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## Comparison between the sponge fauna living outside and inside the coralligenous bioconstruction: A quantitative approach

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### Abstract

Coralligenous habitat results from a multi-stratified accumulation of crustose coralline algae and animal builders in a dynamic equilibrium with disruptive agents. The result is a complex architecture crossed by crevices and holes. Due to this three-dimensional structure, the coralligenous habitat may host a rich and diversified fauna, more abundant than in any other Mediterranean habitat. Unfortunately, very few data are available about the cryptic fauna that lives inside the conglomerate. As already reported for coral reefs, the cryptic fauna plays an important role in the exchange of material and energy between water column and benthic assemblages. In this study, we compare the sponge community present inside and outside the coralligenous framework of the Portofino Promontory (Ligurian Sea) at different depths (15 and 30 meters) not only in terms of taxonomic diversity but, for the first time, also in terms of biomass. Sponges present on the surface of each block were collected, weighed and identified; after the dissolution of blocks in HCl, target cryptic sponges were separated from other organisms, weighed, and identified.

We recorded a total of 62 sponge species. The average number of sponge taxa occurring outside the coralligenous accretions is lower than the number of taxa identified inside. This pattern is confirmed also regarding sponge biomass. These results underline that studies focused on coralligenous functioning should take into account the important contribution of cryptic fauna, as recently evidenced also for tropical reef habitats.

**Keywords:** Porifera, bioconstruction, bioerosion, Ligurian Sea.

### Introduction

The coralligenous habitat is the most important biogenic structure in the Mediterranean Sea in terms of biodiversity, ecological and economic values (Boudourisque, 2004; Ballesteros, 2006; Bertolino *et al.*, 2013).

Coralligenous frameworks result from a multi-stratified accretion due to the accumulation of crustose coralline algae (CCA) and animal builders; these structures are in dynamic equilibrium due to the simultaneous actions of active builders and disruptive agents (Cerrano *et al.*, 2001). The aspect of this bioconstruction results in a spatially complex structure, characterized by holes and cavities supporting different microhabitats (Pica *et al.*, 2014). Consequently, a rich and diversified fauna, both sessile and vagile, excavating, and buried in the soft sediments accumulated inside holes, is hosted in coralligenous accretions (Ballesteros, 2006). As a consequence of this peculiar heterogeneity, the coralligenous habitat harbours a biodiversity higher than any other Mediterranean habitat. Regarding the organisms associated to this habitat, Hong (1980, 1982) defined four categories of invertebrates according to their ecological

role: 1) the fauna helping to develop and strengthen the concretions created by calcareous algae, which contribute to 24% of the total number of species; 2) the crypto fauna, about 7% of the species; 3) the epifauna and endofauna, representing a great number of the species (nearly 67%) and 4) the bioeroders accounting for roughly only 1%.

However, the number of species of the coralligenous has not yet been estimated precisely, because of several factors, such as an extremely rich fauna (Laubier, 1966), the habitat complexity (Pérès & Picard, 1964; Ros *et al.*, 1985), its depth, and the scarcity of studies dealing with the diversity of coralligenous species. The estimates of this biodiversity are far from being updated and exhaustive (Ballesteros, 2006; Bertolino *et al.*, 2013a). Ballesteros (2006) made a census of 1241 invertebrates in the coralligenous habitat, and these data, as underlined by the author, are conservative. Considering also cryptic sponges (Bertolino *et al.*, 2013), the number of species associated with the coralligenous concretions increased to 302 with respect to 142 listed in Ballesteros (2006).

To date studies on the coralligenous biodiversity have usually taken into account the visible epibenthic

layer (Deter *et al.*, 2012; Gatti *et al.*, 2012); only few data are available on the cryptic endolithic fauna of the coralligenous habitat. In contrast, in coral reefs, it is well documented that the cryptic community represents a net sink of DOC and plays an important role in the energy budget of coral reefs (de Goeij & van Duyl, 2007), acting also as a reserve pool and refuge for deep organisms or competitors (Meesters *et al.*, 1991).

The hypothesis of this paper is to test whether, in terms of diversity and biomass, the cryptic sponge community, occurring inside the coralligenous accretions of the Portofino Promontory (Ligurian Sea, Italy) at two depths, is different compared to the outside counterpart.

## Material and Methods

### Sample treatment

Coralligenous samples were collected at Punta del Faro (44°17'55,43"N, 9°13'9,18"E), Portofino Promontory, Eastern Ligurian Sea (Fig. 1) at 15 and 30 m depth. The north-eastern side of the Promontory is characterized by turbid waters (Cerrano *et al.*, 2005), justifying a shallow upper limit for the coralligenous accretions that start to develop at 15 m depth.

For each considered depth, 10 coralligenous blocks, having a volume of about 150-200 ml, were removed by hammer and chisel and immediately fixed in 4% formalin. In the lab, for each block (n=20) the volume was evaluated by water displacement.

The surface of each block was observed under a stereomicroscope. Sponges present therein were photographed, removed with tweezers and weighed (with a precision balance, Radwag Wagi Elektroniczne AS 220/X). The weight of each sample was measured as wet weight after 1 minute of draining. Finally, sponges were preserved in 70 % alcohol for subsequent taxonomic identification.

The blocks, after the collection of the external sponges, were treated separately with diluted hydrochloric acid (HCl 5 %) to retrieve the cryptic sponges present inside the cavities of the coralligenous blocks (Brock & Brock, 1977). The HCl 5 % dissolves the carbonate components of the block, preserving tissues of siliceous sponges and other soft-bodied organisms. Once the blocks had completely dissolved, samples were recovered by filtration through a filter having a 1 mm mesh, gently rinsed in water and then weighed. Target cryptic sponges were separated from other organisms eventually present under a stereomicroscope and weighed (wet weight).

All the blocks (n=20) were used to evaluate the wet weight of the sponges (g) present on the block surface (out) and inside the coralligenous blocks (in). For data analysis the sponge wet weight and the number of the inner (in) and outer (out) sponges were standardized to 1 l of block volume. The cryptic sponges obtained after the dissolution of the blocks were weighed and stored in alcohol (70 %) for taxonomic identification according to Rützler (1978). For

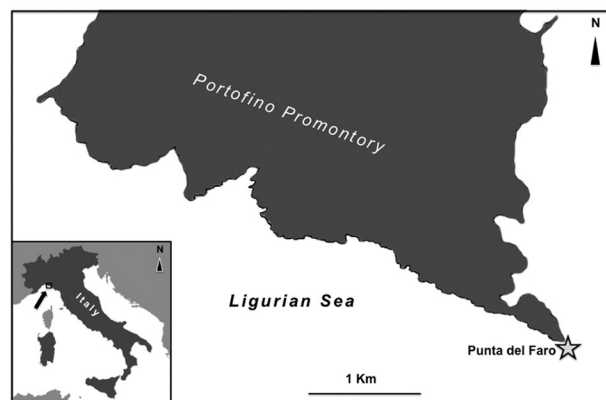


Fig. 1: Map showing the location of the sampling.

this purpose, only 5 blocks per depth were used (n=10).

The acid dissolution technique has the advantage of being quantitative (Brock & Brock, 1977), but since sponges have soft and delicate mass, it is difficult to retain separate sponge tissue and distinguish a single specimen from another (Schönberg, 2001). For this reason, the wet weight of the cryptic sponge species was evaluated as cumulative, and it was not possible to calculate the number of specimens of the cryptic sponge assemblage.

### Data analysis

Two-way analysis of variance (ANOVA) was used to assess differences in sponge biomass and the number of sponge taxa in relation to depth (15 vs 30 m) and habitat in the coralligenous block (out vs in). Prior to running analyses, the homogeneity of variances was tested by Levene's test; whenever necessary, data were  $\log(x+1)$  transformed and re-tested (Underwood, 1997). When transformation did not produce homogeneous variances, we set  $\alpha=0.01$  in order to make up for the increased likelihood of type 1 error (Underwood, 1981).

Sponge assemblage at the two depths along with the occupied habitat were analysed as presence/absence data to minimize the weight of the dominant species and to balance the role of rare species and because the species of sponges living in the coralligenous block after its dissolution were often fragmented into several pieces so that the number of individuals could be overestimated. Sorensen's similarity measure was calculated and data were analysed with ANOSIM and non-metric Multidimensional Scaling (nMDS).

## Results

Our samples showed that the sponges growing on the coralligenous surface were thin and small (about 1-2 cm<sup>2</sup>) with very few species reaching a bigger size (i.e., *Corticium candelabrum* about 20 cm<sup>2</sup>). Cryptic sponges found inside the coralligenous blocks appeared to be coarsely chopped after the dissolution process, so it was very difficult to evaluate the size and shape of each single specimen.

In total, 62 species were recorded in the studied sam-

**Table 1.** Identified taxa according to their depth and presence on the surface (out) and inside the coralligenous blocks (in).

Taxa	15 m		30 m	
	out	in	out	in
<i>Corticium candelabrum</i> Schmidt, 1862	X			
<i>Plakinastrella</i> cf. <i>copiosa</i> Schulze, 1880			X	
<i>Plakortis simplex</i> Schulze, 1880		X		
<i>Samus anonymus</i> Gray, 1867			X	
<i>Stelletta lactea</i> Carter, 1871		X		
<i>Stelletta</i> cf. <i>stellata</i> Topsent, 1893			X	
<i>Jaspis incrustans</i> (Topsent, 1890)		X	X	
<i>Jaspis johnstonii</i> (Schmidt, 1862)		X	X	
<i>Penares euastrium</i> (Schmidt, 1868)			X	
<i>Alectona</i> sp.		X		
<i>Geodia conchilega</i> Schmidt, 1862		X		
<i>Dercitus</i> ( <i>Stoebea</i> ) <i>plicatus</i> (Schmidt, 1868)	X	X	X	
<i>Triptolemma simplex</i> (Sarà, 1959)			X	
<i>Cliona burtoni</i> Topsent, 1932			X	
<i>Cliona janitrix</i> Topsent, 1932			X	
<i>Cliona viridis</i> Schmidt, 1862			X	
<i>Dotona</i> sp.			X	
<i>Pione vastifica</i> (Hancock, 1849)		X	X	
<i>Spiroxya sarai</i> (Melone, 1965)			X	
<i>Diplastrella bistellata</i> (Schmidt, 1862)		X	X	
Suberitidae			X	
<i>Aaptos aaptos</i> (Schmidt, 1864)		X		
<i>Prosuberites longispinus</i> Topsent, 1893			X	X
<i>Timea stellata</i> (Bowerbank, 1866)			X	
<i>Timea</i> cf. <i>stellata</i> (Bowerbank, 1866)		X		
<i>Timea stellifasciata</i> Sarà & Siribelli, 1960		X		
<i>Timea</i> sp.			X	
Lithistida			X	
<i>Clathria</i> ( <i>Clathria</i> ) <i>toxivaria</i> (Sarà, 1959)			X	
<i>Eurypon cinctum</i> Sarà, 1960		X		
<i>Eurypon clavatum</i> (Bowerbank, 1866)			X	
<i>Eurypon</i> cf. <i>major</i> Sarà & Siribelli, 1960			X	X
<i>Eurypon viride</i> (Topsent, 1889)		X	X	
<i>Eurypon</i> sp.			X	X
<i>Rhabdermia gallica</i> Van Soest & Hooper, 1993		X	X	
<i>Rhabdermia</i> cf. <i>topsenti</i> Van Soest & Hooper, 1993		X	X	
<i>Forcepia</i> ( <i>Leptolabis</i> ) cf. <i>luciensis</i> (Topsent, 1888)		X		
<i>Forcepia</i> ( <i>Leptolabis</i> ) sp.			X	
<i>Crambe crambe</i> (Schmidt, 1862)	X	X		
<i>Crella</i> ( <i>Grayella</i> ) <i>pulvinar</i> (Schmidt, 1868)			X	X
<i>Hymedesmia</i> ( <i>Hymedesmia</i> ) <i>rissoi</i> Topsent, 1936		X	X	
<i>Hymedesmia</i> ( <i>Stylopus</i> ) <i>coriacea</i> (Friedt, 1885)			X	X
<i>Phorbas fibulatus</i> (Topsent, 1893)			X	
<i>Phorbas tenacior</i> (Topsent, 1925)	X	X		
<i>Hymerhabdia typica</i> Topsent, 1892		X		
<i>Acanthella acuta</i> Schmidt, 1862			X	X
<i>Halichondria</i> ( <i>Halichondria</i> ) <i>genitrix</i> (Schmidt, 1870)	X	X	X	X

(continued)

**Table 1** (continued)

Taxa	15 m		30 m	
	out	in	out	in
<i>Halichondria</i> ( <i>Halichondria</i> ) <i>panicea</i> (Pallas, 1766)				X
<i>Topsentia glabra</i> (Topsent, 1898)				X
<i>Haliclona</i> ( <i>Halichoclona</i> ) <i>fulva</i> (Topsent, 1893)	X	X		
<i>Haliclona</i> ( <i>Gellius</i> ) sp.				X
<i>Haliclona</i> ( <i>Soestella</i> ) <i>mucosa</i> (Griessinger, 1971)			X	
<i>Haliclona</i> sp.1			X	
<i>Haliclona</i> sp.2				X
<i>Haliclona</i> sp.3	X			
<i>Petrosia</i> ( <i>Petrosia</i> ) <i>ficiformis</i> (Poiret, 1789)			X	
Homoscleromorpha			X	
Dictyoceratida			X	X
Spongiidae	X		X	
<i>Dysidea</i> sp.	X			
<i>Pleraplysilla spinifera</i> (Schulze, 1878)			X	
Calcarea	X			X

ples (Table 1); 28 species from 15 m depth samples and 46 species from 30 m depth samples (Table 1).

Considering the sponge habitus at 15 m depth, we recorded 10 encrusting and 23 cryptic species of which the latter were living in coralligenous crevices. At 30 m depth, 17 species were recorded on the block surface (encrusting) and 38 in the internal part (cryptic) (Table 1). The average number of sponge taxa (standardized to 1 l of substrate volume) present on the block surface (out) was lower than the number of taxa identified inside the blocks (in) (Fig. 2), for both of the considered depths. In fact, at 15 m depth, the number of sponges outside and inside the blocks was  $17.7 \pm 16.8$  vs  $129.1 \pm 89.4$  (mean  $\pm$  SD), respectively; while at 30 m depth, the number of sponge taxa identified outside (out) and inside (in) the blocks was  $31.9 \pm 32.9$  vs  $86.7 \pm 58$  (mean  $\pm$  SD), respectively (Fig. 2). In total, 37 species were exclusively present inside the blocks (Table 1).

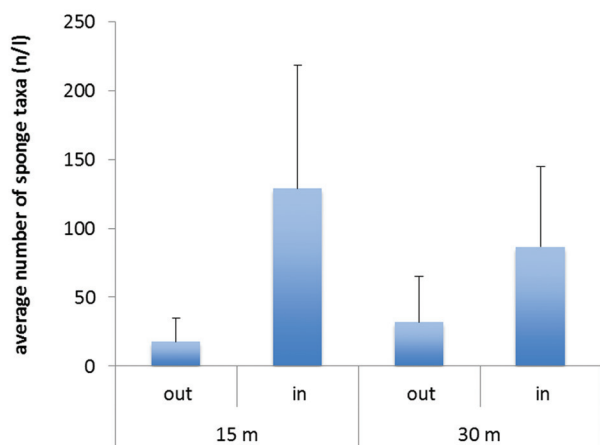
ANOVA shows that the sponge habitat (in/out) had a significant effect on the species number ( $F = 12.6$ ,  $p = 0.002672$ ), while there was no significant effect due to depth.

ANOSIM evidences that the composition of sponge assemblage differed significantly considering the depth (Global R: 0.47,  $p = 0.01\%$ ) and the occupied habitat (in/out) (Global R: 0.349,  $p = 0.3\%$ ).

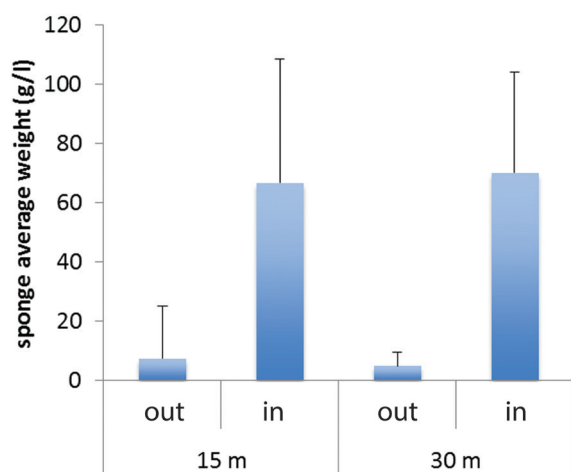
These results are clear in the nMDS ordinations (Fig. 3) where a pattern of clustering is quite apparent, especially according to the habitat occupied (in/out), with more similarity for assemblages inside the blocks.

The comparison between the sponge biomass present inside the coralligenous blocks (in) with that of the outer one (out) evidences the highest values of the sponge biomass inside the coralligenous blocks. In fact, the sponge





**Fig. 2:** Average number of sponge taxa ( $\pm$ SD) identified inside (in) and outside the coralligenous blocks (out) at the two considered depths.



**Fig. 4:** Average weight of the sponges ( $\pm$ SD) present inside (in) and outside (out) the coralligenous blocks at the two considered depths.

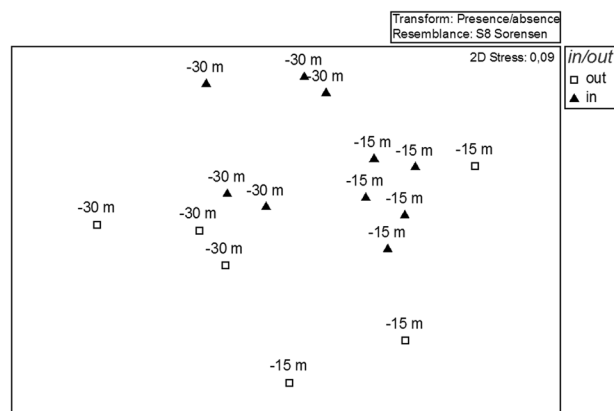
weight was 66.8 g ( $\pm$ 41.7) vs 7.5 g ( $\pm$ 17.6) (mean  $\pm$  SD), respectively inside and outside the blocks at 15 m depth, and 70.1 g ( $\pm$  34) vs 4.8 g ( $\pm$ 4.8) (mean  $\pm$  SD), respectively inside and outside the blocks at 30 m depth (Fig. 4).

Two-way analysis of variance (ANOVA) shows that the sponge habitat (in/out) had a significant effect on sponge biomass ( $F=96.22$   $p = 1.04E-11$ ) and, also in this case, no significant effect was due to the depth.

## Discussion

All around the world, the most diversified marine habitats are related to biobuilders, such as hard corals in tropical areas and crustose coralline algae in temperate waters.

The high biodiversity hosted by bioconstructions is likely related to the three-dimensional structure of the substratum (rich in holes and microhabitats) facilitating the coexistence of several species with different ecological requirements and offering an ideal habitat to escape from predation and from substrate competition. Moreover,



**Fig. 3:** Nonmetric Multidimensional Scaling Ordination plots showing pattern of Sorensen's similarities of the assemblages evaluated at 15 and 30 m and inside and outside the coralligenous blocks, derived from presence/absence data.

in the coralligenous habitat, the presence of pockets of soft-sediments allows the contemporary presence of both hard- and soft-bottom species (Ballesteros, 2006) and the peculiar structure leads to the entrapment of sediments that can represent a sort of black box, telling the story of the ancient assemblages (Bertolino *et al.*, 2014).

Scheffers *et al.* (2010) demonstrated that in Curaçao reefs, cryptic suspension feeders, especially sponges, covered more than 60% of the inner cavity surface. Coral cavities were shown to be important net sinks of bacterioplankton and net sources of dissolved inorganic nitrogen ( $\text{NO}_3$ ) suggesting that coral reefs should be considered as open systems instead of closed ecosystems (de Goeij *et al.*, 2013; Mueller *et al.*, 2014). At the moment, a good map of the cryptic fauna of the coral reefs is still lacking. Hong (1982) showed that the species of the cryptofauna in the coralligenous framework could represent about 7% of the total amount in terms of animal species. Pica *et al.* (2014) calculated, applying X-ray microtomography techniques, an average micro-porosity of about 40% in the coralligenous habitat and about 25% of area occupied by sponges out of the total surface of crevices. At this scale, it was not possible to analyse the micro-patterns of boring sponges (Calcinai *et al.*, 2003) but siliceous spicules were well evident. Just like the coral reefs, also for coralligenous accretions, it is possible to hypothesise that cryptic fauna may play a crucial functional role. The holes and crevices of this biogenic structure support a complex community dominated by suspension feeders (sponges, hydrozoans, tunicates, bryozoans, serpulids, mollusks), with sponges representing the most abundant taxon (Ballesteros, 2006; Bertolino *et al.*, 2013). Bertolino *et al.* (2013), sectioning big coralligenous blocks into slices identified the presence of 27 exclusively endolithic species giving an idea of the richness of this group inside the coralligenous substrata. Moreover, it was stated that the diversity of coralligenous sponges has remained stable over a millennial span of time, and this extended stability may be related to the en-

vironmental stability of the inner habitat of the conglomerates (Bertolino *et al.*, 2014).

Our results show that the number and biomass of the cryptic sponges, living inside the coralligenous substrata in the Ligurian Sea, are significantly higher than those of the epibenthic layer. These data, even if relative to a single benthic taxon, clearly demonstrate the great importance that the cryptic fauna plays in the coralligenous habitat.

While the number of the species and biomass of cryptic specimens do not change significantly with depth, significant differences were recorded according to species composition: in fact, out of 62 species in total, only 12 species (*Jaspis incrustans*, *Jaspis johnstonii*, *Dercitus* (*Stoebea*) *plicatus*, *Pione vastifica*, *Diplastrella bistellata*, *Eurypon viride*, *Rhabderemia gallica*, *R. cf. topsenti*, *Hymedesmia* (*Hymedesmia*) *rissoi*, *Halichondria* (*Halichondria*) *genitrix* and two unidentified species, one belonging to Spongidae and the other to Calcarea, were present at both depths (Table 1), while 16 species were exclusively present at 15 m depth and 34 species were exclusive of the concretions at 30 m depth.

Species assemblages showed differences according to depth and the habitat occupied (in/out), as shown by ANOSIM and nMDS.

Considering both depths, 37 species were exclusively present inside the blocks. These different assemblages, detected inside and outside the blocks, could be explained considering that sponges, especially those living in the more internal part of the coralligenous habitat, need to cope with particular environmental conditions (reduced hydrodynamism above all), and not all the species can do that. In fact, the majority of the inner species were previously recorded in caves or crevices (e. g., *Samus anonymus*, *Jaspis johnstonii*) or were excavating (*Cliona* spp., *P. vastifica*, *Alectona* sp. etc.).

Our results are related to a small spatial scale, and a wider sample design could lead to different patterns (Andrew & Mapstone, 1987) and ratio between inner and outer sponges as evidenced for example in Bertolino *et al.* (2013). In general, coralligenous assemblages can show a wide homogeneity at a large-scale, resulting in a lack of significant differences between separate locations (Piazzi *et al.*, 2004), while at small scale, a high heterogeneity is always evident, limiting the definition of a standardized monitoring protocol valid at Mediterranean level (Zapata *et al.*, 2013). In a coralligenous habitat, spatial variability is scale dependent just like in other marine habitats (Benedetti-Cecchi, 2001; Underwood & Chapman, 1996), asking for an optimization in further sampling designs.

The same sponge species can be found in very different environmental conditions, with different shapes and habitus, they could be either creeping or encrusting or massive (Sarà *et al.*, 1998). As highlighted for sponges living on octocorals (Calcinai *et al.*, 2013), phenotypic plasticity offers to Porifera an important evolutionary opportunity, mediating the relationship between genotype and phenotype

(Pfenning *et al.*, 2010). It is now generally accepted that adaptive plasticity can allow populations to face environmental change. When specimens of the same species occupy different kinds of habitats, a divergent selection can work on different norms of reaction (Grether, 2005). The cryptic habitat exploited by sponges here clearly offers this opportunity, creating a more stable environment where sponges are under different selective pressure compared to the conspecific living outside the coralligenous habitat.

As typically supported by any ecosystem engineer, the creation of stable 3D structures lead to a buffer effect towards micro-environmental conditions (Cerrano *et al.*, 2005, 2010; Notari *et al.*, 2015), enhancing and supporting local biodiversity, as likely performed by CCA. The high habitat complexity and heterogeneity of coralligenous habitat is slowing down the development of a common and shared methodological monitoring approach, asking for new technologies to be applied (Zapata *et al.*, 2013). The incredible richness of cryptic habitats should be considered when coralligenous diversity is evaluated, especially in the planning and the activation of management and conservation measures. Destructive methods should be avoided, but a relationship between epibenthic assemblages' characteristics and the richness of the cryptic component needs to be urgently defined.

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