.
4. Gerovasileiou, V.; Bianchi, C.N. Mediterranean Marine Caves: A Synthesis of Current Knowledge. In *Oceanography and Marine Biology*; CRC Press: Boca Raton, FL, USA, 2021; pp. 1–87.
5. Ingrosso, G.; Abbiati, M.; Badalamenti, F.; Bavestrello, G.; Belmonte, G.; Cannas, R.; Benedetti-Cecchi, L.; Bertolino, M.; Bevilacqua, S.; Bianchi, C.N.; et al. Mediterranean bioconstructions along the Italian coast. *Adv. Mar. Biol.* **2018**, *79*, 61–136.
6. Rossi, S.; Bramanti, L.; Gori, A.; Orejas, C. (Eds.) *Marine Animal Forests. The Ecology of Benthic Biodiversity Hotspots*; Springer Nature: Berlin/Heidelberg, Germany, 2017; p. 1350.
7. Guido, A.; Rosso, A.; Sanfilippo, R.; Miriello, D.; Belmonte, G. Skeletal vs. microbialite geobiological role in bioconstructions of confined marine environments. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2022**, *593*, 110920.
8. Gerovasileiou, V.; Dimitriadis, C.; Arvanitidis, C.; Voultsiadou, E. Taxonomic and functional surrogates of sessile benthic diversity in Mediterranean marine caves. *PLoS ONE* **2017**, *12*, e0183707. [CrossRef]

9. Jones, C.G.; Lawton, J.H.; Shachak, M. Organisms as ecosystem engineers. *Oikos* **1994**, *69*, 373–386.
10. Laborel, J. Le concrétionnement algal "Coralligène" et son importance géomorphologique en Méditerranée. *Rec. Trav. St. Mar. Endoume* **1961**, *23*, 37–60.
11. Cocito, S.; Federghini, F.; Sgorbini, S. Bioconstructions promote biodiversity: Lessons from bryozoans and other invertebrates. *Biol. Mar. Mediterr.* **2001**, *8*, 175–180.
12. Ballesteros, E. Mediterranean coralligenous assemblages: A synthesis of present knowledge. *Oceanogr. Mar. Biol. Annu. Rev.* **2006**, *44*, 123–195.
13. Casellato, S.; Stefanon, A. Coralligenous habitat of the Northern Adriatic Sea: An overview. *Mar. Ecol.-Evol. Persp.* **2008**, *29*, 321–341. [CrossRef]
14. Rossi, S.; Bramanti, L. (Eds.) *Perspectives on the Marine Animal Forests of the World*; Springer Nature: Berlin/Heidelberg, Germany, 2020; p. 530.
15. Onorato, R.; Forti, P.; Belmonte, G.; Costantini, A.; Poto, M. La grotta sottomarina lu Lampiùne: Novità esplorative e prime indagini ecologiche. *Thalass. Salentina* **2003**, *26*, 55–64.
16. Belmonte, G.; Ingrosso, G.; Poto, M.; Quarta, G.; D'elia, M.; Onorato, O.; Calcagnile, L. Biogenic stalactites in submarine caves at the Cape of Otranto (SE Italy): Dating and hypothesis on their formation. *Mar. Ecol.* **2009**, *30*, 376–382. [CrossRef]
17. MacIntyre, I.G.; Videtich, P.E. Pseudostalactites from Submarine Cave Near Columbus Cay, Belize Barrier-Reef Complex-Evidence of Extensive Submarine Lithification. *AAPG Bull.* **1979**, *63*, 489.
18. Guido, A.; Mastandrea, A.; Rosso, A.; Sanfilippo, R.; Russo, F. Micrite precipitation induced by reducing sulfate bacteria in serpulid bioconstructions from submarine caves (Syracuse, Sicily). *Rend. Online Soc. Geol. Ital.* **2012**, *21*, 933–934.
19. Guido, A.; Heindel, K.; Birgel, D.; Rosso, A.; Mastandrea, A.; Sanfilippo, R.; Russo, F.; Peckmann, J. Pendant bioconstructions cemented by microbial carbonate in submerged marine caves (Holocene, SE Sicily). *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2013**, *388*, 166–180. [CrossRef]
20. Sanfilippo, R.; Rosso, A.; Guido, A.; Mastandrea, A.; Russo, F.; Riding, R.; Taddei Ruggiero, E. Metazoan/microbial biostalactites from present day submarine caves in the Mediterranean Sea. *Mar. Ecol.* **2015**, *36*, 1277–1293. [CrossRef]
21. Guido, A.; Jimenez, C.; Achilleos, K.; Rosso, A.; Sanfilippo, R.; Hadjioannou, L.; Petrou, A.; Russo, F.; Mastandrea, A. Cryptic serpulid-microbialite bioconstructions in the Kakoskali submarine cave (Cyprus, Eastern Mediterranean). *Facies* **2017**, *63*, 21. [CrossRef]
22. Sanfilippo, R.; Rosso, A.; Guido, A.; Gerovasileiou, V. Serpulid communities from two marine caves in the Aegean Sea, eastern Mediterranean. *J. Mar. Biol. Assoc.* **2017**, *97*, 1059–1068. [CrossRef]
23. Jimenez, C.; Achilleos, K.; Petrou, A.; Hadjioannou, L.; Guido, A.; Rosso, A.; Gerovasileiou, V.; Albano, P.; Di Franco, D.; Andreou, V.; et al. A dream within a dream: Kakoskali Cave, a unique marine ecosystem in Cyprus (Levantine Sea). In *Marine Caves of the Eastern Mediterranean Sea. Biodiversity, Threats and Conservation*; Öztürk, B., Ed.; Turkish Marine Research Foundation (TUDAV): Istanbul, Türkiye, 2019; Publication no. 53; pp. 91–110.
24. Fagerstrom, J.A. *The Evolution of Reef Communities*; John Wiley & Sons: New York, NY, USA, 1987.
25. Palma, R.M.; Angeleri, M.P. Early Cretaceous serpulid limestones: Chachao Formation, Neuquen Basin, Argentina. *Facies* **1992**, *27*, 175–178. [CrossRef]
26. 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.
27. Friebe, J.G. Serpulid-bryozoan-foraminiferal biostromes controlled by temperate climate and salinity: Middle Miocene of the Styrian Basin, Austria. *Facies* **1994**, *30*, 51–62. [CrossRef]
28. Cirilli, S.; Iannace, A.; Jadoul, F.; Zamparelli, V. Microbial serpulids buildups in Norian–Rhaetian of the Western Mediterranean area: Ecological response of marginal communities to stressed environments. *Terra Nova* **1999**, *11*, 195–202. [CrossRef]
29. Kupriyanova, E.K.; Rzhavsky, A.V.; ten Hove, H.A. Serpulidae Rafinesque, 1815. In *Handbuch der Zoologie, Annelida, Band 2 Pleistoannelida, Sedentaria II*; Westheide, W., Ed.; De Gruyter: Berlin, Germany, 2020; pp. 213–275.
30. Georgieva, M.N.; Paull, C.K.; Little, C.T.S.; McGann, M.; Sahy, D.; Condon, D.; Lundsten, L.; Pewsey, J.; Caress, D.W.; Vrijenhoek, R.C. Discovery of an extensive deep-sea fossil serpulid reef associated with a cold seep, Santa Monica Basin California. *Front. Mar. Sci.* **2019**, *6*, 115. [CrossRef]
31. Rouse, G.W.; Pleijel, F. *Polychaetes*; Oxford University Press: New York, NY, USA, 2001; p. 354.
32. Vinn, O.; ten Hove, H.A.; Mutvei, H.; Kirsimaee, K. Ultrastructure and mineral composition of serpulid tubes (Polychaeta, Annelida). *Zool. J. Linn. Soc.* **2008**, *154*, 633–650. [CrossRef]
33. Vinn, O.; Kupriyanova, E.K. Evolution of a dense outer protective tube layer in serpulids (Polychaeta, Annelida). *Carnets Géol./Noteb. Geol.* **2011**, *5*, 137–147. [CrossRef]
34. Vinn, O. Biomineralization in Polychaete Annelids: A Review. *Minerals* **2021**, *11*, 1151. [CrossRef]
35. Zabala, M.; Riera, T.; Gili, J.M.; Barange, M.; Lobo, A.; Peñuelas, J. Water flow, trophic depletion, and benthic macrofauna impoverishment in a submarine cave from the Western Mediterranean. *Mar. Ecol.* **1989**, *10*, 271–287. [CrossRef]

36. Bussotti, S.; Terlizzi, A.; Fraschetti, S.; Belmonte, G.; Boero, F. Spatial and temporal variability of sessile benthos in shallow Mediterranean marine caves. *Mar. Ecol. Prog. Ser.* **2006**, *325*, 109–119. [CrossRef]
37. Antonioli, F.; Silenzi, S.; Frisia, S. Tyrrhenian Holocene palaeoclimate trends from spelean serpulids. *Quat. Sci. Rev.* **2001**, *20*, 1661–1670. [CrossRef]
38. Rosso, A.; Sanfilippo, R.; Guido, A.; Gerovasileiou, V.; Ruggiero, E.T.; Belmonte, G. Colonisers of the dark: Biostalactite-associated metazoans from "lu Lampiùne" submarine cave (Apulia, Mediterranean Sea). *Mar. Ecol.* **2021**, *42*, e12634. [CrossRef]
39. Onorato, R.; Palmisano, G. Otranto: La grotta sottomarina de "lu Lampiune". *Itiner. Speleol. II* **1990**, *2*, 84–90.
40. Montefalcone, M.; Oprandi, A.; Azzola, A.; Morri, C.; Bianchi, C.N. Serpulid reefs and their role in aquatic ecosystems: A global review. *Adv. Mar. Biol.* **2022**, *92*, 1–54.
41. ten Hove, H.A. Different causes of mass occurrence in serpulids. In *Biology and Systematic of Colonial Organisms, Systematics Association Special*; Larwood, G., Rosen, B.R., Eds.; Academic Press: London, UK, 1979; Volume 11, pp. 281–298.
42. Bianchi, C.N.; Morri, C. Studio bionomico comparativo di alcune grotte marine sommerse; definizione di una scala di confinamento. *Mem. Istit. Ital. Speleolog.* **1994**, *6* (Suppl. S2), 107–123.
43. Fornós, J.J.; Forteza, V.; Martínez-Taberner, A. Modern polychaete reefs in western Mediterranean lagoons: *Ficopomatus enigmaticus* (Fauvel) in the Albufera of Menorca, Balearic Islands. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **1997**, *128*, 175–186.
44. Scicchitano, G.; Monaco, C. Grotte carsiche e linee di costa sommerse tra Capo Santa Panagia e Ognina (Siracusa, Sicilia sudorientale). *Alp. Mediterr. Quat.* **2006**, *19*, 187–194.
45. Bianchi, C.N.; Aliani, S.; Morri, C. Present day serpulid reefs, with reference to an on going research project on Ficopomatus enigmaticus. *Publ. Serv. Géol. Luxemb.* **1995**, *29*, 61–65.
46. Bosence, D.W.J. Recent serpulid reefs, Connemara, Eire. *Nature* **1973**, *242*, 40–41.
47. Denitto, F.; Licciano, M. Recruitment of Serpuloidea (Annelida: Polychaeta) in a marine cave of the Ionian Sea (Italy, central Mediterranean). *J. Mar. Biol. Assoc.* **2006**, *86*, 1373–1380.
48. Harmelin, J.G.; Vacelet, J. Clues to deep-sea biodiversity in a nearshore cave. *Vie Milieu* **1997**, *47*, 351–354.
49. Bianchi, C.N.; Cattaneo-Vietti, R.; Cinelli, F.; Morri, C.; Pansini, M. Lo studio biologico delle grotte sottomarine: Conoscenze attuali e prospettive. *Boll. Mus. Ist. Biol. Univ. Genova* **1996**, *60*, 41–69.
50. Zibrowius, H. Remarques sur la faune sessile des grottes sous-marines et de l'étage bathyal en Méditerranée. *Rapp. Comm. Int. Mer Médit.* **1971**, *20*, 243–245.
51. Vacelet, J.; Boury-Esnault, N.; Harmelin, J.G. Hexactinellid cave, a unique deep-sea habitat in the scuba zone. *Deep. Sea Res. Part I Oceanogr. Res. Pap.* **1994**, *41*, 965–973. [CrossRef]
52. Passelaigue, F. Les Migrations Journalières du Mysidacé Marin Cavernicole Hemimysis Speluncola. Comparaison Avec les Migrations Verticales du Plancton. Ph.D. Thesis, Université d'Aix-Marseille II, Marseille, France, 1989.
53. Rosso, A.; Sanfilippo, R.; Taddei Ruggiero, E.; Di Martino, E. Faunas and ecological groups of Serpuloidea, Bryozoa and Brachiopoda from submarine caves in Sicily (Mediterranean Sea). *Boll. Della Soc. Paleontol. Ital.* **2013**, *52*, 167–176.
54. Belloni, S.; Bianchi, C.N. Policheti di alcune grotte marine della Penisola Sorrentina (Golfo di Napoli). *Boll. Mus. Ist. Biol. Univ. Genova* **1982**, *50*, 118–127.
55. Bianchi, C.N. Policheti Serpuloidei. In *Guide per il Riconoscimento Delle Specie Animali Delle Acque Lagunari e Costiere Italiane, Collana del Progetto Finalizzato "Promozione Della Qualità Dell'ambiente"*; serie AQ/1/96, 5; C.N.R.: Roma, Italy, 1981; p. 187.
56. Guido, A.; Mastandrea, A.; Rosso, A.; Sanfilippo, R.; Tosti, F.; Riding, R.; Russo, F. Commensal symbiosis between agglutinated polychaetes and sulfate reducing bacteria. *Geobiology* **2014**, *12*, 165–175. [CrossRef] [PubMed]
57. Quarta, G.; D'Elia, M.; Belmonte, G.; Braione, E.; Scrimieri, L.; Calcagnile, L. 14C dating on marine bio-constructions from a submarine cave in the Adriatic Sea. *Nucl. Instrum. Methods Phys. Res. B* **2019**, *455*, 234–237. [CrossRef]
58. Quarta, G.; D'Elia, M.; Calcagnile, L.; Belmonte, G.; Ingrosso, G. Reconstructing the formation mechanism of submarine biogenic stalactites: The contribution of AMS. *Nucl. Instrum. Methods Phys. Res. B* **2010**, *268*, 1244–1247. [CrossRef]
59. Sket, B. The ecology of anchihaline caves. *Trends Ecol. Evol.* **1996**, *11*, 221–225. [CrossRef]
60. Harmelin, J.G. Bryozoaires des grottes sous-marines obscures de la région marseillaise, faunistique et écologie. *Téthys* **1969**, *1*, 793–806.
61. Pérès, J.M.; Picard, J. Notes sommaires sur le peuplement des grottes sous-marines de la région de Marseille. *Comptes Rendu Soc. Biogeogr.* **1949**, *227*, 42–45.
62. Pérès, J.M.; Picard, J. Nouveau manuel de bionomie benthique de la Mer Méditerranée. *Rec. Trav. Stn. Marittime Endoume* **1964**, *47*, 3–137.
63. Balduzzi, A.; Bianchi, C.N.; Boero, F.; Cattaneo-Vietti, R.; Pansini, M.; Sarà, M. The suspension-feeder communities of a Mediterranean Sea cave. *Sci. Mar.* **1989**, *53*, 387–395.
64. Harmelin, J.G. Patterns in the distribution of bryozoans in the Mediterranean marine caves. *Stygologia* **1986**, *2*, 10–25.
65. Zibrowius, H. Étude morphologique, systématique et écologique des Serpulidae (Annelida Polychaeta) de la région de Marseille. *Rec. Trav. Stn. Marittime Endoume* **1968**, *43*, 81–252.

66. Ten Hove, H.A.; Kupriyanova, E. Taxonomy of Serpulidae (Annelida, Polychaeta): The state of affairs. *Zootaxa* **2009**, *2036*, 1–126.
67. D'Elia, M.; Quarta, G.; Calcagnile, L.; Belmonte, G. Study of the formation of biogenic speleothems found in submarine caves at the cape of Otranto, Italy, by 14C AMS. *Nucl. Instrum. Methods Phys. Res. B* **2007**, *259*, 395–397. [CrossRef]
68. Gerovasileiou, V.; Chintiroglou, C.C.; Vafidis, D.; Koutsoubas, D.; Sini, M.; Dailianis, T.; Issaris, Y.; Akritopoulou, E.; Dimarchopoulou, D.; Voultsiadou, E. Census of biodiversity in marine caves of the Eastern Mediterranean Sea. *Mediterr. Mar. Sci.* **2015**, *16*, 245–265. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.