

Article

First Speleological and Biological Characterization of a Submerged Cave of the Tremiti Archipelago Geomorphosite (Adriatic Sea)

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Abstract: The Tremiti Islands Archipelago is located in the Central-Southern Adriatic Sea and is characterized by a strong karst activity. Due to their landscape, geological, hydrological, archaeological, and historical value, Tremiti Islands are considered a geomorphosite. We carried out a preliminary, non-destructive survey by studying the speleological and biological features of the submerged sea cave Elle, located at Cala Sorrentino (Capraia Island), representing an EU Natura 2000 Priority Habitat. Topographic (perimeter length, width, height, GPS location, depth) and hydrological parameters (temperature, dissolved oxygen, pH, irradiance, water movement) were measured and its map was produced, based on morphological measurements and biological evidence. Species composition and biotic cover were investigated by image analysis. The benthic community was dominated by poriferans, together with remarkable assemblages of scleractinians and polychaete serpulids and clearly reflected the sharp light and hydrological gradients from the entrance to the cave bottom. Furthermore, different phases were hypothesized for the genesis of the cave during sea regression and flooding periods. To be signaled is the record of *Petrobiona massiliana*, a sponge was protected under both Barcelona and Bern Convention and of some individuals of polychaete Serpulinae, which are still under description.

Keywords: submerged caves; benthic community; dark habitat; cave morphology; speleogenesis; karst system; Mediterranean Sea

1. Introduction

Submerged marine caves are a peculiar aspect of the karst landscape, which extensively characterize the coast of the Mediterranean Sea and are the result of complex speleogenetic processes that have caused their origin. Marine caves are part of a unique fragmented habitat, each characterized by specific topographic features and hydrological gradients, in order to function as ecological islands. For their significant ecological value and ecosystem services, as reservoirs of species, marine caves are listed in the European Community Habitats Directive (92/43/ECC) as “Submerged and partially submerged sea caves (code 8330)”, included in the protected area network Natura 2000 and also in the “Dark Habitats Action Plan” of the Barcelona Convention (UNEP-MAP-RAC/SPA, 2015), together with seamounts, underwater canyons, aphotic hard beds, and chemo-synthetic phenomena, for the conservation of sensitive species and habitats [1,2].

The current scientific interest in submarine caves is evidenced by the progressive increase of research on these topics [2]. Notwithstanding, knowledge on these habitats is far from exhaustive and up-to-date.

In the Mediterranean Sea, these habitats are widespread along rocky coastal areas, counting more than 3000 marine caves [3]. In the Southern Adriatic Sea, a vast karst system extends along the Italian coast and includes several marine caves that have been under study for many years, particularly in the Apulian Salento peninsula [1]. At the junction of the Central-Southern Adriatic Sea, the Tremiti Islands Archipelago lies in a complex geological area. Here, the karst action is evident both on the surface of the islands, with several underground caves, and on underwater carbonated rocks at different bathymetric ranges, where several submerged caves are present [4]. In accordance with its special value in terms of landscape and geological, geomorphological, hydrological, archaeological, and historical features, the Tremiti Archipelago is considered a geomorphosite [5]. Mediterranean caves have been increasingly studied using approaches concerning single caves (see e.g., [6–11]) or synthetic reviews [2,12–18], but a particular attention has been devoted to the karst system of the Adriatic Sea, both in the northern and southern parts (e.g., [1,19–22]). Notwithstanding, the comprehension of the structure and functioning of caves' ecosystems deserves further investigation, especially given the "individuality" model [2], which takes into account both spatial heterogeneity and the unique characteristics of each cave. Although sharing common abiotic and biotic characteristics, which mostly derive from the drastic decrease in light, each single cave has its own morphological and hydrological specificities that, together with stochastic historical events, strongly affect the biotic zonation.

This study aims at contributing to the knowledge of the still poorly known priority habitat represented by submerged caves, by presenting the morphological, hydrological, and biological features of Elle submarine cave (Cala Sorrentino, Capraia Island in the Tremiti Archipelago). The data collected allowed for the drawing up of the topography of the cave, suggesting the possible speleogenetic processes and describing the specific benthic communities along the horizontal development of the cave.

2. Materials and Methods

2.1. Study Area

The Archipelago of Tremiti Islands is located in the Central-Southern Adriatic Sea, about 20 km from the Italian coast, and is formed by four islands, namely San Nicola, San Domino, Capraia, and Cretaccio islands, together with La Vecchia and Pianosa rocks. The islands are aligned along the SW–NE orientation and have a 3 km² surface. Islands show a tabular shape with elevation between 119 m a.s.l. (San Domino) and 55 m a.s.l. (Capraia) and are bound by very steep or vertical cliffs [23].

Bathymetry is characterized by three main flat surfaces at 8–10 m b.s.l., at 20–25 m b.s.l., and at 50–55 m b.s.l. Dolines and potholes are located between 9 and 15 m b.s.l., partly filled with gravel deposits, and intensely reshaped by marine erosion. At greater depth, there are dolines more than 100 m wide, filled by sand and gravel deposits. Caves and dolines are the result of several phases of karst activity starting from the Eocene [24] and developed mainly along the main tectonic discontinuities. In the deep seabed, submarine caves are found along the main subvertical rock escarpments, at depths of 6/18 and 25/45 m b.s.l. Most of them have small entrances and are mostly less than 25 m long without wide chambers [4].

2.2. Survey and Sampling

The investigation of the Elle cave was carried out in August–September 2020. Preliminary investigation of the cave was conducted through photos and videos taken with a GoPro 7 Hero Black (Woodman Labs, San Mateo, CA, USA) and a Nilox F-60 (Esprinet S.p.A., Vimercate, Italy) throughout the cave, to provide preliminary data without damaging the structure or the biota. The areas of topographic discontinuity have been identified and three sampling stations have been established: in the entry sector (ES), in the interme-

diate sector (IS), and in the bottom sector (BS) (Figure 1). The standard underwater cave survey technique was used to map both the trend and dimensions of the cave passages.

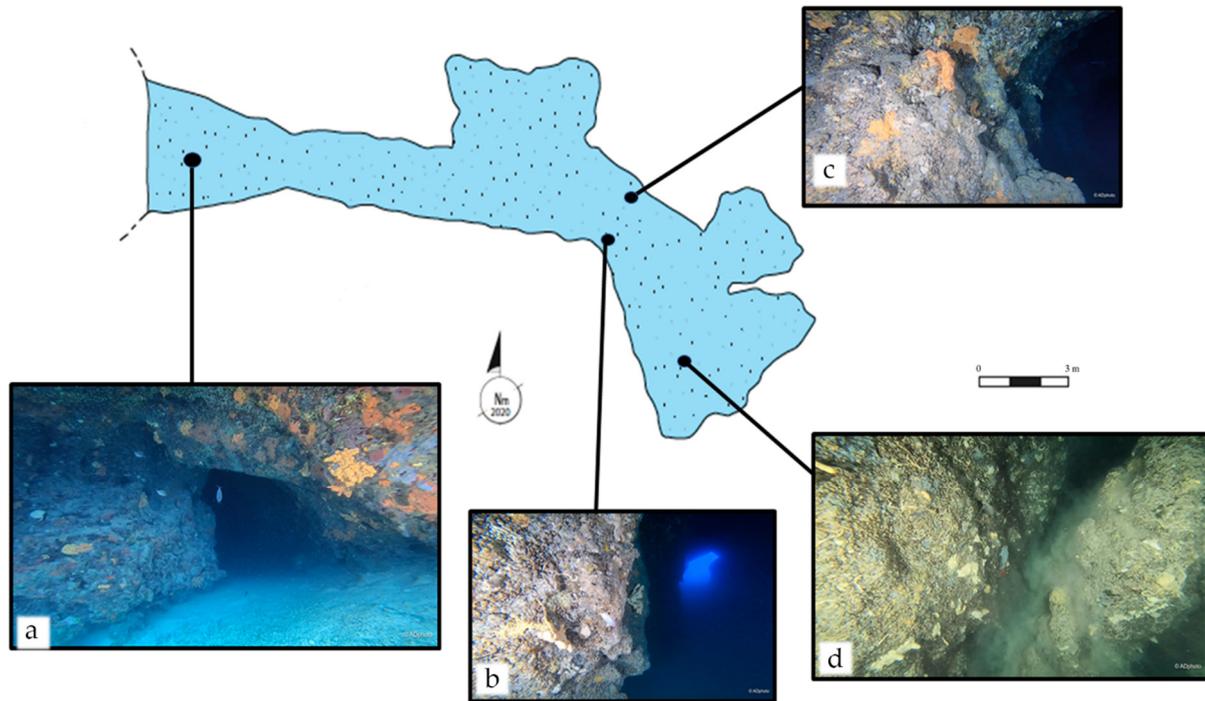


Figure 1. Sampling stations in the cave: (a) Entrance (ES), (b) right wall of the intermediate sector (IS) with the view of the entrance in the background, (c) left wall in the intermediate sector, and (d) crack in the wall in the innermost area of the cave, showing sediments suspended by the outflow of water (BS). Photos by Adelmo Sorci.

The GPS position ($42^{\circ}08'05.8''$ N $15^{\circ}30'28.5''$ E) of the entrance of the cave was taken as the first step in the survey, through a surface marker buoy (SMB). A longitudinal transect was set up from the cave entrance to the innermost zone in the cave. A guideline with a total of 13 iron pegs, bearing numbered plates, was used. The pegs were positioned in the sediment at the major points of discontinuity of the cave perimeter (Figure 2). A tape 50 m long was used to measure the length of the perimeter, width, and height of the cave. The azimuth and distance between consecutive survey stations and depth, width, and height at each station were measured using an underwater compass to build the relief of the cave.

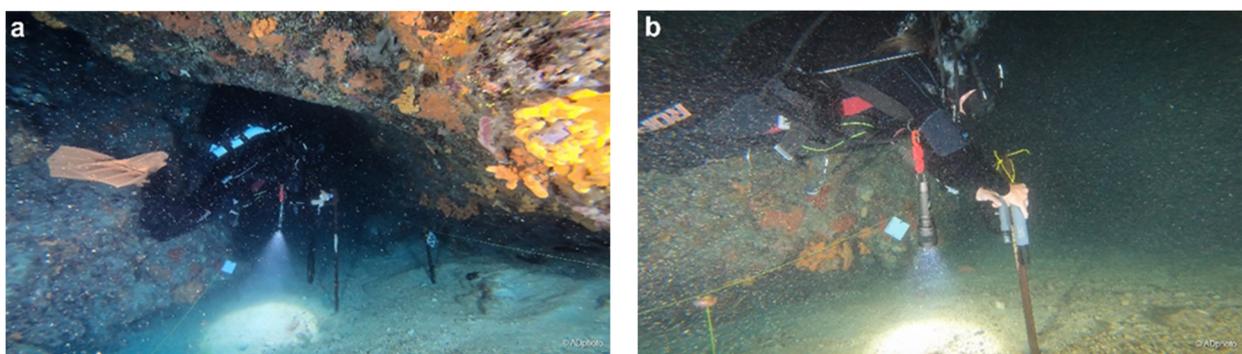


Figure 2. Evidence of outflow of water with sediment particles through the cave entrance during sampling procedures; (a) placement of numbered peg and multiparametric probes for morphological survey in Elle cave (b). Photos by Adelmo Sorci.

2.3. Physical–Chemical Parameters

At each of the sampling stations, the abiotic parameters temperature (T), pH, and dissolved oxygen (DO) were measured by HOBO Onset Data Logger during the period 23 August–16 September 2020. The multiparameter probes were fixed on a stake in the center of the duct in each of the sampling stations, and the physical–chemical parameters were measured continuously for 48 h.

2.4. Irradiance

Light in each station was measured using the HOBO Pendant[®] Temperature/Light 64K Data Logger. The logger was attached to a flat surface on the sediment, and mounted horizontally to ensure that the sensor was pointing upward. The values refer to the subsurface light.

2.5. Water Motion

Water motion in the sampling zones of the cave was derived from the measurement of weight losses of calcium sulphate balls (3.0 cm in diameter). To form the spheres, “Scagliola” gypsum and fresh water were used in proportions of 50 g per 45 cc, in accordance with the methods reported by [25,26]. A numbered iron bar, previously weighed, was inserted into each ball.

The spheres were weighed using a precision scale to calculate the initial weight in grams before placing them in the cave. By means of scuba diving, three steel bars were inserted with the relative three balls on a picket, corresponding to the sampling station. At the same time, the abiotic parameters were measured using multiparameter probes. The spheres were left to soak for 48 h and later dried in the sun for 72 h before weighing them with a precision scale.

2.6. Video and Image Acquisition

Benthic assemblages were investigated using visual census, as a non-destructive method, in agreement with other previous studies [9,19]. Fourteen video transects were planned from the entrance to the bottom of the cave and carried out by technical cave divers equipped with high-definition video cameras (GoPro 7 Hero Black—Woodman Labs, San Mateo, CA, USA) and two high-performance LED strobe illuminators (Weefine Solar Flare 5000—Foshan Weefine Technology Co., Ltd., Foshan (China)). In addition, photo quadrat surveys were performed in the three sectors of the cave: External sector (ES), intermediate sector (IS), and bottom sector (BS) using a digital camera Nilox F-60 (Esprinet S.p.A., Vimercate, Italy) equipped with an underwater case and two strobes. The sampling area was delimited by a 60 × 60 cm (3600 cm²) sampling square mounted on the top of an iron rod trapezoid to keep the camera at a fixed distance from the substrate. For each sector of the cave, three sampling stations have been set to take photos: Two stations on the left and right wall, respectively and one station on the ceiling of the cave. At each station, three replicate photos have been taken for a total of 15 photo-quadrats (12 on the vertical walls and three on the roof) at each station. A total of 45 photos have been taken in the cave and analyzed.

The benthic organisms observed in the photo-quadrat images have been identified at the lowest possible taxonomic level. The quantitative contribution was evaluated by measuring the coverage of each taxon in relation to the total area covered by benthos and referred to as a percentage. ImageJ software was used for the analysis of the photo images.

3. Results

3.1. Morphological Description of the Elle Cave

In accordance with the definition of marine cavities proposed by Cattaneo and Pastorino [27], the marine Elle cave can be considered as an infralittoral cavity, that is, completely submerged cavities. Following the detailed topographic survey, the cave shows a substantially horizontal trend and an overall planimetric development of 50 m was calcu-

lated (perimeter 50 m, planimetric development 25 m, spatial development 30 m), with a height of the vault between 2.4 m at the entrance and 4.5 m at the end. The cave is oriented 90° N–NE in the first part (entrance), 100° N–NE in the intermediate zone, and 140° E–SE in the inner zone. The longitudinal vertical section and the plan of the cave are shown in Figure 3. The floor of the cavity is completely covered by a silty-sand sediment at the entrance and by a sandy-silt sediment in the innermost part, while the walls are characterized by concretion phenomena forming speleothems and by a biological concretion cover. The cave becomes impracticable at its end due to a narrowing that appears as a crack a few centimeters wide.

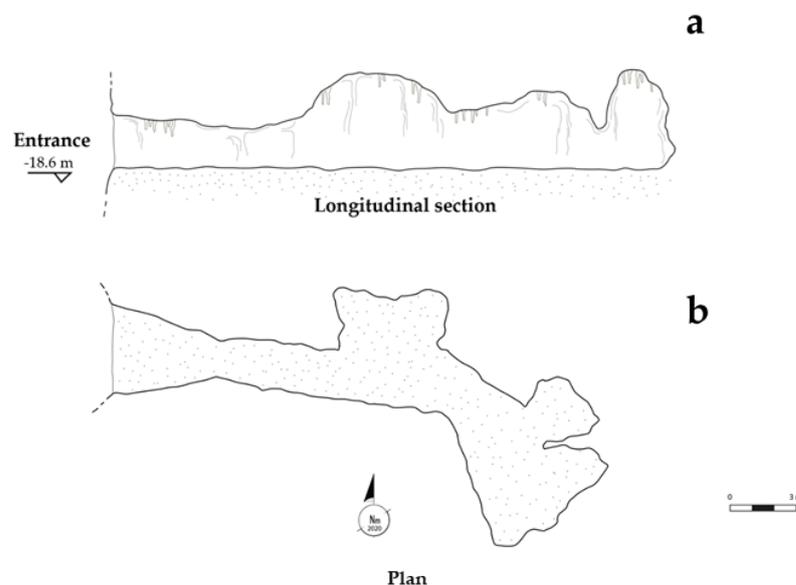


Figure 3. Longitudinal vertical section (a) and plan (b) of the Elle cave.

A notable feature is a water spring from a fissure at the end of the cave, and then a water flow directed towards the entrance that carries muddy sediment from the floor to the outside, as documented by scuba observations (Figures 1d and 2a). Although our preliminary data do not allow for the demonstration of the presence of freshwaters inside the cave, the occurrence of freshwater springs in submerged caves in the Adriatic Sea has been already documented [10]. For this characteristic, the cavity could be considered as active, or partially active, and to all intents and purposes defined as a karst resurgence (Vrulja: For a general description of both submarine resurgences and submarine springs, see [28]).

The cave has been registered in the “Catasto delle Grotte e delle Cavit  artificiali” of the Region Apulia (Italy), number PU-2670 Lat N 42.134944 Lon E 15.507917, by Martina Mazzetti on 1 September 2020.

3.2. Hydrological Parameters

Water movement progressively decreased along the cave following an outer-inner gradient. In Table 1a, the weight losses of CaSO_4 balls after 72 h are reported, both in terms of mean grams (\pm SD) and average percentage, representing a measure of water movement at the three investigated zones of the cave. The differences of weight losses between the three zones were significant, in accordance with the ANOVA test (Table 1b).

Table 1. (a) Mean (\pm SD) weight losses (g) of calcium sulfate balls after 72 hours and respective average percentage as measure of water movement at the three zones investigated. (b) Results of analysis of variance (ANOVA) on the weight losses of calcium sulfate balls as measure of water movement at the three investigated zones of the cave.

(a)					
Zone	Date	Mean (\pm SD)	Average %		
1	23-25 August	5.041 (\pm 0.039)	39.7		
2	3-5 September	4.288 (\pm 0.116)	33.6		
3	14-16 September	3.933 (\pm 0.059)	31.0		
(b)					
	Sum of Sqrs	df	Mean Square	F	<i>p</i>
Between groups	1.914	2	0.957	262.6	<0.001
Within groups	0.022	6	0.004		
Total	1.936	8			

The highest pH values were recorded in zone 2 and the lowest in the innermost zone, while at the entrance of the cave intermediate pH values were measured (Table 2a). The ANOVA test showed significant differences between the three zones on repeated measurements during the time of 48 h (Table 2b).

Table 2. (a) Mean (\pm SD) values of pH measured in the three zones of the cave. (b) Results of analysis of variance (ANOVA) on the pH repeated measures in the three zones of the cave.

(a)					
Zone	Date	Mean (\pm SD)			
1	23–25 August	8.13 (\pm 0.01)			
2	3–5 September	8.23 (\pm 0.01)			
3	14–16 September	8.06 (\pm 0.02)			
(b)					
	Sum of Sqrs	df	Mean Square	F	<i>p</i>
Between groups	0.659	2	0.3294	1760	<0.001
Within groups	0.025	138	0.0002		
Error	0.017	92	0.0002		
Between hours	0.008	46	0.0002		
Total	0.684	140			

The dissolved oxygen concentration and percent of saturation were highest at the entrance of the cave and progressively decreased in the intermediate and inner zones (Table 3a); the differences between the three zones were significant by the ANOVA test (Table 3b).

The average temperature values were the lowest at the entrance and one degree higher in the inner zones. The ANOVA test showed significant differences between the three zones, but the results of Tukey's pairwise test were significant between zone 1 in comparison with zone 2 and 3, respectively, while zones 2 and 3 were not significantly different (Table 4a–c).

Table 3. (a) Mean (\pm SD) values of dissolved oxygen measured in the three zones of the cave. (b) Results of analysis of variance (ANOVA) on repeated measures of dissolved oxygen in the three zones of the cave.

(a)					
Zone	Date	mg/L	% Saturation		
1	23–25 August	8.5 (\pm 0.4)	100.1 (\pm 3.5)		
2	3–5 September	7.74 (\pm 0.73)	92.5 (\pm 7.4)		
3	14–16 September	7.36 (\pm 0.61)	88.5 (\pm 7.2)		
(b)					
	Sum of Sqrs	df	Mean Square	F	<i>p</i>
Between groups	31.95	2	15.97	47.15	<0.001
Within groups	48.58	138	0.35		
Error	31.17	92	0.34		
Between subjects	17.42	46	0.38		
Total	80.53	140			

Table 4. (a) Mean (\pm SD) values of temperature ($^{\circ}$ C) measured in the three zones of the cave. (b) Results of analysis of variance (ANOVA) on temperature repeated measures in the three zones of the cave. (c) Results of Tukey's pairwise test below the diagonal; *p* values above the diagonal (N.S. = not significant).

(a)					
Zone	Date	Mean (\pm SD)			
1	23–25 August	23.6 (\pm 0.8)			
2	3–5 September	24.4 (\pm 1.0)			
3	14–16 September	24.6 (\pm 0.2)			
(b)					
	Sum of Sqrs	df	Mean Square	F	<i>p</i>
Between groups	29.64	2	14.82	31.2	<0.001
Within groups	78.93	138	0.57		
Error	43.71	92	0.48		
Between hours	35.23	46	0.77		
Total	108.57	140			
(c)					
	ST1	ST2	ST3		
ST1		<i>p</i> < 0.001	<i>p</i> < 0.001		
ST2	8.35		N.S.		
ST3	10.6	2.26			

The innermost zones (2 and 3) of the cave remained dark throughout the day, while only the zone at the entrance of the cave received some light during for seven hours a day from 11:30 to 19:30. The average light intensity in zone 1 was 29.6 lux.

3.3. Biodiversity Pattern of the Benthic Assemblages

The benthic assemblage detected on the walls and ceiling of the Elle cave comprised 63 living taxa belonging to nine phyla: Two algae and seven invertebrate phyla (Table 5). The assemblages varied in both the species/taxa composition and cover values from the entrance to the inner cave sector (Figures 4 and 5). Rhodophyta and Chlorophyta algae were found only at the entrance together with all of the other taxonomic groups (Figure 6a), except for Brachiopoda, which were observed in the innermost area of the cave. In addition to sponges, which were the most represented, cnidarians and polychaetes included multiple species, while molluscs, brachiopods, and ascidians were observed with

a single species identified. Porifera encompass 39 taxa, as follows: One taxon of Calcarea, two of Homoscleromorpha, and 36 of Demospongiae, with 20 of them identified to the species level (Table 5). Sponges were not uniformly distributed along the sectors of the cave. Most of the recorded species were concentrated at the ES (87.1%), while their number progressively decreased towards the IS (64.1%) and the BS (51.3%) with a preferential distribution on the walls rather than on the ceiling (Figure 5). Sponge cover was 56.9% of the substrate at the ES, reached a maximum in the IS (64.8%), and decreased to 25.9% at the BS (Figure 4). At the ES, the sponge assemblage was rich and diversified. The most common species were *Spirastrella* spp., which covered a wide portion of the substrate both on the wall and ceiling of the cave, *Agelas oroides*, *Petrosia ficiformis*, and *Phorbas tenacior*, found mainly close to the entrance of the cave (Figure 6a). In the IS *Spirastrella* spp., together with *Dendroxea* cf. *lenis* and the Homoscleromorpha cf., *Pseudocorticium jarrei* dominated the sponge assemblage of the walls (Figure 6c). The abundance of *Spirastrella* spp. and *D. cf. lenis* decreased sharply on the ceiling and was completely replaced by cf. *P. jarrei*, which was clearly dominant in the IS. In the BS, excluding cf. *P. jarrei*, this pattern was completely replaced by a few other species. In particular, the encrusting blue sponge (Demospongiae 10, Table 5), a few specimens of cf. *P. jarrei* and other small encrusting sponge species characterized most assemblages of this cave sector. *Petrobiona massiliana* was the only calcareous sponge species recorded in the cave (Figure 6d). The sponge was abundant mainly in the BS with preferential distribution on the walls.

Table 5. List of the species/taxa identified from the photos taken on the wall and ceiling of the external (ES), intermediate (IS), and bottom (BS) sectors of the Elle cave. *: Long small tubes up to 10 mm; **: Medium tubes longer than 10 mm up to about 30 mm; ***: Bryozoans with small erect colonies; ****: Bryozoans with encrusting colonies.

	ES-Wall	ES-Ceiling	IS-Wall	IS-Ceiling	BS-Wall	BS-Ceiling
RHODOPHYTA						
Encrusting Coralline Algae	x	x				
CHLOROPHYTA						
Chlorophyta ind. spp.	x					
<i>Palmophyllum crassum</i> (Naccari Rabenhorst, 1868)	x					
PORIFERA						
<i>Petrobiona massiliana</i> (Vacelet and Lévi, 1958)	x		x	x	x	x
cf. <i>Pseudocorticium jarrei</i> (Boury-Esnault, Muricy, Gallissian and Vacelet, 1995)			x	x	x	
<i>Oscarella</i> spp.			x			
<i>Erylus discophorus</i> (Schmidt, 1862)			x		x	
<i>Dercitus plicatus</i> (Schmidt, 1868)		x				
<i>Cliona schmidtii</i> (Ridley, 1881)	x					
<i>Spirastrella</i> spp.	x	x	x	x	x	
<i>Diplastrella bistellata</i> (Schmidt, 1862)			x	x	x	
<i>Pseudosuberites sulphureus</i> (Bowerbank, 1866)	x		x		x	
<i>Terpios gelatinosus</i> (Bowerbank, 1866)	x	x	x			
<i>Timea</i> sp.	x					
<i>Chondrosia reniformis</i> (Nardo, 1847)	x	x				

Table 5. Cont.

	ES-Wall	ES-Ceiling	IS-Wall	IS-Ceiling	BS-Wall	BS-Ceiling
<i>Antho incostans</i> (Topsent, 1925)	x					
<i>Phorbas tenacior</i> (Topsent, 1925)	x					
cf. <i>Merlia normani</i> (Kirkpatrick, 1908)	x					
<i>Axinella damicornis</i> (Esper, 1794)	x					
<i>Didiscus</i> sp.	x					
<i>Acanthella acuta</i> (Schmidt, 1862)	x		x			
<i>Dictyonella</i> sp.	x					
<i>Agelas oroides</i> (Schmidt, 1864)	x	x	x		x	
<i>Dendroxea</i> cf. <i>lenis</i> (Topsent, 1892)	x	x	x	x	x	x
<i>Haliclona</i> (<i>Soestella</i>) <i>mucosa</i> (Griessinger, 1971)			x		x	
<i>Petrosia</i> (<i>Petrosia</i>) <i>ficiformis</i> (Poiret, 1789)	x	x	x		x	
<i>Ircinia oros</i> (Schmidt, 1864)	x					
<i>Ircinia variabilis</i> (Schmidt, 1862)	x					
<i>Cacospongia mollior</i> (Schmidt, 1862)	x					
<i>Fasciospongia cavernosa</i> (Schmidt, 1862)	x					
<i>Spongia</i> sp. 1	x					
<i>Spongia</i> sp. 2		x				
<i>Hexadella</i> cf. <i>pruvoti</i> (Topsent, 1896)	x	x	x			
Demospongiae 1	x					
Demospongiae 2	x					
Demospongiae 3			x		x	
Demospongiae 4			x		x	x
Demospongiae 5			x			
Demospongiae 6			x			
Demospongiae 7			x	x	x	x
Demospongiae 8					x	
Demospongiae 9					x	x
CNIDARIA						
Hydrozoa ind.	x	x				
<i>Madracis pharensis</i> (Heller, 1868)	x	x		x		
<i>Hoplangia durotrix</i> (Gosse, 1860)	x	x		x		
<i>Leptopsammia pruvoti</i> (Lacaze–Duthiers, 1897)	x	x		x		
Scleractinia ind.	x					
ANNELIDA						
Sabellidae ind. spp.			x			
<i>Placostegus crystallinus</i> (Zibrowius, 1968)					x	
<i>Protula tubularia</i> (Montagu, 1803)	x	x	x	x	x	
<i>Vermiliopsis infundibulum</i> (Philippi, 1844)	x					
<i>Vermiliopsis labiata</i> (O. G. Costa, 1861)			x	x		

Table 5. Cont.

	ES-Wall	ES-Ceiling	IS-Wall	IS-Ceiling	BS-Wall	BS-Ceiling
<i>Spiraserpula massiliensis</i> (Zibrowius, 1968)			x		x	x
Serpulinae gen. sp1			x		x	
Serpulinae gen. spp. *	x	x	x	x	x	x
Serpulinae gen. spp. **	x		x	x	x	x
Spirorbinae gen. spp.	x	x	x	x	x	x
MOLLUSCA						
<i>Rocellaria dubia</i> (Pennant, 1777)	x					
BRACHIOPODA						
<i>Novocrania anomala</i> (O. F. Müller, 1776)					x	x
Articulata gen. sp.					x	x
BRYOZOA						
Bryozoa ind. ***			x			
Bryozoa ind. ****			x	x	x	
Ascophorina gen. spp.	x	x				
CHORDATA						
<i>Halocynthia papillosa</i> (Linnaeus, 1767)	x					

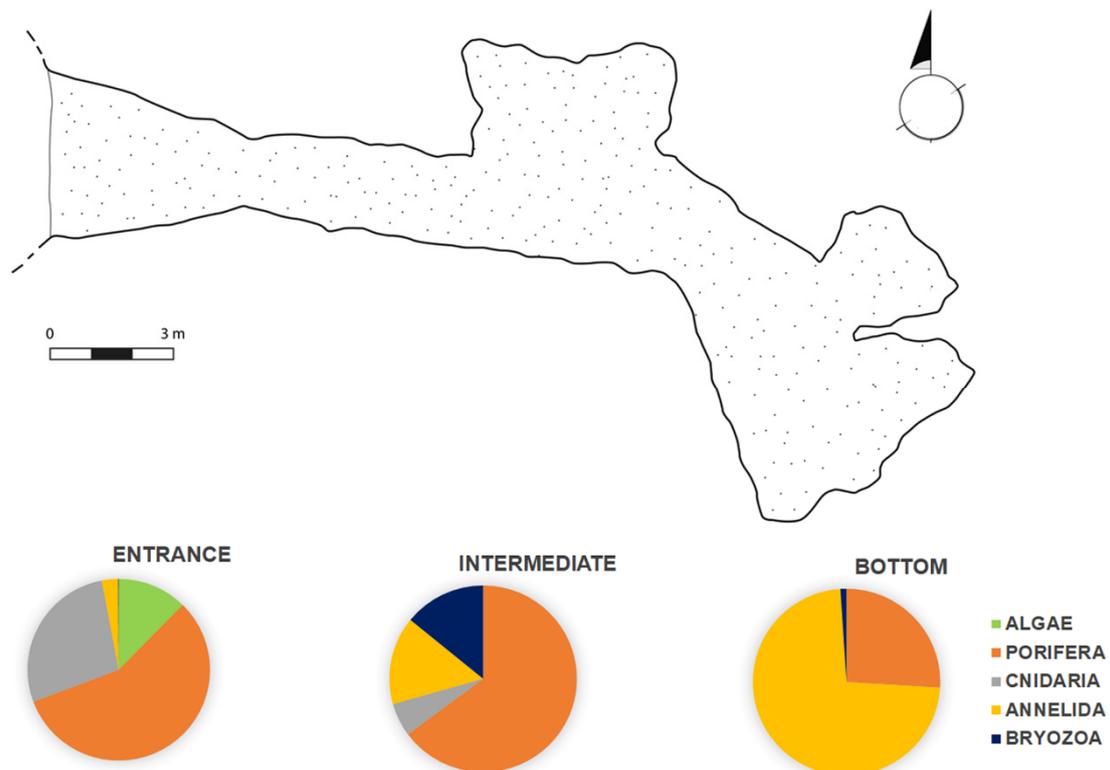


Figure 4. Percent cover of the benthic organisms in the three sectors of the Elle cave: Entrance, intermediate, and bottom sector.

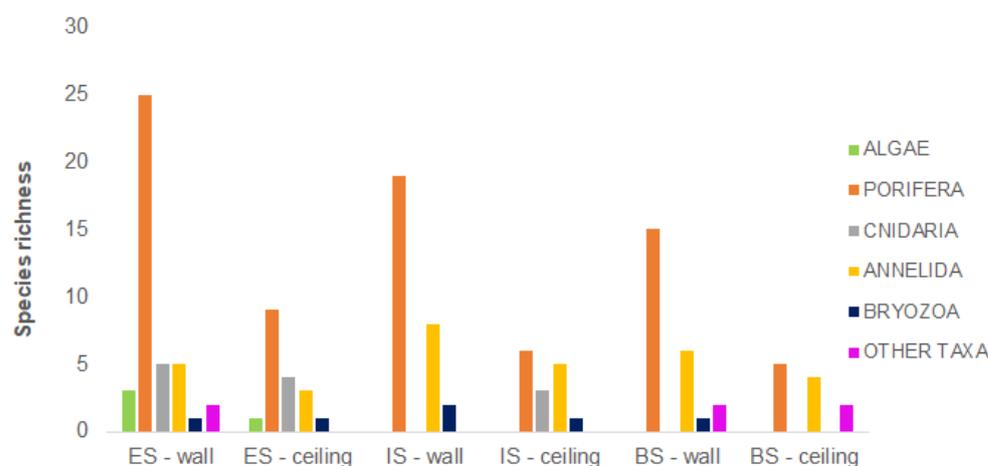


Figure 5. Species richness of the benthic organisms on the wall and ceiling of the three sectors of the Elle cave: ES: Entrance; IS: Intermediate sector; BS: Bottom sector.

Cnidaria counted 5 taxa belonging to the classes Hydrozoa and Anthozoa (Table 5). Hydroids were abundant mainly in the ES, penetrating up to the initial portion of the IS. Anthozoa, with 4 taxa, was represented exclusively by the order Scleractinia. All of the four species detected were distributed primarily in the ES, penetrating only up to the initial portion of the IS. *Leptopsammia pruvoti* coupled with *Hoplanguia durotrix* were more abundant near the entrance, while *Madracis pharensis* created true facies both on the wall and ceiling of the innermost section of the ES (Figure 6b).

Polychaetes included 10 taxa: In addition to the sabellids found only in the intermediate sector, all of the other worms were serpulids, six of which were identified at the species level, while most of them were distinguished by size grouping the smallest, comprehensive of spirorbinae, and the largest of 10 mm (Table 5). Two species were dominant: *Protula tubularia* had the highest frequency as it was present in most samples albeit with few individuals (Figure 6a,c); *Spiraserpula massiliensis* was the most abundant species forming the typical tube assemblages, due to its gregarious behavior, in the bottom sector of the cave (Figure 6e). Indeterminate serpulids especially occurred in the bottom sector and exhibited remarkably long tubes, with a peculiar parallel alignment along the same definite direction (Figure 6e). Particularly noteworthy in the dark sectors of the cave is the record of a few individuals of a species still under description, which has been identified as Serpulinae sp. 1. Moreover, few scattered individuals of *Vermiliopsis infundibulum* and *V. labiata* have been detected, the first at the entrance of the cave and the latter in the intermediate sector. *Placostegus crystallinus* was exclusively found with a few individuals in one sample in the innermost sector. Very numerous individuals of serpulids smaller than 10 mm, including spirorbinae, have been observed particularly in the intermediate and innermost sectors of the cave, both on the walls and on the ceiling (Figure 6d,f). Conversely, they were found in fewer numbers in the samples at the entrance. Some bivalves of the boring species *Rocellaria dubia* and of the sciaphilic ascidian *Halocynthia papillosa* have been observed in few samples at the entrance. In addition, many individuals of Articulata (not identified) brachiopods together with some individuals of *Novocrania anomala* were found exclusively in the innermost area of the cave (Figure 6f).

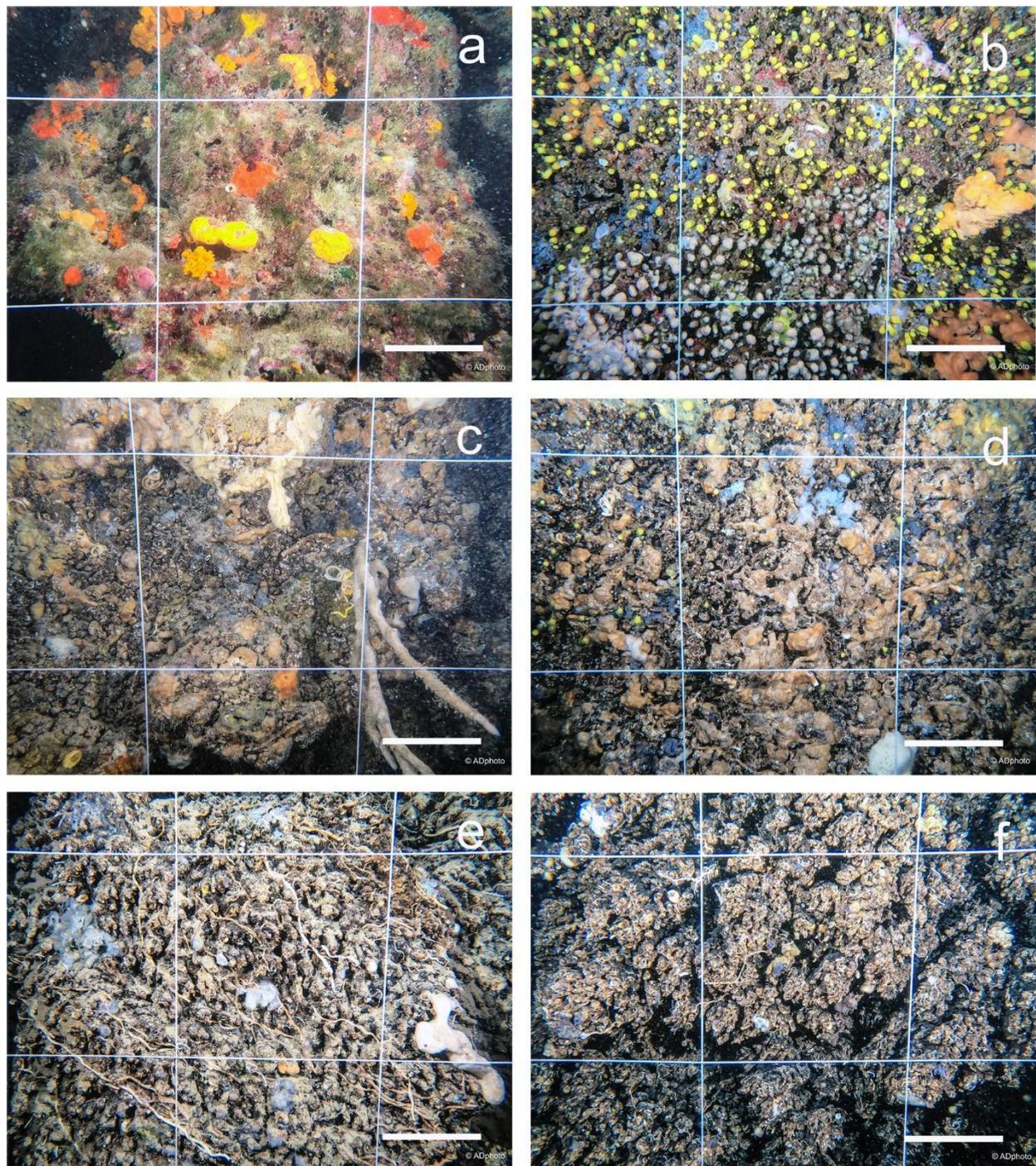


Figure 6. Details of the wall and ceiling of the three sectors of the Elle cave: (a) Entrance wall covered by algae mixed with coralligenous sponge species, i.e., *Spirastrella cuntatrix*, *Agelas oroides*, *Petrosia ficiformis*, and *Achantella acuta*; (b) entrance ceiling covered by scleractinians *Madracis pharensis*, *Leptopsammia pruvoti*, and *Hoplanguia durotrix*; (c) wall of the intermediate sector with specimen of cf. *Pseudocorticium jarrei* (on the top) associated with other encrusting and erect Demospongiae and coiled doughnuts of tubes of *Protula tubularia*; (d) ceiling of the intermediate sector with sparse specimens of *L. pruvoti*, numerous tubes of small serpulids, the coiled doughnuts of *P. tubularia*, and the Calcareo sponge *Petrobiona massiliana* (at the bottom of the image); (e) wall of the bottom sector with few encrusting sponges and numerous long serpulid tubes aligned parallel to the water flow; (f) ceiling of the bottom sector with numerous specimens of brachiopods *Novocrania anomala* and not identified Articulata.

4. Discussion

4.1. Proposal of Speleogenetic Processes of the Cave

The data collected through both direct survey and photo-video documentation regarding the Elle cave at Cala Sorrentino allowed us to describe the geomorphology of the cave itself and to propose some hypotheses on the characteristics of this karst phenomenon and on the formative processes that may have interested the cavity. A particularly relevant aspect of the cave is the outflow of water observed at the bottom of the cavity. Although our preliminary data do not allow for evidence of freshwater presence, they do suggest that Elle cave could be a further example of submarine springs (vruljas) that are common all along the Adriatic coasts [15]. This finding would exclude the possibility that Elle cave originated exclusively by the erosive action of the sea. Therefore, it would not be an isolated or marginal marine cavity, as an example of the complex landscape of the karst coastal environment [29]. In addition, the morphology and length of the cavity, approximately 25 m of linear development, also suggest the presence of a phreatic duct. If the outgoing freshwater activity will be confirmed by further analyses, it would classify the Elle cave as an active karst resurgence, which is likely connected with underground developments currently not practicable by humans due to the small size of the terminal bottleneck.

A further relevant morphological feature is the presence of concretions especially in the innermost sector of the cave. They are in a state of conservation, which is not clearly detectable by the analysis of images, and thus, hinders the identification of their composition and origin. The presence of speleothems in a karst environment currently completely invaded by seawater suggests that the concretions have formed at a time when the cavity had not yet flooded. In other words, the speleogenetic phenomenon lasted for a period during which the cavity was not invaded by seawater flow or karst: This period could be traced back to a completely fossil phase of development, which favored the formation of concretion on the ceiling. Consequently, due to the presence of concretions, the possibility of a mixed situation with partial flooding of freshwater should also be excluded. In fact, in the case of cavities strongly affected by water flow, the development of concretions would be prevented. On the other hand, the formation of a fossil duct with an almost circular section (similar to the Elle cave) unequivocally originates during a phase characterized by the erosive activity of fresh groundwater; this cavity would only subsequently be subjected to a considerable reduction in flow and exposed to a fossil-type regime. In this regard, the formation phase of a phreatic duct is an aspect that deserves attention since it dates back to the period of activity that precedes the fossil phase (at least in the early stage of the caves' origin, the possibility of a mixing corrosion phase is excluded, as assumed by Bayari [30]). Therefore, two hypotheses can be advanced for the formation of the phreatic duct: (a) It would have taken place during the phase of marine regression, (b) it would have occurred in the presence of seawater, in a submerged condition. Despite the difficulty in defining the situation of the water regime at the time of its formation with certainty, evidence of this study supports the hypothesis of the formation phase of the cave in the absence of seawater, during the sea level regression phase. At the present state of knowledge, it is not even possible to establish how much time elapsed between the phreatic formative phase of the cave in a flooded condition and its transformation into a fossil cavity, in which the concretions phenomenon began. In the Elle cave, the presence of concretions traces the chronological *terminus*, after which the phenomenon of concretion began, to a time in which the sea was not present. Based on the depth of the current entrance, at 18 m below sea level, the fossil period of the cavity would be dated back to the time when the sea had regressed to the extent that the cavity was not flooded. As a direct consequence, the current situation as a submerged cave should have arisen following a phase of reactivation of the groundwater flow conjointly with the rise of the sea level. Finally, it is reasonable to explain the large size of the entrance, compared with the duct, in accordance with the well-defined model by Maylroie and Carew [31], which highlights that the erosive phenomena are enhanced along the contact area between fresh and saltwater.

Our preliminary data therefore allow us to hypothesize that at least three phases can be defined for the genesis of the Elle cave at Cala Sorrentino: The first formative phase of the phreatic type, which was followed by the second fossil phase during which the concretions were formed; both of these phases most likely occurred during the marine regression period; finally, the sea level rise in the third phase determined the current condition of the submerged cave.

However, the presence of biostalactites has been reported in Mediterranean submarine caves, as bioconstructions formed in submerged conditions by the action of different organisms [20,32–36]. Investigation of their origin is relevant in the study of underwater caves, but unfortunately destructive sampling is required. Given the preliminary nature of this study, we proposed the speleogenetic hypothesis of the Elle cave based on our field experimentations, observations, and measurements. In-depth studies on the nature of the concretions observed could shed light on new aspects regarding the origin of the cave.

4.2. Biodiversity Pattern

The epibenthic assemblage pattern found in the Elle cave clearly reflected the sharp environmental gradient. Indeed, the hydrological parameters measured in the different sectors of the cave highlighted remarkable variations from the entrance to the inner dark zone, which mainly consisted of the disappearance of the light and the reduction of water movement. In particular, the light intensity dropped sharply within a short distance, probably due to the dimension of the entrance, and was totally absent in the interior, thus its reduction strongly affected the benthic zonation in the cave. In addition, the variations in the other hydrological parameters showed lower values of dissolved oxygen, pH, water movement, and higher values of temperature in the internal zone, compared with the external zone. This environmental zonation pattern is consistent with the presence of the submarine spring inside the cave, which feeds the outflow of water observed from the cave. The environmental changes recorded in the Elle cave fall within the variability of the general pattern described for the Mediterranean caves, particularly for tunnel shaped caves [2,8,12,37–39]. In accordance with these environmental trends, the epibenthic community revealed a remarkable zonation, showing marked changes in its composition and structure along the outer-inner gradient. As a consequence, the aspect of the semi-dark cave biocoenosis was limited to the entrance area and was clearly recognizable in the facies with scleractinians, i.e., *Madracis pharensis*, *Leptopsammia pruvoti*, *Hoplangia durotrix* [40], which was found only on the ceiling and part of the right wall in this sector. Regarding this small-scale heterogeneity between the even neighboring benthic assemblages, it is reasonably attributable to the morphology of the cave itself, which in turn strongly affected the environmental and biological gradients of cave peculiar morphology. This agrees with the heterogeneity pattern, which has been attributed to the cave specific topography in other Mediterranean caves [2]. On the other hand, the extensive cover of scleractinians on the ceiling of the entrance cave was explained by the higher water movement present in this sector that provides food, such as plankton, to these passive suspension feeders [41]. Consistently with this, the assemblage of the entrance sector can be framed in the confinement zone III of the zonation model by [42], where along with cnidarians, bryozoans with erect colonies, ascidians, and bivalves are still found. Within a short distance of the entrance, the richness and diversity of the community are severely depleted, due to the disappearance of algae , as well as erect colonies of bryozoans and hydroids, and species characteristic of the coralligenous, as the sciaphilic ascidian *H. papillosa* and the bivalve *R. dubia*, which is a typical component of the boring fauna and found in some other submarine caves of the Salento peninsula [1,19].

From that time onwards, the community was dominated by the poriferans that constituted the predominant element of the benthic assemblages. Sponges were widespread in all three sectors of the cave, with species richness and covering higher in ES to IS and lower in BS of the cave, similarly to what has been observed in other Mediterranean caves [43–46]. The increase of sponge coverage from the outer zone to the middle zone is related to the

sharp light depletion and the consequent disappearance of macroalgae competing in space for the benefit of sciaphilous species, including sponges [47]. The almost total absence of competition for space would explain the high coverage values recorded in the IS. This is primarily related to the dominance of encrusting species that tend to colonize large portions of substrate, such as the *Spirastrella cunctatrix* and *Dendroxea* cf. *lenis* dominant species in this sector. On the contrary, a decrease in sponge coverage is evident on the ceiling of the ES and IS, possibly related to the presence of the *facies* of scleractinians, which prevented colonization of this portion of substrate by the sponges. The decrease in species richness and sponge abundance found in the innermost sector of the cave is attributable to the progressive reduction in the trophic resources available to the filter-feeding community, caused by the reduced water movement [45]. However, other studies [6,8,48] showed an inverse trend in benthic community diversity along the horizontal axis of caves and suggested the influence of other cave-specific factors, such as topographic features, in shaping patterns of cave biodiversity [49]. A different pattern in species distribution was observed along the axis of the Elle cave in the three sectors ES, IS, BS, where more than half of the sponge species were concentrated in the outer portion of the cavity. This pool of species is mainly represented by *S. cunctatrix*, *Agelas oroides*, *Petrosia ficiformis*, *Chondrosia reniformis*, *Axinella damicornis*, *Acanthella acuta*, and *Phorbas tanacior* and is typically related to the coralligenous or other sciaphilous communities. In the IS and BS, the sponge assemblage of the walls and ceiling was replaced by typically dark cave species, such as the *Calcarea* sponge *Petrobiona massiliana* [50] and the *Homoscleromorpha* cf. *Pseudocorticium jarrei*. The transition from semi-dark to dark cave communities reflects taxonomic selection based on specific adaptations to the increasingly severe environmental requirement, such as scarcity of trophic resources [13], that hexactinellida, lithistids, and carnivorous sponges exhibit [51–55]. Sponge community composition showed high heterogeneity both at the regional and local scale [6,56]. The sponge taxocoenosis of Elle cave consists of elements that are common to most caves in the Mediterranean basin except for a pool of species concentrated in the BS, whose determination requires further taxonomic investigations. Indeed, few elements of uniqueness were detected, in comparison with the spongofauna of neighboring caves of the Tremiti Archipelago [43–45] and other Mediterranean caves [56–58]. However, it must be considered that photographic approaches often do not allow for a complete census of the community, since they prevent the identification of cryptobiotic species [56].

The described zonation pattern was confirmed by the serpulid population, which was consistent with three different assemblages. Briefly, the first zone was characterized by sparse and isolated tubes, mainly of the species *P. tubularia*; here serpulids resulted as occasional components, when compared with other benthic organisms. In the second zone, intermediate sector, progressively larger aggregations of small sized tubes colonized the substratum; here the species *P. tubularia* often had the typical coiled “doughnuts” of tubes. This coiled form was described from a cave in the Aegean Sea [33] and then reported for the cave dark sector [2], but it deserves further investigation. Moreover, *V. labiata*, a sciaphilic species commonly found in bio-concretions, characterized this sector similarly to other caves along the Salento peninsula [1] and the Aegean Sea [33]. Finally, a third zone characterized the innermost dark sector, where dense aggregates of tubes distinguished the species *S. massiliensis* as a typical component of the dark cave habitat [10]. This species is shared with the faunal assemblages of other submarine caves in the Southern Adriatic Sea, Ionian Sea, and Aegean Sea [1,33,36]. In this sector, serpulids were particularly abundant and partially replaced sponges in the extent of cover. It is noteworthy that the dark zone of Elle cave did not exhibit a marked decrease in biotic cover, contrary to what typically occurs in the habitat of dark caves [13,59]. In fact, here the rock appeared only partially coated by black oxide crusts, which are typically found on the rocky walls of the dark caves. In the Elle cave, the black coating of ferromanganese oxides only partially covered the nodular formations, which was confined in the crevices and interstices between the nodules themselves, whilst most of the exposed surface was extensively colonized by

serpulids. The observed colonization pattern agrees with the recorded water movement that provides suspended material as food to support an extensive cover of the active suspension-feeding serpulids. Concerning serpulids, it is worth noting the record of few specimens attributable to *Protula tubularia* for the morphology and size of their tube, which are devoid of ornamentation. Notwithstanding, the observed specimens differed from *P. tubularia* for the other characteristics, while matched with individuals, found in the dark sector of other submerged caves in the Southern Adriatic Sea, Ionian Sea, Aegean Sea, and Eastern Mediterranean Sea, which are still to be described [32,34–36].

Finally, the biological evidence of the present study supports the speleogenetic hypothesis proposed for the cave. Indeed, the benthic coverage, which was total at the entrance, remained close to 100% even in the innermost sector, thus indicating a significant degree of water circulation in the cave [13]. This fact is also confirmed by the arrangement of some faunal components, such as the serpulids. Indeed, in the innermost sector of the cave, most of their largest tubes were particularly long and parallel along a definite alignment in the same direction, as an indication of the existence of a water flow from the bottom towards the entrance of the cave.

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