Mediterranean Red Macro Algae Mats as Habitat for High Abundances of Serpulid Polychaetes

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Abstract: The Mediterranean Sea harbors more than 17,000 eukaryotic marine species, with several ecosystems recognized as biodiversity hotspots, such as *Posidonia oceanica* meadows. Recent research indicates that benthic mats formed by the fleshy red alga *Phyllophora crispa* are also associated with high species richness. Among key groups found in these mats are sessile polychaetes, which live as epiphytes on the red algae thalli. Knowledge of abundance, species richness, and spatial variation of polychaetes associated with these habitats is still scarce. We carried out a comparative assessment focusing on serpulid polychaetes within samples from *P. crispa* mats and neighboring *P. oceanica* meadows at six different sampling sites around Giglio Island (Tyrrhenian Sea, Italy). A total of 17 serpulid taxa were identified. The abundance of serpulids (5665 individuals m^{-2} of *P. crispa* mat) were similar to neighboring *P. oceanica* meadows (2304 individuals m^{-2} leaves and 5890 individuals m^{-2} shoots). The number of serpulid taxa was significantly higher in *P. crispa* mats (average 6.63 ± 1.32 taxa) compared to *P. oceanica* beds (average 1.56 ± 0.63 and 1.84 ± 1.04 taxa in leaves and shoots, respectively). Within habitat type, there were no significant differences in species richness between sites. The most abundant species found was *Josephella marenzelleri* (61% of individuals), while *Vermiliopsis* spp. and *Bathyvermilia* sp. were exclusively found in *P. crispa* samples. Our results highlight that *P. crispa* mats host an exceptional diversity and that these habitats should be included in conservation strategies. Further research should focus on the significance of other important taxonomic groups within these mats and evaluate the distribution of *P. crispa* in different regions of the Mediterranean Sea.

Keywords: *Phyllophora crispa*; phytal habitat; hard-bottom communities; ecosystem engineer; Serpulidae; Mediterranean Sea; sessile epifauna

1. Introduction

With almost 17,000 described eukaryotic species [1], the Mediterranean Sea harbors high levels of biological diversity accompanied by a high rate of endemism (estimated 20%) accounting for the term "biodiversity hotspot" [1–3]. Its relatively small size compared to the world's oceans (less than 1%), in combination with this prevalent endemism, underlines the Mediterranean Sea's ecological relevance [4]. In particular, many Mediterranean biogenic habitats' structural complexity (e.g., *Posidonia oceanica* seagrass meadows) facilitates their role as biodiversity hotspots by providing spawning grounds, nurseries, and permanent settling space for many species [5–9]. Furthermore, this structural complexity results in a wide range of ecological niches formed by temporal and spatial gradients (e.g., light and temperature). These gradients are often created by engineering or foundation species [10] that modify their environment through their growth (e.g., macroalgae), while others alter local conditions collectively (e.g., tropical corals). This habitat modification often results in a shift of ecological zonation (e.g., seaweeds providing wet layers in tidal zones) by mitigating stressors for inhabiting species [11]. Finally, associated mobile species

often enhance the engineer's growth by driving out competitors or protecting against grazers [12,13].

Some of the most studied biodiversity hotspots in the Mediterranean basin include *Posidonia oceanica* (L.) Delile, 1813 meadows (Figure 1C–E) and coralligenous habitats. The marine angiosperm *P. oceanica* promotes high biodiversity and provides nursery grounds for commercially important vertebrate species [14]. Furthermore, the structural complexity of the meadow serves as a secondary substrate for diverse communities [15] of sessile invertebrates (e.g., Serpulidae) [5]. Coralligenous habitats are bioconstructions primarily built by encrusting red algae and secondarily by calcifying invertebrate species, which also build a structurally complex habitat and provide biogenic substrate for a wide range of epibiota [7].
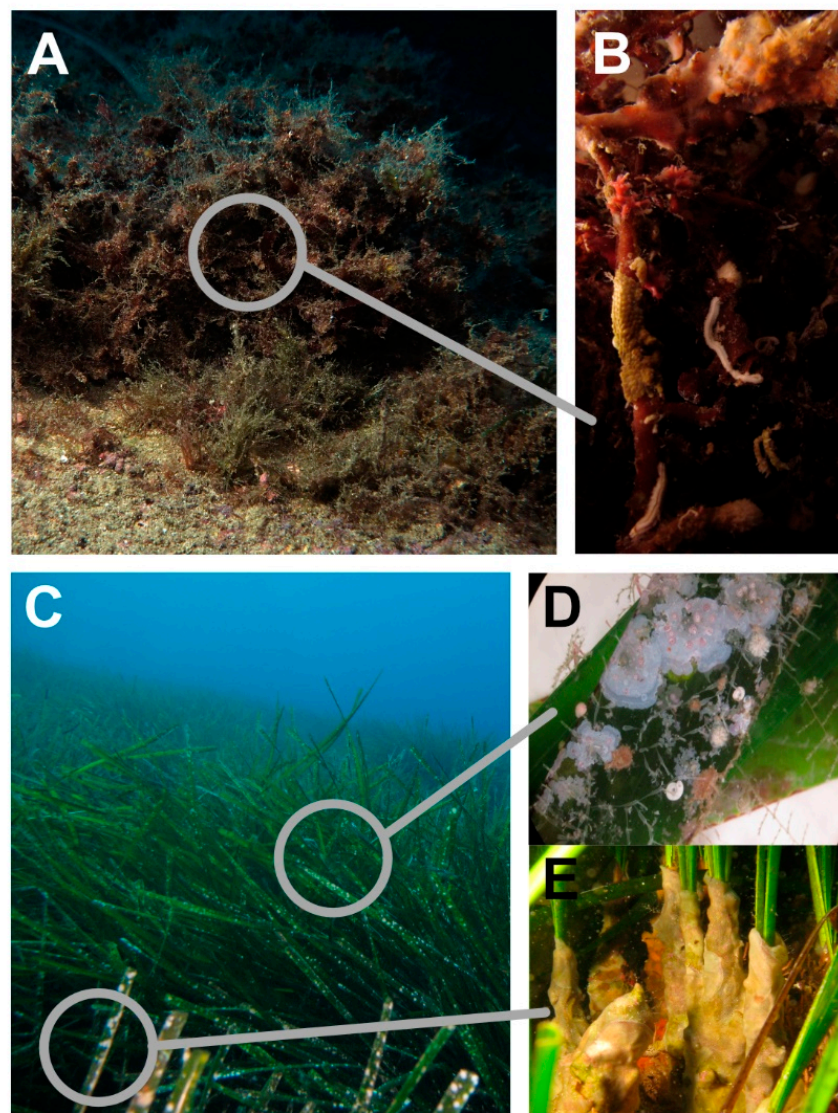

Figure 1. Top: *P. crispa* mat in full view (**A**) and close up including sessile polychaetes and other epifauna (**B**). Bottom: *P. oceanica* mat as seen from the water column (**C**) and close-ups of the leaves (**D**) and shoots (**E**), showing different sessile epifauna. Pictures: F. I. Rossbach.

An additional potential and relatively unexplored biodiversity hotspot is the habitat created by mat-forming, fleshy red alga *Phyllophora crispa* (Hudson) P.S.Dixon, 1964 (Figure 1A,B) [16,17]. In the Black Sea, *P. crispa* mats harbor a rich community of associated fauna, including a diverse invertebrate epifauna [17]. The documented importance of these

habitats led to the establishment of a marine reserve [18]. Marine polychaetes are one of the main groups of associated invertebrate epifauna of these mats [19].

P. crispa mats colonize light-exposed rock surfaces all around Giglio Island. Together with *P. oceanica* meadows and coralligenous reefs, it represents one of the most abundant biogenic habitats in this area. Hemisciaphilic assemblages dominated by *P. crispa* were reported in the Tyrrhenian Sea, both along Giglio Island [16,17] and the North-East coast of Sardinia [20,21]. While differences in diversity and abundance of associated epifauna between *P. oceanica* meadows and other sciaphilic (adapted to low light conditions) hard-bottom communities have been reported [22,23], only a few studies describe the associated biodiversity of *P. crispa* mats.

Polychaetes are among the most diverse metazoan groups, with an estimated number of 2481 species in the Mediterranean Sea, accounting for 20.5% of the 12,088 species reported globally [24]. They show a wide functional variety and adaptation to different environmental conditions [25–27]. Polychaete abundance and diversity are often used to assess benthic communities' states and dynamics [28].

Serpulid polychaetes are sessile organisms that colonize various marine habitats, from the shallow infralittoral to abyssal depths [29] and contain great taxonomic diversity [30]. Key drivers for the abundance and diversity of sessile polychaetes are environmental gradients (e.g., light and depth), as well as suitable space for larval settlement [19,31]. They contribute a considerable amount of carbonate bioconstructions from tropical to boreal latitudes, and shape the seafloor by acting as secondary builders [32,33]. Their ability to precipitate carbonate emphasizes their pivotal role as bioengineering species [34]. In the Mediterranean Sea, serpulids have been relatively well-studied [35–38], and several species have been recognized as lessepsian migrants (i.e., immigrated species from the Red Sea) [30,39–41]. Furthermore, the habitats' structural heterogeneity provided by the algae influences serpulid assemblages (i.e., by offering settling ground and accumulating food particles), as demonstrated by Casoli et al. (2016) [19]. Therefore, the study of serpulids distribution as proxy groups for the occurrence of spatial microhabitats [42,43] is pivotal to understanding the biological diversity hosted in poorly investigated habitats, such as *P. crispa* mats. However, the distribution and role of serpulid worms in Mediterranean *P. crispa* mats are still not well understood. With this work, we aim to answer the following research questions:

(1) What are the abundance and species richness of serpulid polychaetes in *P. crispa* mats compared to *P. oceanica* meadows?
(2) Which species are found in both habitats and which species are unique to *P. crispa* mats?
(3) What is the spatial variability of serpulid polychaetes associated with red algae mats?

2. Materials and Methods

2.1. Study Area and Sampling Activities

The study was carried out at five sites distributed along the North-eastern and North-western coasts of Giglio Island, in the Tuscan Archipelago National Park (42°21′19.4″ N 10°54′06.1″ E, Tyrrhenian Sea) (Figure 2). The islands' underwater seabeds are characterized by granite slopes, alternating with sand bottoms, where *P. oceanica* meadows, *P. crispa* mats and coralligenous habitats colonize infralittoral seabeds. All samples were collected by SCUBA divers at a water depth of 30 m. Samplings were carried out at three sites with *P. crispa* mats (Site PC1, PC2 and PC3), one with only *P. oceanica* meadows (Site PO), and one site (Site mix) with both habitats being present (Figure 2).

Temporally randomized sampling took place during late spring, between May and July 2019. For sampling *P. crispa* mats, a standardized maximum mat thickness of 5 cm was defined. After randomly defining a sampling spot in the target area, a metal frame (size 30 × 30 cm) was placed in the mat and all algal thalli within, including the holdfasts, were carefully removed using a spatula. Sampled material was then placed into 1 L Kautex jars (each holding approx. 1/3 algae, 2/3 seawater). Every site was sampled four times, resulting in a total of 16 replicate samples for this habitat.
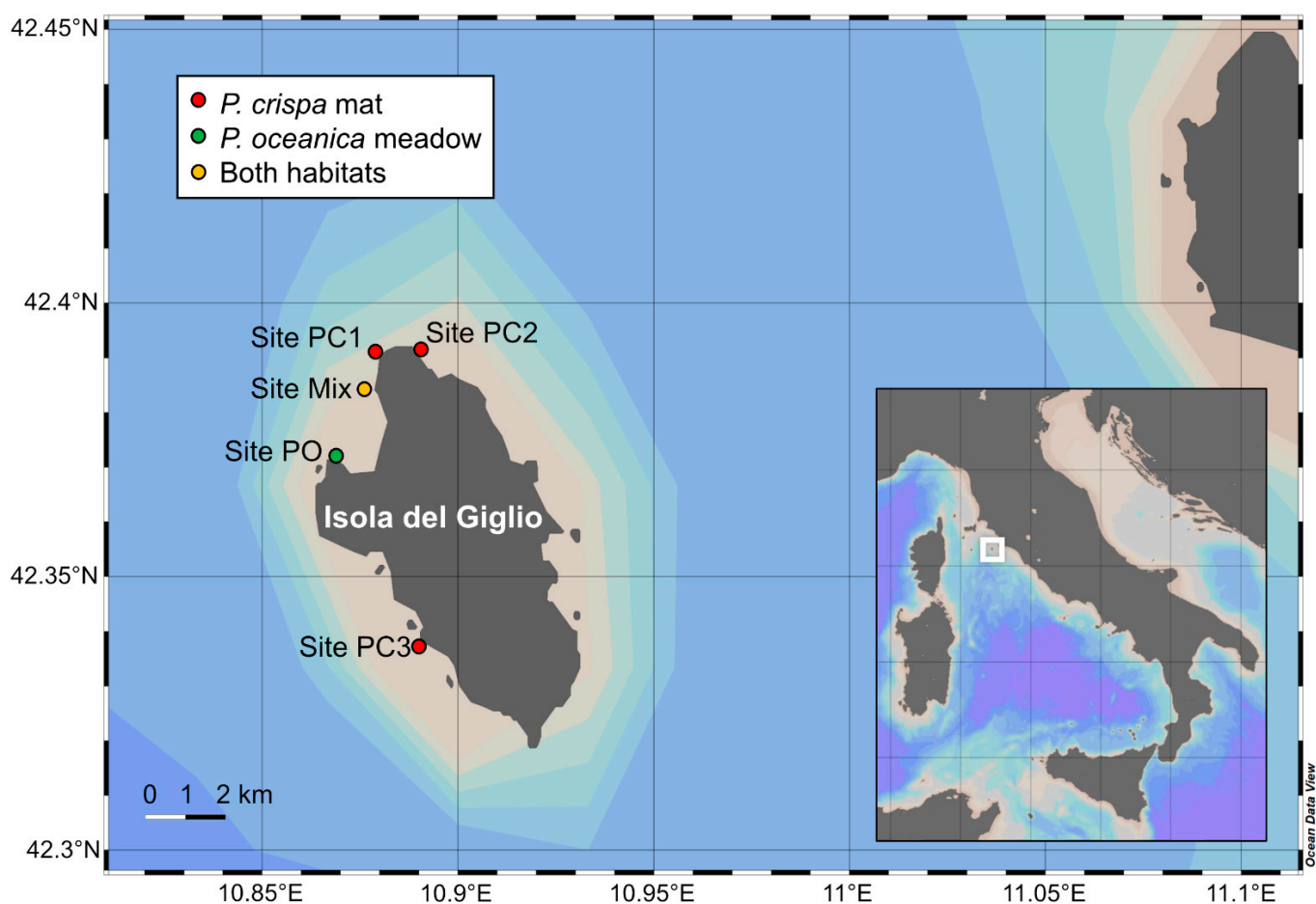
Figure 2. Location of Giglio Island in the northern part of the Tyrrhenian Sea and sampling sites, for *P. crispa* mats (red), *P. oceanica* meadows (green) and sites where both habitats were sampled (yellow).

In total, 19 *P. oceanica* shoots and 17 leaves (site PO: 10 shoots and 9 leaves; site mix: 9 shoots and 8 leaves) were sampled separately into 1 L Kautex jars. The leaves were cut at the sheath of the shoot and shoots were cut at the rhizome node. Samples were immediately transferred into seawater holding tanks at the Institute for Marine Biology (IfMB, Campese, Italy) and kept at a constant temperature of 18 °C. Shoots and leaves were treated as separate subhabitats taking into account their different ecological traits, particularly regarding their longevity as a fundamental trait for serpulid settlement [44,45]. With this approach, we followed recent studies on *P. oceanica* epifauna [46–48]. The number of samples at each of the sites represents the whole epiphytic community, as demonstrated by previous studies on epiphytic communities associated with *P. oceanica* meadows that used 15 shoots as a significative sampling effort [49,50]. We used this number as a minimum goal for our sampling efforts. Sample completeness was confirmed using the approach of Chao et al. (2016) (Appendix A Figure A1).

In addition, we counted the number of *P. oceanica* shoots per m^2 (total $n = 74$ quadrats; using a plastic tube frame of 40×40 cm) and leaves per shoot (total $n = 32$ shoots) in the sampling area.

2.2. Species Identification

For the analysis of *P. crispa* associated serpulid polychaetes, a subsample of approximately 10 g wet weight was taken from the main sample. All replicated samples were processed within three days after collection. Algal thalli were transferred into small bowls and cut into single phylloids for analysis under a stereomicroscope (maximum $40\times$ magnification). Following the analysis, the wet weights of the main and subsamples were

measured after removing adherent water. The *P. oceanica* shoots were analyzed as a whole under a stereo microscope, while leaves were cut into pieces of approximately 8 cm for easier handling and to avoid double counting.

All specimens were identified using relevant literature (Appendix A Table A1) and crosschecking with online resources (WORMS; marinespecies.org, accessed on 31 July 2019). The number of individuals per species were also recorded for quantitative analysis. Counting and sample processing were standardized, and every observer was trained to a high level of taxonomic proficiency prior to processing the samples.

The data were then correlated to the surface area, as individuals per m^2 surface of *P. crispa* and *P. oceanica*. Thalli of *P. crispa* were placed in a bowl of water on a laminated graph paper sheet and flattened with a glass pane. Pictures were taken from a 90° angle using a Canon G12 camera and a tripod stand to ensure a constant distance and angle to the sample. The surface area was then calculated from the picture with ImageJ (version 1.52o, https://imagej.nih.gov/ij/, accessed on 23 April 2019) and multiplied by two to account for both sides of the thalli. The measured surface area was then extrapolated to the main sample, using the wet weight measured after the analysis. This relation allowed for the extrapolation of the density of serpulids to the surface area of underlying rock (Appendix A Formula (A1)). For *P. oceanica* shoots, we assumed a cylindrical shape and calculated the surface area using diameter and length. For *P. oceanica* leaves, we assumed a rectangular shape, calculating its surface area with length and width multiplied by two to account for both sides of the leaf. The total surface area of *P. oceanica* substrate was then extrapolated using the field observations of leaves per shoot and shoots per m^2 (Appendix A Formulas (A2) and (A3)), to assess the number of serpulids per m^2 of seafloor.

2.3. Diversity Descriptors

Diversity was assessed using four descriptors: total numbers of serpulid taxa per site and habitat, total abundances of individuals per m^2 of substrate, Shannon diversity index [51] and Pielou evenness index [52]. The descriptors were calculated as means per site and then reported with the respective standard error.

2.4. Statistical Analysis

Pairwise Wilcoxon–Mann–Whitney tests were carried out to assess differences in diversity descriptors among sites and habitats (Appendix A, Table A2). Differences in the composition of serpulid assemblages among sites and habitats were tested through multivariate permutational analysis of variance (PERMANOVA [53]). The northern sites' data were pooled to compare among habitats and further analyze differences among *P. crispa* sites (Table 1). Pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test. Data was not transformed to stress the importance of the abundance of taxa in determining the differences among habitats. The Serpulid assemblages were hierarchically clustered with Spearman ranked correlation (average linkage) using the software 'heatmapper' [54] to reveal differences in the species composition among sites and visually highlight variances in the serpulid assemblages among habitats. Analyses and plots were made with R (version 3.5.3) [55].

Table 1. Results of permutational multivariate analysis of variance (PERMANOVA) of Serpulid assemblages among *P. oceanica* subhabitats (shoots and leaves), and pairwise comparison of all habitats on the northern sites (site PC1, PC2, PO and mix). Significant results are indicated in bold.

Serpulid Assemblages on *P. oceanica* Subhabitats					
Source	**Df**	**SS**	**R2**	**F**	***p***
Habitat	1	6.1885	0.5350	50.946	**0.001**
Site	1	0.2829	0.0267	2.329	0.094
Habitat:Site	1	0.2474	0.2333	2.037	0.117
Residuals	32	3.8871	0.3665		
Total	35	10.6059	1.0000		
Pairwise Comparison (All Habitats, N Sites)					
Pairs		***p***	***p* adj**		
P. crispa mat	*P. oceanica* leaf	**0.001**	**0.003**		
P. crispa mat	*P. oceanica* shoot	**0.001**	**0.003**		
P. oceanica leaf	*P. oceanica* shoot	**0.001**	**0.003**		

3. Results

3.1. Diversity Descriptors

A total of 2403 Serpulidae specimens belonging to 17 taxa were collected. Overall abundances of individuals m^{-2} and numbers of taxa were significantly higher in *P. crispa* mats than in *P. oceanica* shoot and leaf samples (Figure 3).

The density of individuals was highest in *P. oceanica* shoots at site mix (average 8197 ± 1549 individuals m^{-2}) and lowest in *P. oceanica* leaves at site PO (average 1714 individuals m^{-2}). The comparison with *P. oceanica* leaves showed significantly higher densities for *P. crispa* mats ($p < 0.001$). The shoots of *P. oceanica* hosted a similar number of individuals (average 5890 ± 815 individuals m^{-2}) compared to *P. crispa* samples (average 5664 ± 622 individuals m^{-2}) (Figure 3A). The calculated numbers of serpulids per m^2 seafloor showed a similar trend of highest values for *P. crispa* and *P. oceanica* shoots (average $1,239,728 \pm 784,455$ and $864,444 \pm 1,081,419$, respectively), and a lower density for *P. oceanica* leaves (average $238,567 \pm 179,055$). The combined averages of both *P. oceanica* subhabitats add up to $1,103,011 \pm 1,096,142$ individuals per m^2 seafloor.

All of the 17 identified taxa were found in *P. crispa* samples and only 10 taxa were found in *P. oceanica* samples. The highest number of *P. crispa* associated taxa were found in the samples from Site PC2 (average 8 ± 1 taxa) and the lowest in samples from site mix (average 6 ± 0 taxa) and site PC3 (average 6 ± 2 taxa). In comparison, *P. oceanica* samples harbored 2 ± 1 different taxa on the leaves and shoots, respectively (Figure 3B).

Shannon diversity index was higher in *P. crispa* mats (average 1.1 ± 0.09) compared to *P. oceanica* habitats (average leaves: 0.2 ± 0.06; average shoots: 0.2 ± 0.07; Figure 3C). Site PC3 showed a significantly lower diversity compared to other *P. crispa* samples ($p < 0.05$).

Pielou evenness index was highest in *P. crispa* samples (average 0.6 ± 0.05) compared to *P. oceanica* habitats (average leaves: 0.4 ± 0.09; average shoots: 0.4 ± 0.04; Figure 3D). Site PC3 showed a significantly lower evenness compared to other *P. crispa* samples ($p < 0.05$).
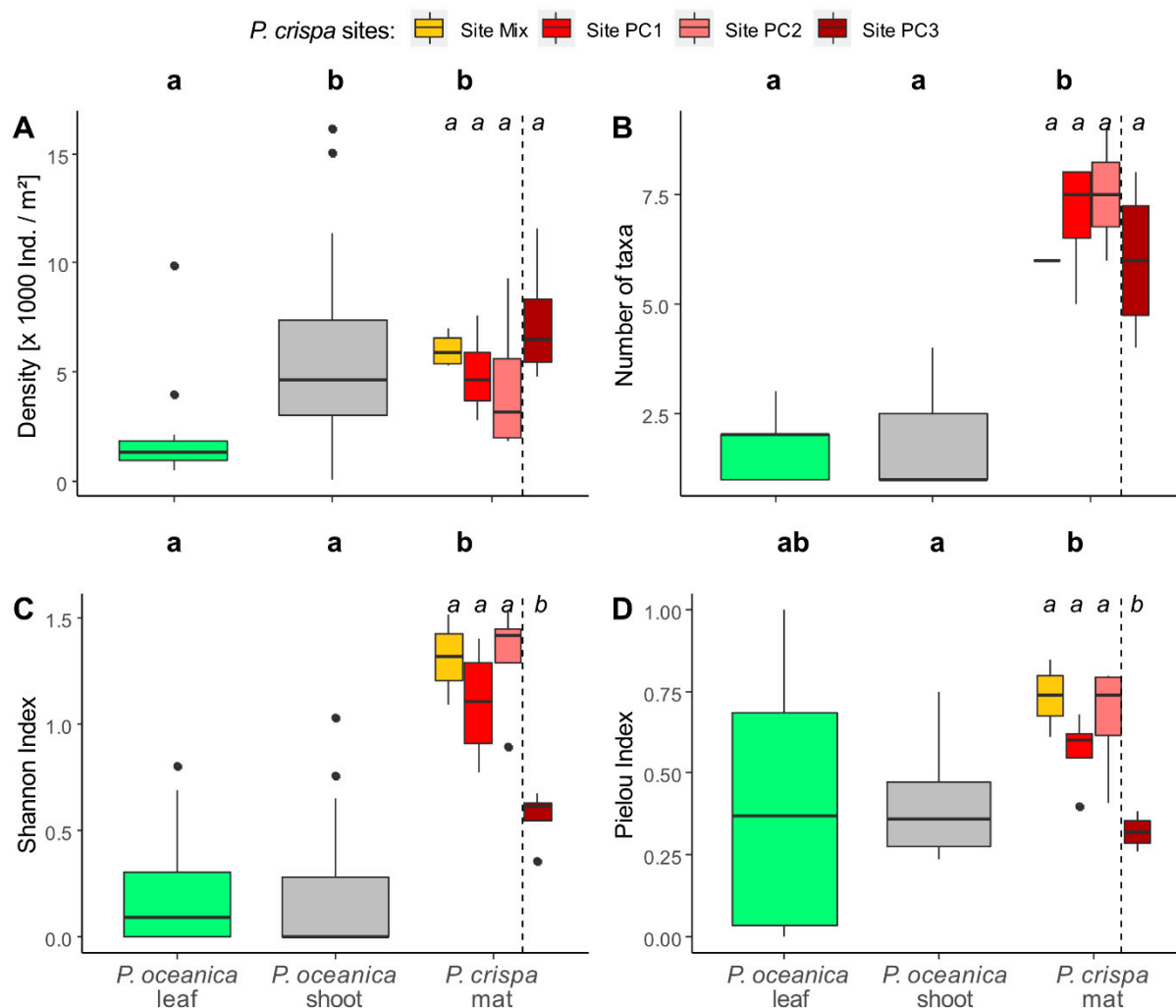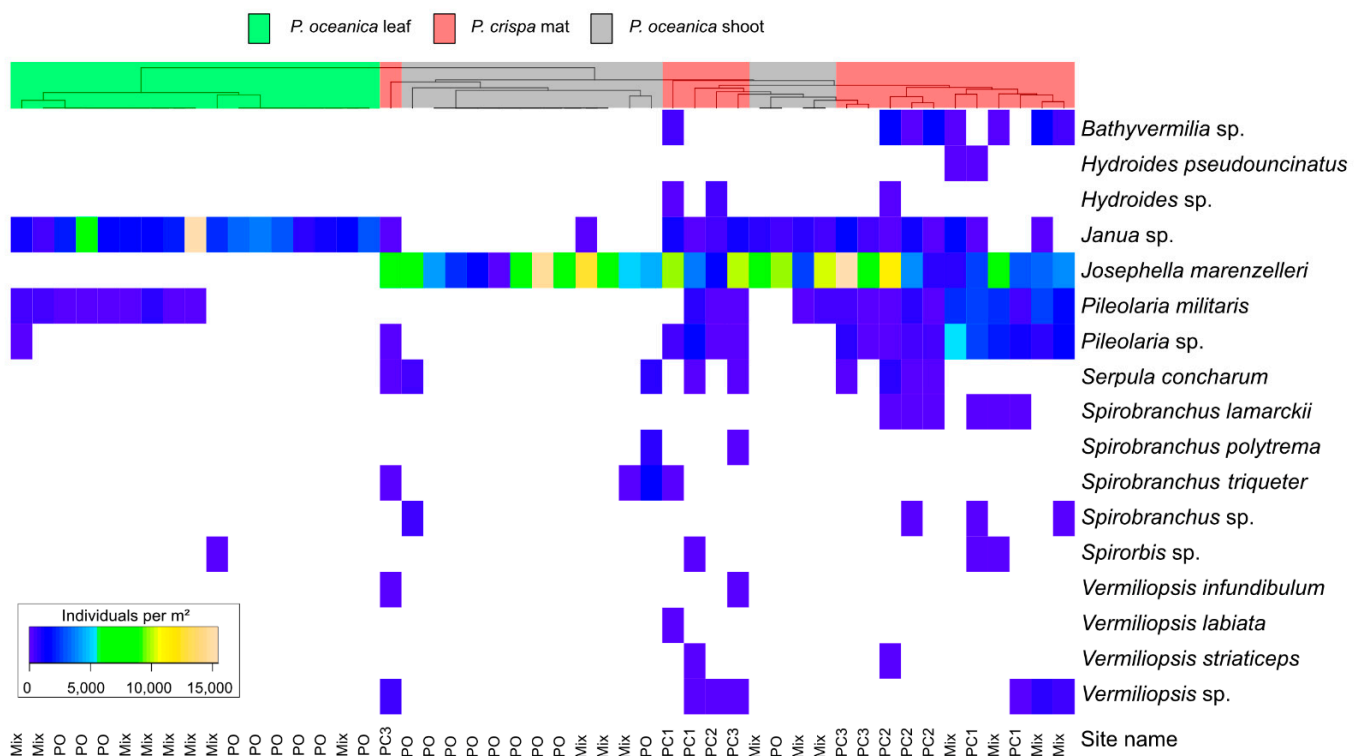
Figure 3. (**A**) Total numbers of identified species per site, (**B**) mean densities of serpulids per m² substrate per site, (**C**) Shannon diversity index and (**D**) Pielou evenness index reported for the investigated *P. crispa* (red) sites in comparison to *P. oceanica* leaves (green) and shoots (grey). Letters a and b indicate the results of pairwise Wilcoxon–Mann–Whitney tests performed for comparison among habitats in northern sites (excluding PC3; bold letters) and among all *P. crispa* sites. Black dots resemble outliers.

3.2. Analysis of Serpulid Assemblages

The multivariate analyses (PERMANOVA) showed significant differences among the two *P. oceanica* habitats without an effect of the site (Table 1). The analysis of *P. crispa* sites showed a significant difference among sites; however, the pairwise comparison did not confirm this result (Table 2). We further analyzed differences among the habitats for the northern sites only, avoiding potential effects of the location around the island. The cluster analysis confirms these results and shows differences in the species composition of the different habitats (Figure 4). The dendrogram of Spearman rank correlation across sites and habitats shows clustering according to habitat, with a lower coefficient for *P. crispa* and *P. oceanica* shoots ($r < 0.6$) than *P. oceanica* leaves compared to *P. oceanica* shoots and *P. crispa* mat ($r > 0.8$).

Table 2. Results of permutational multivariate analysis of variance (PERMANOVA) and pairwise comparison of Serpulid assemblages among *P. crispa* sites. Significant results are indicated in bold.

Serpulid Assemblages on *P. crispa* Mats					
Source	Df	SS	R2	F	*p*
Site	3	0.9182	0.4066	2.7407	**0.012**
Residual	12	1.3401	0.5934		
Total	15	2.2583	1.0000		
Pairwise Comparison (*P. crispa* Sites)					
Pairs		*p*	*p* adj.		
Mix	PC1	0.444	1.000		
Mix	PC2	0.032	0.192		
Mix	PC3	0.033	0.198		
PC1	PC2	0.351	1.000		
PC1	PC3	0.104	0.624		
PC2	PC3	0.062	0.372		

Figure 4. Hierarchical clustering (average linkage, Spearman ranked correlation) of taxa abundances among sites (individuals per m^2) found in *P. crispa* (red) and *P. oceanica* habitat northern sites (green = leaf; grey = shoot).

The most frequent species found in all samples of *P. crispa* mats was *Josephella marenzelleri*. This species was also largely present on *P. oceanica* shoots, however, it was not found on *P. oceanica* leaves. In contrast, the most abundant species on the leaves was *Janua* sp., which also occurred in 13 *P. crispa* replicates and on 7 investigated *P. oceanica* shoots. In addition, *J. marenzelleri* was also the most abundant species in terms of individuals found m^{-2} of *P. crispa* and *P. oceanica* shoots, while *Janua* sp. showed the highest density on *P. oceanica* leaves. Out of the 17 species, two were exclusively found on *P. crispa* samples: *Bathymermilia* sp. and *Vermiliopsis labiata* (Figure 4). The species *Pileolaria militaris* showed similar abundances in *P. crispa* and *P. oceanica* samples.

4. Discussion

This study highlights the high diversity and density of serpulid polychaetes for all investigated habitats. The total number of species found associated with *P. crispa* mats (17 species) was consistent with previous studies from other areas in the Mediterranean Sea [37,56], including the results of Casoli et al. (2016), which investigated *P. crispa* mats along a water depth gradient and found 16 species (Table 3) [19]. Here, we focused on the water depth range with the highest *P. crispa* mat development and identified potential differences among sites. Species richness and abundance were high across all sites; however, the southernmost site (Site PC3) showed a lower diversity and evenness than the northern sites.

Table 3. Serpulidae species found in the samples compared to other habitats, x indicates presence; Data from [1] Casoli et al. (2016) [19] and [2] this study (grey columns).

Species	Shallow Infralittoral [1]	*P. crispa* Mat [1],[2]	Coralligènous Habitat [2]	*P. oceanica* Meadow [2]
Bathyvermilia sp.			x	
Filograna implexa (Berkeley)	x	x		
Hydroides spp. (Gunnerus)	x	x	x	x	x
Janita fimbriata (Delle Chiaje)				x	
Janua sp.	x		x	x	x
Josephella marenzelleri (Caullery and Mesnil)	x	x	x	x	x
Metavermilia multicristata (Philippi)				x	
Pileolaria spp.	x	x	x	x	x
Protula sp. (Risso)				x	
Semivermilia crenata (O. G. Costa)		x		x	
Semivermilia cribrata (O. G. Costa)				x	
Serpula spp.	x	x	x	x	x
Serpula vermicularis (Linnaeus)		x		x	
Spiraserpula massiliensis (Zibrowius)				x	
Spirobranchus spp.	x	x	x	x	x
Spirorbis spp.			x	x	x
Vermiliopsis spp.		x	x	x	

The extrapolated abundances per m^2 seafloor were comparable between *P. crispa* (average 1,239,728 ± 784,455) and *P. oceanica*, (1,103,011 ± 1,096,142) with the shoots mainly contributing to these numbers. However, the calculations likely cause some bias due to the extrapolation based on average values, as indicated by the large standard deviations.

Comparing *P. crispa* mats and neighboring *P. oceanica* meadows revealed significant differences in serpulid assemblage composition. By modifying the substratum through the creation of spatial microhabitats and influencing feeding habits, seagrass beds and algae mats appear to favor the establishment of different polychaete assemblages [43,57,58]. Habitat formation and the substantial improvement of food source accessibility are pivotal ecological drivers that influence the epiphytic serpulid assemblages' diversity and structure. Overall abundance, species richness, diversity and evenness were significantly lower in most *P. oceanica* samples compared to *P. crispa*. Exceptions are *P. oceanica* shoots, where similar densities of individuals were found compared to *P. crispa* mats. The species composition was also similar, with the same dominant species (*J. marenzelleri*), which resulted in a shared cluster disparate from the *P. oceanica* leaf samples (Figure 4). This clustering could be related to the two habitats' semi-hemisciaphilious conditions, which leads to reduced competition with algal epiphytes and influences rhizome communities [31]. Lower light conditions inside *P. crispa* mats and *P. oceanica* shoots are also reflected by the lower numbers of the photophilic Spirorbinae (e.g., *Janua* sp.) [59], mostly found on the leaves of *P. oceanica*. The perennial *P. crispa* thalli provide an extension of colonizable surfaces that are less flexible and long-lived compared to *P. oceanica* leaves. Constant motion and a one-year life cycle favor species with well-developed strategies to cope with stresses on *P. oceanica* leaves, such as Spirorbidae (*Janua* sp.). These are recognized as pioneer species,

characterized by fast and consistent recruitment on smaller surfaces [60,61]. Thus, dense red algae mats composed of both prostrate and erect thalli (heterotrichous species) [17] constitute a sheltered and long-lived habitat rather than oscillating structures of shallow algae or *P. oceanica* leaves.

In comparison to previous reports of neighboring (i.e., coralligenous reefs) habitats, we see that *J. marenzelleri* is a ubiquitous species that is found from the shallow infralittoral to deeper coralligenous habitats (Table 3). This species is known to be a pioneer species with the ability to colonize available settling grounds quickly and efficiently [36]. Furthermore, *J. marenzelleri,* with its articulate tube, is particularly adapted to colonize rigid algal thalli as epiphytes, being found both on *Cystoseira* spp. canopy and encrusting red algae [59,62]. Other widely distributed taxa characterized the serpulid assemblage on *P. crispa* samples. However, the presence of species with dark or deep-habitat affinity (*Bathyvermilia* sp. and *V. infundibulum*) confirmed the hemisciaphilic conditions provided within the algal mat.

In contrast, serpulids with a larger and more erect growth form are only found in coralligenous habitats (e.g., *Protula* sp., Table 3). These slow-growing species are sensitive to water movement and prefer hard settling grounds over flexible algae and plants [59]. However, the overall high abundance of serpulids on *P. crispa* thalli supports their rigid structure and long-term stability as key characteristics for the establishment of these species. The laminal thalli of *P. crispa* represent a living substratum suitable for the colonization of sessile solitary species, increasing the surface available for settlement. Sedimentation processes on horizontal and gently sloped sea beds, where *P. crispa* is abundant, can help understand the patterns described in this study [17]. Serpulidae comprise exclusively filter-feeding species that can benefit from organic and inorganic particle sedimentation as trophic sources.

Communities of the brown algae *Cystoseira* sp. in the Ionian Sea host a notably higher diversity of serpulids (Shannon of 2.4) than we found in *P. crispa* mats (average Shannon of 1.1) [59]. This shows dominance of a few species in the *P. crispa* mats, as also seen in the abundance values. Interestingly, the abundances exhibit a trend of higher numbers on the southern site (PC3) while diversity and evenness are significantly lower (Figure 3). This variation could reflect the serpulid assemblage's heterogeneity over a larger spatial scale due to the physical exposure of different *P. crispa* mats. While the diversity was still considerably high in both areas, it also points out that the western Peninsula (Figure 2) separates the southern *P. crispa* mats from the northern patches. This effect could be driven by the rather exposed situation of PC3 to the prevalent southern currents, while the northern sites are situated on the leeward side of the island [63]. Thus, this situation could also impact larval as well as food particle supply.

We conclude that *P. crispa* mats harbor a rich serpulid assemblage, comparable to or even exceeding other neighboring biodiversity hotspots (i.e., *P. oceanica* meadows). Furthermore, we found two species that were unique to this habitat in our study. This high diversity underlines the vital function of *P. crispa* as a habitat-forming species and host for exceptional biodiversity. Therefore, we recommend evaluating this habitat for future conservation actions to prevent habitat and biodiversity loss. Future research should also address the role of other taxonomic groups associated with the *P. crispa* mats and assess differences in the composition of infauna communities on a regional scale to confirm its role as an essential benthic habitat in the (western) Mediterranean Sea. Our results revealed potential regional differences in the *P. crispa* associated fauna that need to be further addressed by sampling other areas along the Mediterranean coast. This information is crucial to explore how connected or isolated the different patches of *P. crispa* mats are in terms of exchange of biodiversity and larval settlement. Potential drivers of connectivity for serpulid communities are prevalent water currents [33] as indicated by the differences between the northern sites and the rather exposed southern site [64].
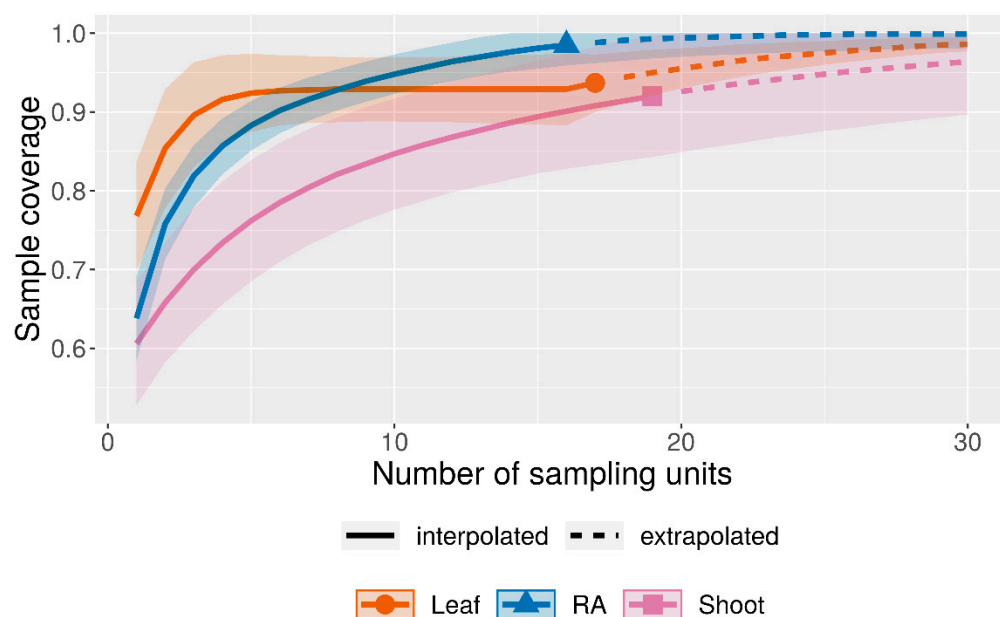
Appendix A

Figure A1. Sample completeness curve based on incidence data according to Chao et al. (2016) [64]. Number of bootstraps used: 500; level of confidence: 0.95. RA = *P. crispa* mats, Leaf = *P. oceanica* leaves, Shoot = *P. oceanica* shoots with sample coverages (C.hat) of 0.98, 0.93 and 0.92, respectively.

Table A1. Literature used for identification of species.

Author(s)	Year	Title
Riedl, R.	2011	Fauna und Flora des Mittelmeeres
Stresemann, E.	1992	Wirbellose
Hayward, P.J. and Ryland, J.S.	1999	Handbook of the Marine Fauna of North-West Europe
Bianchi et al.	1981	Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane
Ten Hove et al.	2009	Taxonomy of Serpulidae (Annelida, Polychaeta): the state of affairs
Zibrowius H.	1968	Etude morphologique, systématique et écologique des Serpulidae (Annelida Polychaeta) de la région de Marseille
Zibrowius H.	1972	Mise au point sur les especes mediterraneennes de Serpulidae (Annelida Polychaeta) déecrites par Stefano delle Chiaje (1822–1829, 1841–1844) et Oronzio Gabriele Costa (1861)

Table A2. Results of univariate tests (Wilcoxon–Mann–Whitney) among *P. crispa* sites (ns = $p > 0.05$; * = $0.01 < p < 0.05$).

	Group 1	Group 2	*p*-Value	*p*-Signif
Density	Site Mix	Site PC1	0.34	ns
	Site Mix	Site PC2	0.34	ns
	Site Mix	Site PC3	0.69	ns
	Site PC1	Site PC2	0.69	ns
	Site PC1	Site PC3	0.34	ns
	Site PC2	Site PC3	0.20	ns
No of taxa	Site Mix	Site PC1	0.278	ns
	Site Mix	Site PC2	0.069	ns
	Site Mix	Site PC3	1.000	ns
	Site PC1	Site PC2	0.766	ns
	Site PC1	Site PC3	0.454	ns
	Site PC2	Site PC3	0.306	ns
Shannon index	Site Mix	Site PC1	0.486	ns
	Site Mix	Site PC2	0.686	ns
	Site Mix	Site PC3	0.029	*
	Site PC1	Site PC2	0.200	ns
	Site PC1	Site PC3	0.029	*
	Site PC2	Site PC3	0.029	*
Pielou index	Site Mix	Site PC1	0.057	ns
	Site Mix	Site PC2	0.886	ns
	Site Mix	Site PC3	0.029	*
	Site PC1	Site PC2	0.200	ns
	Site PC1	Site PC3	0.029	*
	Site PC2	Site PC3	0.029	*

Formula (A1): Calculation of serpulid individuals on *P. crispa* per m^2 seafloor (Ind_{SF}) from individuals per m^2 substrate (Ind_{SS}), using wet weights (WW) and surface area of the sub sample (SA_{SS}) (0.09 m^2 corresponds to the size of the sampling frame:

$$Ind_{SF} = \frac{\frac{WW_{MS} \times SA_{SS}}{WW_{SS}}}{SA_{SS}} * Ind_{SS} * \frac{1 \text{ m}^2}{0.09 \text{ m}^2} \tag{A1}$$

Formula (A2): Calculation of serpulid individuals on *P. oceanica* leaves per m^2 seafloor using the average leaf surface area (SA_{leafAVG}), average number of leaves per m^2 (162),

surface area of investigated leaf sample (SA_{leafSS}) and individual count per m² substrate (Ind_{SS}):

$$Ind_{SF} = \frac{162 \times SA_{leafAVG}}{SA_{leafSS}} * Ind_{SS} \tag{A2}$$

Formula (A3): Calculation of serpulid individuals on *P. oceanica* shoots per m² seafloor using the shoot surface area ($SA_{shootSS}$), average number of shoots per m² (40.5) and individual count per m² substrate (Ind_{SS}):

$$Ind_{SF} = \frac{40.5 \times SA_{shootSS}}{SA_{shootSS}} * Ind_{SS} \tag{A3}$$

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