**Exploring the genetic diversity and the population structure of the mesophotic
Paramuricea macrospina in the Menorca Channel**

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Keywords: Anthozoa, connectivity, Mediterranean Sea, microsatellite loci,
management

28

29 **Abstract**

30 Gorgonians play an important structural and functional role promoting high diversity
31 and biomass of associated fauna. Up to now, studies on gorgonian ecology in the
32 Mediterranean Sea have been focused mainly on the SCUBA diving depth range.
33 Although increased availability of remotely operated vehicles allowed access to the
34 deeper areas, gorgonian assemblages located on continental shelf and slopes are still
35 barely known. Gorgonian assemblages on continental shelves are extremely vulnerable
36 to anthropogenic impacts, especially bottom trawling and longline fishing. To
37 implement effective management and conservation policies it is crucial to understand
38 patterns of genetic structure among populations since connectivity enhances the
39 resilience of populations. *Paramuricea macrospina* is a key structuring gorgonian in the
40 Menorca Channel's (Balearic Archipelago) outer continental shelf where it covers vast
41 extensions, reaching very high densities. Combining two mitochondrial makers and 10
42 microsatellites, this study gives the first insight into the genetic diversity and population
43 structure of *P. macrospina* between 60 and 100 meters depth in the Menorca Channel
44 and at a horizontal spatial scale of about 60 Km. Overall, we demonstrate a low genetic
45 diversity and a lack of genetic structure among populations, which may be explained by
46 the geomorphology and hydrodynamic features of the Menorca Channel (e.g. internal
47 waves, high currents). Our study suggests some connectivity among *P. macrospina*
48 populations in accordance with the high recruitment rates observed. This connectivity
49 may increase the resilience and foster the recovery of impacted populations since the
50 study area will become a Marine Protected Area of the Natura 2000 network in the near
51 future. Nevertheless, complementary studies based on a larger sample size should be
52 conducted to complement our results. In addition, temporal genetic monitoring of these

populations should be envisaged to monitor the potential reduction of genetic diversity of this mesophotic species.

Introduction:

In the past few years there has been a substantial increase in the ecological characterization of benthic mesophotic communities located between 30 m and 150 m depth dominated by suspension feeders (e.g. Bo et al., 2011; Kahng et al., 2014). In tropical environments, these communities are mainly characterized by the presence of light dependent anthozoans (e.g. Kahng et al., 2017), while in temperate environments they are mostly composed by coralligenous algae that generate hard substrates (i.e. coralligenous outcrops and maërl beds (Barberá et al., 2012)), supporting high-density of gorgonians and sponges (Grinyó et al., 2016; Santín et al., 2018).

Mesophotic communities provide habitat for a wide variety of species, many of which of commercial interest (Bo et al., 2014). Consequently, these communities have been severely impacted by different fishing practices such as bottom trawling (Ordines et al., 2017; Ferrigno et al., 2018). These practices damage and remove structuring organisms (Mytilineou et al., 2014) disrupting the metapopulation structure and the connectivity among populations (*sensu* Cowen et al., 2000). Connectivity plays a fundamental role in local and metapopulation dynamics, enhancing the resilience, the recovery and/or recolonization of the populations after a disturbance (Hastings and Harrison, 1994; Padrón et al., 2018a). Therefore, understanding the patterns of genetic structure and the levels of connectivity among populations is crucial to implement effective management and conservation strategies. Connectivity can be assessed through different methods such as direct field observations, mark-recapture techniques, geochemical and genetic markers analysis and biophysical models (Andrello et al., 2013). However,

characterizing connectivity in sessile species with planktonic dispersive stages, such as sponges or octocorals, is quite challenging (Costantini et al., 2018), especially at depths preventing the use of air SCUBA diving. In these cases, population genetics represent the most suitable approach.

Polymorphism analyses of DNA nuclear sequence and microsatellite loci are powerful to assess ecological connectivity among octocoral populations (Costantini et al., 2007a,b, Aurelle et al., 2011, Ledoux et al., 2010a,b). Recently, microsatellite loci analyses have been used to characterize the patterns of genetic structure in shallow Mediterranean gorgonian populations generally revealing a significant genetic differentiation at fine spatial scales (from tens of meters to kilometers), which suggest a restricted connectivity among populations (e.g. Aizmendi-Mejía et al., 2015, Aurelle et al., 2011; Costantini et al., 2016; Ledoux et al., 2010a; Masmoundi et al., 2016; Padrón et al., 2018a,b). Nevertheless, diversity and population structure on Mediterranean gorgonians in mesophotic environments (i.e. below 40 m depths) still received limited attention (but see Perez-Portela et al., 2016). Perez-Portela et al. (2016) described the genetic structure of several *Paramuricea clavata* populations in three canyon heads on the Catalan continental margin (50-60 m depth). They encountered a genetic isolation among all canyons (separated by 50 km) and among populations within one of the canyons (separated by 4 km).

Paramuricea macrospina (Koch, 1882) has been recently reported as one of the most frequent and abundant gorgonian species in Mediterranean mesophotic ecosystems (Bo et al., 2011; Topçu and Öztürk, 2015), dominating maërl beds on the outer continental shelf of the Menorca Channel at 65–100 m depth (Grinyó et al., 2016). *Paramuricea macrospina* presents a high morphological plasticity based on its coloration and sclerite morphology (Grinyó et al., 2018a; Pica et al., 2018). On the Menorca Channel's

continental shelf a yellow (M1) and purple (M2) morphotypes were observed occurring side by side (Grinyó et al., 2016). Sclerites in both morphotypes present very different morphologies, M1 sclerites are large with very ramified roots and covered with plenty of tuberculations, whereas M2 sclerites' are smaller and their roots are smooth and poorly ramified (Grinyó et al., 2018a). The aim of this study was to assess the level of genetic diversity and to describe the pattern of spatial genetic structure among populations of *P. macrospina* across the Menorca Channel continental shelf. To reach this goal, molecular markers with different level of polymorphisms (two mitochondrial markers and 10 microsatellites) were used. For the first-time a cross-amplification of ten microsatellite loci specifically developed for *P. clavata* was carried out to test their efficiency on *P. macrospina*.

The genetic characterization of *P. macrospina* colonies will be useful to characterize the level of isolation among populations, to investigate the processes affecting its dispersal and its resilience and to increase our knowledge of the Mediterranean mesophotic ecosystems.

MATERIAL AND METHODS

Sampling design

The sampling sites were localized in the Menorca Channel (39°53'0.73"N, 3°29'51.16"E) (Fig. 1). The collection of samples was made using three methods: i) the remotely operated vehicles (ROV) NEMO (Gavin Newman), equipped with a grabber, ii) SCUBA diving and iii) removing colonies from trammel nets.

Ten sites separated by less than 1 km to 56 km were sampled. Overall a total of 76 colonies were collected (7.4 ± 2.03 colonies/site; mean $\pm$ standard error). The number of sampled colonies has been constrained due the deep distribution of the species and

rough weather conditions. Branch fragments were preserved in 100% ethanol and stored at 4°C for the following analysis.

Figure 1. Sampling map of the sites where *Paramuricea macrospina* colonies were collected in the Menorca Channel. Continuous lines indicate the isobaths.

Molecular techniques

From each colony fragments of 3-5 polyps were collected using a microscope and, from them, DNA was extracted following the Cetyl Trimethyl Ammonium Bromide (CTAB) procedure (Hillis and Moritz, 1990).

Amplification of a portion of the mitochondrial *mutS* homolog gene (homolog of the bacterial mismatch repair gene) (mtMUTs, Pont-Kingdon et al., 1995) was performed using the primers MUT4759f and MSH5376r following France and Hoover (2001)).

Based on the published mitogenome of *P. macrospina* (LT576168, Polisenio et al., 2017) new primers for the mitochondrial Intergenic Region (IGR) between ND4 and ND5 mitochondrial genes, was specifically designed using the online PRIMER3 version 4.0 software (Rozen and Skaletsky, 2000) (<http://primer3.ut.ee>). The designed primers

were: IGR 14221_F: 5'-TTAGGCCCCACTGGAATA-3' and IGR 14704_R: 5'-GAGACCACTCTAACGCTT-3'. PCR reactions were carried out in 24 µl volume reaction containing 2.5 µl amplification buffer (10X), 2.5 µl of each primer (forward and reverse) (10 mM), 2.5 µl of MgCl₂ (25 mM), 1 µl of dNTP mix (10 mM), 0.2 µl of 1 U Taq polymerase, and 2.5 µl of DNA. Amplification proceed with an initial denaturation step at 94°C for 2 min, then 30 cycles of denaturation at 94°C for 30 s, annealing to 60°C for 15 s and extension at 72°C for 1 min followed by a final extension at 72°C for 10 min. PCR products were purified with ExoSap-IT Cleanup kit (USB Corp., Cleveland, OH, USA).

As a first step, eleven microsatellite loci specifically developed for *P. clavata* (Abdoullaye et al., 2010; Ledoux and Garrabou, unpublished) were tested using different multiplex combination and/or using single PCR protocol. Of the eleven microsatellites, ten were selected based on their amplifiability and polymorphism. These loci were amplified in two multiplex PCR using QIAGEN® Multiplex PCR Kit: Pcla-10, Pcla-12, and Pcla-17 were amplified following the PCR conditions described in (Abdoullaye et al., 2010); Pcla-20, Pcla-21, Pcla-23, Pcla-25, Pcla-27, Pcla-28, Pcla-29 were amplified following the protocol provided by Ledoux and Garrabou (unpublished).

Resulting PCR products were then visualised with an ABI 3730 XL genetic analyser, using an internal size standard (GeneScan 500 LIZ), at Macrogen Inc. (Spain).

Mitochondrial and microsatellite genetic variability and structuring

MtMutS sequences and IGR sequences were analysed and aligned using MEGA v.5.0.5. DNAsp v.5.10.1 (Rozas and Rozas, 1999) were used to calculate the number of haplotypes (H), the haplotypes diversity (h) and the nucleotide diversity (Pi). The

haplotypes were plotted to build the haplotype network, using HAPLOTYPE VIEWER (<http://www.cibiv.at/~greg/haploviewer>).

PEAK SCANNER software v.1.0 was used for microsatellite allele scoring. We looked for identical multilocus genotypes (MLGs) in GenAlEx v.6.41 (Peakall and Smouse, 2006) by requiring complete matches at all loci.

Linkage disequilibrium among all pairs of microsatellite loci was tested in GENEPOP v.3.4 (Raymond and Rousset, 1995) with significance levels determined by the Markov chain method (dememorization = 5,000, batches = 500, iterations = 10,000).

With GENETIX v.4.05 (Belkhir, 2004), we computed the numbers of alleles per locus and population (N_a), the expected heterozygosity (H_E), the observed heterozygosity (H_O), and the inbreeding index (F_{IS}) per locus and populations using Weir and Cockerham's f estimator (Weir and Cockerham, 1984). Significant departures from the Hardy–Weinberg equilibrium were tested using an exact test in GENEPOP with the significance determinate by a Markov-chain randomization. For these analyses 1,000 steps of dememorization, 100 batches and 1,000 iterations per batch were used.

We used the Robust Multilocus Estimation of Selfing (RMES) (David et al. 2007) to test the relative impact of null alleles and inbreeding on the significant departures from Hardy-Weinberg (see results). The occurrence of null alleles is specific of each locus while inbreeding should equally affect all markers. A selfing rate (s), considered as biparental inbreeding rate in gonochoric species (e.g., Ledoux et al. 2012), is deduced from an estimator of the 2-locus heterozygosity disequilibrium over all pairs of loci (g^2) under the assumption of inbreeding and linkage equilibrium. We tested the null hypothesis $s = g^2 = 0$ (i.e., no biparental inbreeding) by resampling single-locus genotypes independently 1,000 times.

Considering the limited sample size of the population under study, we assessed the statistical power of our microsatellite dataset to detect low F_{ST} values. We used POWSIM 4.0 (Ryman et al., 2006). Simulations were run using various combinations of effective population size (N_e) and time since divergence (t), leading to F_{ST} values ranging from 0 to 0.08 as observed in the data (see below). Two hundred replicates were run, and the power of the analysis was indicated by the proportion of tests that were significant at $p < 0.05$ using the respective allele frequencies at the 10 loci.

For both markers, genetic differentiation between population pairs was estimated using pairwise F_{ST} and its significance determined using a permutation test (10,000 permutations) using ARLEQUIN v.3.5 (Excoffier and Lisher, 2010). Moreover, a hierarchical Analysis of the Molecular Variance (AMOVA; Excoffier et al., 1992) was conducted, to test the genetic differentiation between the two morphotypes. Jost's actual measure of differentiation, $Dest$ (Jost, 2008) was calculated for the microsatellite dataset using DEMETICS v.0.8.3 (Gerlach et al. 2010) within the statistical package R v2.13.1 (R core team 2014).

Isolation by distance (IBD) model among populations was tested through a Mantel test ($n=10,000$ permutations) in GENEPOP on the web (Rousset, 2008).

For microsatellite dataset, a clustering analysis based on the Bayesian approach using STRUCTURE v.2.3.1 (Pritchard et al., 2000) was performed to infer the optimal number of homogeneous genetic units (K). The software was run with the whole data set considering K values from 1 to 10. For each K , ten independent replicates were tested considering 500,000 Markov chain Monte Carlo repetition (burnin period 50,000).

Because sampling location information set as prior information can assist clustering for data sets with weak structure (Hubisz et al., 2009), the LOCPRIOR option was used. The optimal K values were determined using STRUCTURE HARVESTER web v.0.6.94 (Earl and vonHoldt, 2012). Results of all the runs were averaged with CLUMPP (Jakobsson and Rosenberg, 2007) and visualized with DISTRUCT (Rosenberg, 2004) using the online CLUMPAK server (Kopelman et al., 2015). A discriminating analysis of principal components (DAPC) was performed in ADEGENET v.1.3 (Jombart et al., 2010). It is used to extract information from genetic dataset performing a PCA on pre-defined populations. SPAGeDi v.1.3 (Hardy and Vekemans, 2002) was used to conduct spatial autocorrelation analyses. This analysis allows calculating a genetic relatedness coefficient for all pairs of individuals and bins individuals into a set of predefined distance intervals to test for spatial dependency in the distribution of individual genotypes (Epperson and Li, 1996). The Loiselle's kinship coefficient was used since it is free from assumption on Hardy–Weinberg (HW) equilibrium and it has been previously recommended (Vekemans and Hardy, 2004).

When multiple tests were performed, the level of significance was corrected using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

Results

Mitochondrial sequence variation and genetic structure

Fragments of 609 bp in length of the mtMutS gene were obtained for 54 individuals. All the individuals host the same haplotype. These sequences are identical to the sequence of *P. macrospina* retrieved in GenBank (accession number LT576168) and sampled in

Corsica (North Western Mediterranean). *P. macrospina* mtMutS sequence vary only of 11 nucleotides (598/609 bp identity) from the sequence of the congeneric species *P. clavata* (accession number: LT576167, Polisen et al., 2017).

Across all the 76 analyzed colonies, the fragment of the mitochondrial region including a portion of the Nad5, Nad4 and the Intergenic Region (IGR) was 351 bp in length. Sequence alignment showed the presence of three nucleotide substitutions defining five haplotypes. The number of haplotypes (H), the haplotypes diversity (h) and the nucleotide diversity (P_i) for each site, are shown in Table 1. Number of haplotypes ranges from 1 (MAL6) to 3 (MAL3, MAL4 and MAL9). The haplotypes HAP1 and HAP2 are the most abundant, with HAP1 present in all the sites excluding MAL8. MAL8 is also the only site hosting a private haplotype (HAP5) (Fig. 2).

Table 1. Number of individuals sequenced per site (N); depth (m) at which *P. macrospina* colonies were collected; total number of haplotypes (H); haplotype diversity (h); standard deviation of the haplotype diversity (S. Dev.); nucleotide diversity (P_i).

Site	N	Depth (m)	H	h	S. Dev.	P_i
MEN1	24	68	2	0.522	0.030	0.00149
MAL1	11	72	2	0.436	0.133	0.00124
MAL2	4	67	2	0.500	0.265	0.00142
MAL3	5	69	3	0.833	0.222	0.00285
MAL4	5	80	3	0.833	0.222	0.00332
MAL5	4	73	2	0.500	0.265	0.00285
MAL6	7	70	1	0.000	0.000	0.00000
MAL7	5	78	2	0.286	0.196	0.00081
MAL8	2	105	2	1.000	0.500	0.00570
MAL9	9	88	3	0.417	0.191	0.00127

Figure 2. Haplotype network of the five haplotypes (HAP1 to HAP5) obtained for IGR sequence of *P. macrospina*. Circle area is proportional to the haplotype frequency and different colors represent different sites as defined in the legend. Each line represents a single nucleotide substitution step.

Focusing on IGR marker, the pairwise estimates of F_{ST} range from -0.078 (MAL9 vs. MAL6) to 0.55 (MAL6 vs. MAL3) (Table 3). Higher pairwise values were observed between populations geographically distant (MAL6 and MAL8; 25 km distant) but also between very close populations (MAL6 and MAL3; 1.35 km distant). These pairwise comparisons were not significant at the level of $p = 0.05$ after FDR correction (Table 2). AMOVAs attributed the majority of the genetic variation to differentiation within individuals (88.78 % $p = 0.034$). No significant difference was observed between the two morphotypes ($p = 0.67$).

Table 2. Pairwise estimates of F_{ST} (Weir and Cockerham, 1984) between all the *Paramuricea macrospina* populations. No significant value was observed after FDR correction.

	MEN1	MAL1	MAL2	MAL3	MAL4	MAL5	MAL6	MAL7	MAL8
MAL1	0.0365								
MAL2	-0.0261	-0.2038							
MAL3	0.0066	0.2549	0.1429						
MAL4	0.2745	0.1959	0.0476	0.0370					
MAL5	0.0661	-0.0629	-0.0200	0.0000	-0.2381				
MAL6	0.3064	0.0852	0.0625	0.5522	0.2857	0.0625			
MAL7	0.3241	0.1171	0.0345	0.4735	0.1163	-0.0584	-0.0553		
MAL8	0.1362	0.1843	-0.0018	-0.8111	0.0330	-0.0811	0.4737	0.3973	
MAL9	0.2000	-0.0138	-0.1258	0.3493	0.0786	-0.1310	-0.0778	-0.1010	0.2671

The relationship between genetic differentiation and geographical distance was not significant ($p = 0.36$).

Microsatellite loci variability and population structure

No identical multilocus genotypes were identified so the final dataset included 76 individuals from 10 sites. No significant linkage disequilibrium was observed between any pair of loci (all $p > 0.05$ after FDR correction). The main genetic descriptors obtained from the ten sites are listed in Table 3. Mean expected heterozygosity was 0.482 ± 0.047 SD, while mean observed heterozygosity was 0.413 ± 0.073 SD (Table 3). Significant deviation from HWE was observed in MEN1, MAL1 and MAL9 with F_{IS} values higher than 0.2. None of the biparental inbreeding rates (s) were significantly different from 0 after FDR correction (not shown) suggesting that null alleles are the main factor behind the positive F_{IS} .

Table 3. Genetic descriptors for *Paramuricea macrospina* for the ten locations. N: number of individuals per site, A: mean number of alleles, H_O : observed heterozygosity, H_E : expected heterozygosity, F_{IS} : Weir and Cockerham's (1984) estimate fixation index with ten microsatellites. Asterisks indicate significant deviations from HWE; * $p < 0.01$.

	N	A	H_O	H_E	F_{IS}
MEN1	24	5.1	0.353	0.477	0.260*
MAL1	11	3.6	0.370	0.487	0.240*
MAL2	4	2.4	0.408	0.429	0.049
MAL3	5	2.7	0.375	0.506	0.259
MAL4	5	2.4	0.435	0.472	0.079
MAL5	4	2.0	0.433	0.400	-0.083
MAL6	7	2.8	0.395	0.558	0.293
MAL7	5	2.7	0.603	0.527	-0.146
MAL8	2	1.9	0.350	0.450	0.222
MAL9	9	3.7	0.410	0.515	0.205*

A power analysis conducted in Powsim indicated that our set of markers had a 100% chance to detect F_{ST} values of ≥ 0.05 . For lower values ($F_{ST} = 0.01$ and $F_{ST} = 0.005$) power decreased to 63% to 26%, respectively.

Pairwise multilocus estimates of F_{ST} ranged from -0.079 (between MAL5 and MAL8) to 0.074 (between MEN1 and MAL2) with 5 pairwise comparisons higher than 0.05 and 16 pairwise comparisons with values between 0.01 and 0.05. All the comparisons are not significant after the FDR correction (Table 4). Dest values ranged from -0.002 (between MAL1 and MAL7) to 0.180 (between MAL2 and MEN1) and all pairwise comparisons were not significant after Bonferroni correction ($p > 0.01$; Table 4).

Table 4. Pairwise multilocus estimates of F_{ST} (Weir and Cockerham, 1984) below the diagonal, and Dest above the diagonal between all the *P. macrospina* populations. All

the pairwise comparisons (F_{ST}) are not significant after FDR correction and after Bonferroni correction (Dest).

	MEN1	MAL1	MAL2	MAL3	MAL4	MAL5	MAL6	MAL7	MAL8	MAL9
MEN1		0.061	0.180	0.110	0.097	0.066	0.055	-0.031	-0.015	0.059
MAL1	0.019		0.005	0.030	0.148	0.098	-0.009	-0.002	0.063	0.030
MAL2	0.074	0.006		0.073	0.055	0.066	0.043	0.148	0.088	0.056
MAL3	0.071	0.053	0.015		0.138	0.058	0.047	-0.073	0.092	-0.010
MAL4	-0.041	0.028	-0.015	0.015		0.013	0.040	0.075	-0.006	0.074
MAL5	0.038	0.068	-0.006	-0.030	-0.065		0.035	0.033	-0.067	0.014
MAL6	0.016	-0.006	0.045	0.061	-0.029	0.018		-0.074	0.009	0.029
MAL7	-0.046	-0.026	0.049	-0.070	0.025	0.012	0.002		-0.023	-0.061
MAL8	-0.006	0.047	0.006	0.040	-0.043	-0.079	0.036	0.022		-0.031
MAL9	0.021	0.001	-0.016	0.002	-0.110	-0.043	-0.004	-0.065	-0.034	

AMOVAs attributed the majority of the genetic variation to differentiation within individuals (97.14% $p = 0.19$). A small percentage of variation was observed by the differences between morphotypes (1.92% $p = 0.05$). Based on Mantel test, there is no relationship between genetic differentiation and geographical distance ($p > 0.05$). The clustering analysis performed in STRUCTURE shown two genetic clusters as more plausible based on the Evanno's method ($K = 2$; $\Delta K = 193.71$). MAL2, MAL3, MAL5, MAL8 and MAL9 are characterized only by individuals coming from the first genetic cluster; while a mix of the two gene pools was observed in the remaining sites (Fig. 3).

Figure 3. Results of the clustering analysis conducted in STRUCTURE for $K = 2$. In the bar plot, each of 76 individuals is represented by a vertical bar indicating its estimates proportion of membership to each cluster (identified by different colors).

The DAPC analysis did not show a clear geographical pattern (Fig. 4). The two first axes of the PCA represent more than 50% of the overall variation (28.96% and 21.60%, respectively).

Figure 4. Graphical representation of the number of clusters of genetically related individuals using discriminant analysis of principal components (DAPC) when populations were used a priori as groups. Dots represent individuals and the second number in the population name is the number of individuals per population.

Spatial autocorrelation analyses did not detect significant patterns between relatedness and geographic distance (Fig. 5).

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349 Figure 5. Spatial autocorrelation analysis of Loiselle kinship coefficient (Loiselle et al.,
 350 1995). Grey lines represent 95% confidence intervals.

351

352 Discussion

353 This study provides the first data on the genetic variability and genetic structure of
 354 mesophotic populations of *Paramuricea macrospina* (60-110 m depth) on the Menorca
 355 Channels continental shelf. For this purpose, two mitochondrial markers with different
 356 evolutionary rates were combined to ten *P. clavata* cross-amplified microsatellites. The
 357 results show:

- 358 1) low genetic variability in all populations with all markers;
- 359 2) *P. macrospina* populations in the Menorca Channel are genetically connected.

In the Menorca Channel all *P. macrospina* colonies host the same mitochondrial MutS homolog (mtMutS) haplotype. This is an expected result, since mitochondrial DNA is highly conservative in octocorals (Shearer et al., 2002; Calderón et al., 2006). In the Mediterranean Sea, a study on deep populations of the red coral several mtMutS haplotypes were found, with the occurrence of a private mtMutS “deep-water” haplotype, which was never found in shallow-water populations (Costantini et al., 2010, Costantini et al., 2013).

Conversely to the mtMutS, the intergenic region between the Nad5 and the Nad4 mitochondrial genes, showed a slight variability with the identification of five haplotypes. Two of the five haplotypes were widespread across all populations and differed from the others by a single substitution. This low variability did not allow to reveal a clear geographical pattern of the haplotype distribution.

Cross-species amplification of microsatellite loci in the genus *Paramuricea* has been successfully attempted. In octocorals, cross-amplification between close related species have been successfully used (e.g. between *Corallium laauense* and *C. rubrum* (Costantini et al., 2010); within the *Eunicella* genus (Costantini et al., 2016)). The level of microsatellite polymorphism found in *P. macrospina* was generally low (mean number of alleles ranging from 2 to 5.1), also compared to values observed in *P. clavata* (from ten to 35, depending on the locus, in Mokhtar-Jamai et al., (2011); from 4 to 8 in the loci developed by Ledoux and Garrabou (unpublished data)). This low polymorphism is expected for cross-amplified microsatellites (Costantini et al., 2016; Barbara et al., 2007) and can potentially limit the statistical power of the following analysis.

Almost all *P. macrospina* populations showed high inbreeding coefficient mainly due to the presence of null alleles instead than biparental inbreeding. Despite the low

polymorphism and the low number of individuals per populations, these results suggest a random mating within populations over the study area, facilitated by the intense hydrodynamics on the Menorca Channel continental shelf (Druet et al., 2017), supporting dispersal of larvae and gametes. Up to now, no information on larval dispersal of this species exists. Larvae have been found inside the female polyps during September and October (Grinyó et al., 2018b), suggesting that they could be released in late and mid autumn when intense hydrodynamism occurs in the study area (storms events) (Grinyó et al., 2017). Overall low genetic differentiation among *P. macrospina* populations were found using both the estimators of population divergence (F_{ST} and $Dest$) at the geographical scale studied (from 1 to 60 km) (global F_{ST} = 0.001; mean $Dest$ = 0.04 ± 0.06) indicating some connectivity within the Menorca Channel. This value is lower than those reported (at the same spatial scale used here) for *P. clavata* populations across the whole Mediterranean Sea (global F_{ST} = 0.116, global F_{ST} = 0.14; Mokhtar-Jamai et al., 2011; 2013, respectively), in the Ligurian Sea (global F_{ST} = 0.154; global F_{ST} = 0.118; Padrón et al., 2018a, Pilczynska et al., 2016) across submarine canyons in the Catalan continental margin (global F_{ST} = 0.097, Perez-Portela et al., 2016), and to those observed across populations of *P. clavata* above 60 m depth in the Balearic Islands, (global F_{ST} = 0.035, Arizmendi-Mejía et al., 2015) (Table 5).

Species	Study area	Microsatellites	Spatial scale (km)	Depth (m)	Global F_{ST}	Genetic structure	Reference
<i>Paramuricea macrospina</i>	Menorca Channel (Spain)	Pcla-10, Pcla-12, Pcla-17, Pcla-20, Pcla-21, Pcla-23, Pcla-25, Pcla-27, Pcla-28, Pcla-29	60	60-100	0.001	no	This study
<i>Paramuricea clavata</i>	Ligurian Sea	Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Par_d	250	25-40	0.154	yes	Padrón et al., 2018a

<i>Paramuricea clavata</i>	Ligurian Sea	Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Par_a, Par_b, Par_d, Par_f, Par_m	60	18-30	0.118	yes	Pilczynska et al., 2016
<i>Paramuricea clavata</i>	Catalan continental margin (Spain)	Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Pcla-81, Par_a, Par_d, Par_f	50	12-63	0.097	yes	Perez-Portela et al., 2016
<i>Paramuricea clavata</i>	Balearic Islands	Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, PC 3-81, Par_a	5	30-55	0.035	yes	Arizmendi-Mejía et al., 2015
<i>Paramuricea clavata</i>	Whole Mediterranean Sea	Pcla-09, Pcla-12, Pcla-10, Pcla-14, Pcla-17, Par_d,	0.2	20	0.140	no	Mokhtar-Jamai et al., 2013
<i>Paramuricea clavata</i>	Whole Mediterranean Sea	Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Par_d	3000	10-40	0.116	yes	Mokhtar-Jamai et al., 2011

Table 5. Summary table comparing the current study with previous studies of *Paramuricea clavata* that used microsatellite markers to investigate population genetic structure in the Mediterranean Sea at different spatial and bathymetric scales.

The results of the pairwise F_{ST} , Structure and DAPC support a lack of genetic structure among populations. In fact, the clustering analysis performed with Structure show the predominance of one genetic cluster, characterizing all the populations, and a second one that includes only few individuals from different sites. Moreover, the DAPC does not evidence a geographical differentiation among sites. The genetic homogeneity is then confirmed by the absence of a significant correlation between genetic and geographical distances among colonies. The hydrodynamic conditions of the Menorca Channel (e.g. internal waves, high currents) (Druet et al., 2017), and fishing practices exerted by local fisherman could explain the observed pattern. In the study area artisanal fisherman traditionally clean their nets in situ, returning *P. macrospina* colonies attached to rhodolites few minutes after nets have been reeled (Díaz et al., 2015). Up to 95% of returned colonies land in an upward position potentially increasing

survival rates (Díaz et al., 2015) however, landing is likely to happen several meters away from their original position artificially increasing mixing between populations. Additionally, this elevated connectivity is in agreement with the abundance of the species (reaching densities of up to 33 colonies m⁻²), and with the dominance of small size classes that suggest a high recruitment rates in the study area (Grinyó et al., 2016). Nevertheless, considering the low sample size combined with the low polymorphism of the genetic markers, complementary studies genotyping larger number of colonies per populations and/or increase the geographical range of the analysis is needed to go further in these results.

AMOVA results evidence that there is no correlation between genetic differentiation and colonies morphotypes agreeing with Grinyó et al. (2018a) and Pica et al. (2018). Regarding this last point, the high morphological variability at sclerite level and colony pigmentation observed by Grinyó et al., (2018a) seems related to phenotypic plasticity (that occur frequently in corals and in gorgonians (Todd, 2008; Costantini et al., 2016)) rather than to genetic variability.

This study provides a first characterization of the genetic makeup and connectivity pattern of the ecological important mesophotic Mediterranean gorgonian *Paramuricea macrospina*. This information is useful to investigate biological processes affecting dispersal and population resilience. Based on this preliminary genetic characterization, populations in the Menorca Channel seem to be “genetically healthy” with a likelihood of being resilient to human impacts due to trawling practices on the continental shelf of the Menorca Channel at 50 – 100 m depth (Grinyó et al., 2018c). The ban of trawling in the near future when this area will become an MPA of the Natura 2000 network, should improve the conservation status of these populations. Increasing the number of microsatellite loci by developing species-specific markers (work in progress) and/or

using next generation sequencing tools, together with a more intense sampling efforts in the area will be helpful to monitor the genetic variability and structure of this mesophotic ecosystem engineering species once the MPA will be implemented.

Acknowledgements

We would like to thank the fishermen from the artisanal fishing boat GOGA (Cala Ratjada, Mallorca) and CURNIOLA (Ciutadella, Menorca). Thank to Jaume Mora Stefano Ambroso, Carlos Domínguez-Carrió, Sandra Mallol and the crew of O/V SOCIB for their help in the collection of the samples. *P. clavata* microsatellite development was done in the framework of the Project PERFECT of the TOTAL Foundation. The genetic analyses were carried out at the Department of Biological, Geological and Environmental Science (BiGeA) of the University of Bologna. JBL was supported by a postdoctoral grant (SFRH/BPD/74400/2010) from Fundação para a Ciência e a Tecnologia (FCT).

Fundings: The study has been partially supported by two Research Projects of National Interest (PRIN), funded by the Italian Ministry of University and Research: ‘Coastal bioconstructions: structures, functions, and management’ (Call 2010–2011; Prot. 2010Z8HJ5M; 2013–2015) and ‘Reef ReseArch Resistance and resilience of Adriatic mesophotic biogenic habitats to human and climate change threats’ (Call 2015; Prot. 2015J922E; 2017 –2020). Sampling was funded by the LANBAL project and the EU-ENPI CBC-Med project ECOSAFIMED ENPI-ECOSAFIMED - II-B/2.1/1073 (“Towards Ecosystem Conservation and Sustainable Artisanal Fisheries in the Mediterranean basin”) (2014-15).

References:

- Abdoullaye, D., Acevedo, I., Adebayo, A.A. et al., 2010. Permanent genetic resources added to the molecular ecology resources database 1 August 2009–30 September 2009. *Molecular Ecology Resources*, 10, 232–236.
- Arizmendi-Mejía, R., Linares Prats, C., Garrabou, J., Antunes, A., Ballesteros, E., Cebrian, E., Díaz, D., Ledoux, J.B., 2015. Combining genetic and demographic data for the conservation of a Mediterranean habitat-forming species. *PLoS One*, 10, e0119585.
- Andrello, M., Mouillot, D., Beuvier, J., Albouy, C., Thuiller, W., Manel, S., 2013. Low connectivity between Mediterranean marine protected areas: a biophysical modeling approach for the dusky grouper *Epinephelus marginatus*. *Plos One*, 8, e68564.
- Aurelle, D., Ledoux, J. B., Rocher, C., Borsa P., Chenuil, A., Feral J. P., 2011. Phylogeography of the red coral (*Corallium rubrum*): inferences on the evolutionary history of a temperate gorgonian. *Genetica*, 139, 855–869.
- Barbarà, T., Palma-Silva, C., Paggi, G.M. et al. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. *Molecular Ecology*, 16, 3759–3767. doi:10.1111/j. 1365-294X.2007.03439.x.
- Barberá, C., Moranta, J., Ordines, F., Ramón, M., Mesa, A., Díaz-Valdés, M., Grau, A.M., Massutí, E., 2012. Biodiversity and habitat mapping of Menorca Channel (western Mediterranean): implications for conservation. *Biodiversity and Conservation*, 21, 701–728. doi:10.1007/s10531-011-0210-1.
- Belkhir, K., 2004. GENETIX V. 4.0, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier (2004). 37.

- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, 57, 289–300.
- Bo, M., Bava, S., Canese, S., Angiolillo, M., Cattaneo-Vietti, R., & Bavestrello, G., 2014. Fishing impact on deep Mediterranean rocky habitats as revealed by ROV investigation. *Biological Conservation*, 171, 167–176.
- Bo, M., Bertolino, M., Borghini, M., Castellano, M., Harriague, A.C., Di Camillo, C.G., Gasparini, G., Misic, C., Povero, P., Pusceddu, A., 2011. Characteristics of the mesophotic megabenthic assemblages of the Vercelli seamount (North Tyrrhenian Sea). *PLoS One* 6, e16357.
- Calderón, I., Garrabou, J., Aurelle, D. 2006 Evaluation of the utility of COI and ITS markers as tools for population genetic studies of temperate gorgonians. *Journal of Experimental Marine Biology and Ecology*, 336, 184–197.
- Costantini, F., Fauvelot, C., Abbiati, M. 2007a. Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. *Molecular Ecology*, 16, 5168–5182. doi:10.1111/j.1365-294X.2007.03579.x
- Costantini, F., Fauvelot, C., Abbiati, M. 2007b. Fine-scale genetic structuring in *Corallium rubrum*: evidence of inbreeding and limited effective larval dispersal. *Marine Ecology Progress Series* 340, 109–119.
- Costantini, F., Abbiati, M. 2016. Into the depth of population genetics: pattern of structuring in mesophotic red coral populations. *Coral Reefs* 35, 39–52.
- Costantini, F., Ferrario, F., & Abbiati, M. 2018. Chasing genetic structure in coralligenous reef invertebrates: patterns, criticalities and conservation issues. *Scientific Reports*, 8, 5844.

- 523 Costantini, F., Carlesi, L., Abbiati, M. 2013. Quantifying spatial genetic structuring in
 524 mesophotic populations of the precious coral *Corallium rubrum*. PloS one 8,
 525 e61546.
- 526 Costantini, F., Taviani, M., Remia, A., Pintus, E., Schembri, P.J., Abbiati, M. 2010.
 527 Deep-water *Corallium rubrum* (L. 1758) from the Mediterranean Sea: preliminary
 528 genetic characterisation. Marine Ecology: an evolutionary perspective 31, 261–269.
- 529 Cowen, R.K., Lwiza, K.M.M., Sponaugle, S., Paris, C.B., Olson, D.B. 2000.
 530 Connectivity of marine populations: open or closed? Science, 287, 887-859.
 531 Doi10.1126/science.287.5454.857
- 532 David, P., Pujol, B., Viard, F., Castella, V., Goudet, J. 2007. Reliable selfing rate
 533 estimates from imperfect population genetic data. Molecular Ecology, 16, 2474–
 534 2487.
- 535 Díaz, D., Bo, M., Gaamour, A., Ambroso, S., Bavestrello, G., Bed Abdallah, L., et al.
 536 2015. Towards Ecosystem Conservation and sustainable artisanal fisheries in the
 537 Mediterranean basin. In: ICES Annual Science Conference 2015. Copenhagen,
 538 Denmark.
- 539 Druet, M., Acosta, J., Muñoz, A., Barberá, C., et al. 2017. Sedimentary bedforms in the
 540 Menorca Channel Region, Balearic Islands (Western Mediterranean). In Atlas of
 541 Bedforms in the Western Mediterranean, 151-157.
- 542 Earl, D.A., vonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program
 543 for visualizing STRUCTURE output and implementing the Evanno method.
 544 Conservation Genetic Resources, 4, 359–361.
- 545 Epperson, B.K., Li, T. 1996. Measurement of genetic structure within populations using
 546 Moran's spatial autocorrelation statistics. Proceedings of the National Academy of
 547 Sciences, 93, 10528–10532.

- 548 Excoffier, L., Lischer, H.E. 2010. Arlequin suite ver 3.5: a new series of programs to
549 perform population genetics analyses under Linux and Windows. *Molecular*
550 *Ecology Resources*, 10, 564–567.
- 551 Excoffier, L., Smouse, P. E., & Quattro, J. M. 1992. Analysis of molecular variance
552 inferred from metric distances among DNA haplotypes: application to human
553 mitochondrial DNA restriction data. *Genetics*, 131, 479–491.
- 554 Ferrigno, F., Appolloni, L., Russo, G. F., & Sandulli, R., 2018. Impact of fishing
555 activities on different coralligenous assemblages of Gulf of Naples (Italy). *Journal*
556 *of the Marine Biological Association of the United Kingdom*, 98, 41–50.
- 557 France, S.C., Hoover, L.L. 2001. Analysis of variation in mitochondrial DNA sequences
558 (ND3, ND4L, MSH) among Octocorallia (=Alcyonaria) (Cnidaria: Anthozoa).
559 *Bulletin of the Biological Society of Washington* 10, 110–118.
- 560 Gerlach, G., Jueterbock, A., Kraemer, P., Deppermann, J., Harmand, P. 2010.
561 Calculations of population differentiation based on G_{ST} and D : forget G_{ST} but
562 not all of statistics! *Molecular Ecology* 19, 3845–3852.
- 563 Grinyó, J., Gori, A., Ambroso, S., Purroy, A., Calatayud, C., Dominguez-Carrió, C.,
564 Coppari, M., Lo Iacono, C., López-González, P.J., Gili, J.M. 2016. Diversity,
565 distribution and population size structure of deep Mediterranean gorgonian
566 assemblages (Menorca Channel, Western Mediterranean Sea). *Progress in*
567 *Oceanography*, 145, 42–56. doi:10.1016/j.pocean.2016.05.001
- 568 Grinyó, J., Isla, E., Peral, L., Gili, J.M. 2017. Composition and temporal variability of
569 particle fluxes in an insular canyon of the northwestern mediterranean Sea.
570 *Progress in Oceanography*, 159, 323–339. doi:10.1016/j.pocean.2017.11.005
- 571 Grinyó, J., Gori, A., López-González, P.J., et al. 2018a. Morphological features of the
572 gorgonian *Paramuricea macrospina* on the continental shelf and shelf edge

- 573 (Menorca Channel, Western Mediterranean Sea). *Marine Biology Research*, 14, 30-
574 40
- 575 Grinyó, J., Viladrich, N., Díaz, D., Muñoz, A., Mallol, S., Salazar, J., Castillo, R., Gili
576 J.M., Gori, A. 2018b. Reproduction, energy storage and metabolic requirements in
577 a mesophotic population of the gorgonian *Paramuricea macrospina*. *PloS one* 13,
578 e0203308.
- 579 Grinyó, J., Gori, A., Greenacre, M., Requena, S., Canepa, A., Lo Iacono, C., Ambroso,
580 S., Purroy, A., Gili, J.M. 2018c. Megabenthic assemblages in the continental shelf
581 edge and upper slope of the Menorca Channel, Western Mediterranean Sea.
582 *Progress in Oceanography*, 162, 40-51. Doi. 10.1016/j.pocean.2018.02.002
- 583 Hardy, O.J., Vekemans, X. 2002. Spagedi: a versatile computer program to analyse
584 spatial genetic structure at the individual or population levels. *Molecular Ecology*
585 *Notes*, 2, 618–620.
- 586 Hastings, A., Harrison, S., 1994. Metapopulation dynamics and genetics. *Annual*
587 *Review of Ecology and Systematics*, 25, 167-188.
- 588 Hillis, D.M., Moritz, C. 1990. *Molecular systematics*. Sinauer, Sunderland, MA
589 Huelsenbeck
- 590 Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K. 2009. Inferring weak population
591 structure with the assistance of sample group information. *Molecular Ecology*
592 *Resources*, 9, 1322–1332.
- 593 Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation
594 program for dealing with label switching and multimodality in analysis of
595 population structure. *Bioinformatics*, 23, 1801–1806.
- 596 Jombart, T., Devillard, S., Balloux, F. 2010. Discriminant analysis of principal
597 components: a new method for the analysis of genetically structured populations.

- 598 BMC Genetics, 11, 94.
- 599 Jost, L. 2008- G_{ST} and its relatives do not measure differentiation. Molecular Ecology
600 17, 4015–4026.
- 601 Kahng, S.E., Copus, J.M., Wagner, D. 2017. Mesophotic coral ecosystems. Marine
602 animal forests: the ecology of benthic biodiversity hotspots. Springer International
603 Publishing, Cham, Switzerland, 185-206.
- 604 Kahng, S., Copus, J., Wagner, D. 2014. Recent advances in the ecology of mesophotic
605 coral ecosystems (MCEs). Current Opinion in Environmental Sustainability, 7, 72–
606 81. doi:10.1016/j.cosust.2013.11.019
- 607 Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I. 2015.
608 CLUMPAK: a program for identifying clustering modes and packaging population
609 structure inferences across K. Molecular Ecology Resources, 15, 1179–1191.
- 610 Ledoux, J. B., Mokhtar-Jamai, K., Roby, C., Feral, J.P., Garrabou, J., Aurelle, D. 2010a.
611 Genetic survey of shallow populations of the Mediterranean red coral [*Corallium*
612 *rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping
613 nuclear diversity and implications for conservation. Molecular Ecology, 19, 675–
614 690
- 615 Ledoux, J.-B., Garrabou, J., Bianchimani, O. et al. 2010b. Fine-scale genetic structure
616 and inferences on population biology in the threatened Mediterranean red coral,
617 *Corallium rubrum*. Molecular Ecology, 19, 4204–4216.
- 618 Ledoux, J-B., Tarnowska, K., Gerard, K., Lhuillier, E., Jacquemin B, Weydmann A,
619 Feral J-P, Chenuil, A. 2012. Fine-scale spatial genetic structure in the brooding sea
620 urchin *Abatus cordatus* suggests vulnerability of the Southern Ocean marine
621 invertebrates facing global change. Polar Biology 35, 611 – 623.

- Loiselle, B.A., Sork, V.L., Nason, J., Graham, C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, 82, 1420–1425.
- Masmoudi, M.B., Chaoui, L., Topçu N.E. Hammami, P., Kara, M.H., Aurelle, D. 2016. Contrasted levels of genetic diversity in a benthic Mediterranean octocoral: Consequences of different demographic histories?. *Ecology and Evolution*, 6, 8665–8678.
- Mokhtar-Jamaï, K., Pascual, M., Ledoux, J.B., et al. 2011. From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. *Molecular Ecology*, 20, 3291–3305.
- Mokhtar-Jamaï, K., Coma, R., Wang, J., et al. 2013. Role of evolutionary and ecological factors in the reproductive success and the spatial genetic structure of the temperate gorgonian *Paramuricea clavata*. *Ecology and Evolution*, 3, 1765–1779.
- Ordines, F., Ramón, M., Rivera, J., Rodríguez-Prieto, C., Farriols, M. T., Guijarro, B., et al. 2017. Why long term trawled red algae beds off Balearic Islands (western Mediterranean) still persist?. *Regional Studies in Marine Science*, 15, 39–49.
- Padrón, M., Costantini, F., Bramanti, L., Guizien, K., & Abbiati, M. 2018a. Genetic connectivity supports recovery of gorgonian populations affected by climate change. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28, 776–787.
- Padrón, M., Costantini, F., Baksay, S., Bramanti, L., & Guizien, K. 2018b. Passive larval transport explains recent gene flow in a Mediterranean gorgonian. *Coral Reefs*, 37, 495–506.
- Peakall, R., Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288–295.

- 647 Pérez-Portela, R., Cerro-Gálvez, E., Taboada, S., Tidu, C., Campillo-Campbell, C.,
 648 Mora, J., Riesgo, A. 2016. Lonely populations in the deep: genetic structure of red
 649 gorgonians at the heads of submarine canyons in the north-western Mediterranean
 650 Sea. *Coral Reefs*, 35:1013-1026.
- 651 Pica, D., Calcinai, B., Polisenò, A., Trainito, E., & Cerrano, C. 2018. Distribution and
 652 phenotypic variability of the Mediterranean gorgonian *Paramuricea macrospina*
 653 (Cnidaria: Octocorallia). *The European Zoological Journal*, 85: 392-408.
- 654 Pilczynska, J., Cocito, S., Boavida, J., Serrão, E., Queiroga, H. 2016. Genetic diversity
 655 and local connectivity in the Mediterranean red gorgonian coral after mass
 656 mortality events. *PloS one*, 11, e0150590.
- 657 Polisenò, A., Altuna, A., Cerrano, C., Wörheide, G., Vargas S. 2017. Historical
 658 biogeography and mitogenomics of two endemic Mediterranean gorgonians
 659 (Holaxonia, Plexauridae). *Organisms Diversity & Evolution*, 17, 365-373.
- 660 Pont-Kingdon, G.A., Okada, N.A., Macfarlane, J.L., et al. 1995. A coral mitochondria
 661 *lmutS* gene. *Nature* 375, 109–11.
- 662 Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using
 663 multilocus genotype data. *Genetics*, 155, 945–959.
- 664 Raymond, M., Rousset, F. 1995. An exact test for population differentiation. *Evolution*
 665 49, 1280–1283.
- 666 R Core Team. 2014. R: a language and environment for statistical computing. R
 667 Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>.
- 668 Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population
 669 structure. *Molecular Ecology Notes* 4, 137–138.
- 670 Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP
 671 software for Windows and Linux. *Molecular Ecology Resources*, 8, 103–106.

- 672 Rozas, J., Rozas, R. 1999. DnaSP version 3: an integrated program for molecular
673 population genetics and molecular evolution analysis. *Bioinformatics* (Oxford,
674 England), 15, 174-175.
- 675 Rozen, S., Skaletsky, H.J. 2000. Primer3 on the WWW for general users and for
676 biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics methods and
677 protocols: methods in molecular biology*. Humana Press, Totowa, pp 365–386.
- 678 Ryman, N., Palm, S. 2006. POWSIM: a computer program for assessing statistical
679 power when testing for genetic differentiation. *Molecular Ecology Notes* 6, 600–
680 602.
- 681 Santín, A., Grinyó J., Ambroso, S., Uriz, M.J., Gori, A., Dominguez-Carrió C., Gili JM.
682 2017. Sponge assemblages on the deep Mediterranean continental shelf and slope
683 (Menorca Channel, Western Mediterranean Sea). *Deep Sea Research Part I:
684 Oceanographic Research Papers*, 131, 75-86.
- 685 Shearer, T.L., van Oppen, M.J., Romano, S.L., Worheide, G. 2002. Slow mitochondrial
686 DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, 11,
687 2475–2487.
- 688 Todd, P.A. 2008. Morphological plasticity in scleractinian corals. *Biological reviews of
689 the Cambridge Philosophical Society* 83, 315–337.
- 690 Topçu, E.N., Öztürk, B. 2015. Composition and abundance of octocorals in the Sea of
691 Marmara, where the Mediterranean meets the Black Sea. *Scientia Marina*, 79, 125-
692 135.
- 693 Vekemans, X., Hardy, O.J. 2004. New insights from fine-scale spatial genetic structure
694 analyses in plant populations. *Molecular Ecology*, 13, 921–935 DOI
695 10.1046/j.1365-294X.2004.02076.x.

696 Weir, B.S., Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population
697 structure. *Evolution* 38, 1358–1360.

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Highlight

Paramuricea macrospina lives in the Menorca Channel between 60 and 100 meters depth.

Genetic diversity and structure of *P. macrospina* have been explored.

Connectivity among Menorca Channel' populations of *P. macrospina* were observed.

No genetic differentiation was observed among morphotypes.

This is in accordance with the high recruitment rates observed in the area.