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

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Abstract

Gorgonians play an important structural and functional role promoting high diversity and biomass of associated fauna. Up to now, studies on gorgonian ecology in the Mediterranean Sea have been focused mainly on the SCUBA diving depth range. Although increased availability of remotely operated vehicles allowed access to the deeper areas, gorgonian assemblages located on continental shelf and slopes are still barely known. Gorgonian assemblages on continental shelves are extremely vulnerable to anthropogenic impacts, especially bottom trawling and longline fishing. To implement effective management and conservation policies it is crucial to understand patterns of genetic structure among populations since connectivity enhances the resilience of populations. *Paramuricea macrospina* is a key structuring gorgonian in the Menorca Channel's (Balearic Archipelago) outer continental shelf where it covers vast extensions, reaching very high densities. Combining two mitochondrial makers and 10 microsatellites, this study gives the first insight into the genetic diversity and population structure of *P. macrospina* between 60 and 100 meters depth in the Menorca Channel and at a horizontal spatial scale of about 60 Km. Overall, we demonstrate a low genetic diversity and a lack of genetic structure among populations, which may be explained by the geomorphology and hydrodynamic features of the Menorca Channel (e.g. internal waves, high currents). Our study suggests some connectivity among *P. macrospina* populations in accordance with the high recruitment rates observed. This connectivity may increase the resilience and foster the recovery of impacted populations since the study area will become a Marine Protected Area of the Natura 2000 network in the near future. Nevertheless, complementary studies based on a larger sample size should be 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 ± 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, Poliseno 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'-TTAGGCCCACTGGAATA-3’
- **IGR 14704_R**: 5’-GAGACCCTCTAACCCTT-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 (Na), the expected heterozygosity (He), the observed heterozygosity (Ho), and the inbreeding index (FIS) 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 (Ne) 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 DEMEthics 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 extracts 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). \textit{P. macrospina} mtMutS sequence vary only of 11 nucleotides (598/609 bp identity) from the sequence of the congeneric species \textit{P. clavata} (accession number: LT576167, Poliseno 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 \textit{P. macrospina} colonies were collected; total number of haplotypes (H); haplotype diversity (h); standard deviation of the haplotype diversity (S. Dev.); nucleotide diversity (\( P_i \)).

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

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

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.

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

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).

<table>
<thead>
<tr>
<th></th>
<th>MEN1</th>
<th>MAL1</th>
<th>MAL2</th>
<th>MAL3</th>
<th>MAL4</th>
<th>MAL5</th>
<th>MAL6</th>
<th>MAL7</th>
<th>MAL8</th>
<th>MAL9</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN1</td>
<td>0.061</td>
<td>0.180</td>
<td>0.110</td>
<td>0.087</td>
<td>0.066</td>
<td>0.055</td>
<td>0.031</td>
<td>0.015</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>MAL1</td>
<td>0.019</td>
<td>0.005</td>
<td>0.030</td>
<td>0.148</td>
<td>0.098</td>
<td>0.009</td>
<td>0.002</td>
<td>0.063</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>MAL2</td>
<td>0.074</td>
<td>0.006</td>
<td>0.073</td>
<td>0.055</td>
<td>0.066</td>
<td>0.043</td>
<td>0.148</td>
<td>0.088</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td>MAL3</td>
<td>0.071</td>
<td>0.053</td>
<td>0.015</td>
<td>0.138</td>
<td>0.058</td>
<td>0.047</td>
<td>0.073</td>
<td>0.092</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>MAL4</td>
<td>-0.041</td>
<td>0.028</td>
<td>-0.015</td>
<td>0.015</td>
<td>0.013</td>
<td>0.040</td>
<td>0.075</td>
<td>-0.006</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>MAL5</td>
<td>0.038</td>
<td>0.068</td>
<td>-0.006</td>
<td>-0.030</td>
<td>-0.065</td>
<td>0.035</td>
<td>0.033</td>
<td>-0.067</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>MAL6</td>
<td>0.016</td>
<td>-0.006</td>
<td>0.045</td>
<td>0.061</td>
<td>-0.029</td>
<td>0.018</td>
<td>-0.074</td>
<td>0.009</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>MAL7</td>
<td>-0.046</td>
<td>-0.026</td>
<td>0.049</td>
<td>-0.070</td>
<td>0.025</td>
<td>0.012</td>
<td>0.002</td>
<td>-0.023</td>
<td>-0.061</td>
<td></td>
</tr>
<tr>
<td>MAL8</td>
<td>-0.006</td>
<td>0.047</td>
<td>0.006</td>
<td>0.040</td>
<td>-0.043</td>
<td>-0.079</td>
<td>0.036</td>
<td>0.022</td>
<td>-0.031</td>
<td></td>
</tr>
<tr>
<td>MAL9</td>
<td>0.021</td>
<td>0.001</td>
<td>-0.016</td>
<td>0.002</td>
<td>-0.110</td>
<td>-0.043</td>
<td>-0.004</td>
<td>-0.065</td>
<td>-0.034</td>
<td></td>
</tr>
</tbody>
</table>

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

Discussion

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

1) low genetic variability in all populations with all markers;

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 \(D_{est}\)) at the geographical scale studied (from 1 to 60 km) (global \(F_{ST}\) = 0.001; mean \(D_{est} = 0.04 \pm 0.06\)) 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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Study area</th>
<th>Microsatellites</th>
<th>Spatial scale (km)</th>
<th>Depth (m)</th>
<th>Global (F_{ST})</th>
<th>Genetic structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paramuricea macrospina</em></td>
<td>Menorca Channel (Spain)</td>
<td>Pcla-10, Pcla-12, Pcla-17, Pcla-20, Pcla-21, Pcla-23, Pcla-25, Pcla-27, Pcla-28, Pcla-29</td>
<td>60</td>
<td>60-100</td>
<td>0.001</td>
<td>no</td>
<td>This study</td>
</tr>
<tr>
<td><em>Paramuricea clavata</em></td>
<td>Ligurian Sea</td>
<td>Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Par_d</td>
<td>250</td>
<td>25-40</td>
<td>0.154</td>
<td>yes</td>
<td>Padrón et al., 2018a</td>
</tr>
<tr>
<td>Paramuricea clavata</td>
<td>Ligurian Sea</td>
<td>Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Par_a, Par_b, Par_d, Par_f, Par_m</td>
<td>60 18-30 0.118 yes</td>
<td>Pilczynska et al., 2016</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>-------------------------------------------------------------------------------</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paramuricea clavata</td>
<td>Catalan continental margin (Spain)</td>
<td>Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, Pcla-81, Par_a, Par_d, Par_f</td>
<td>50 12-63 0.097 yes</td>
<td>Perez-Portela et al., 2016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paramuricea clavata</td>
<td>Balearic Islands</td>
<td>Pcla-09, Pcla-10, Pcla-12, Pcla-14, Pcla-17, PC 3–81, Par_a</td>
<td>5 30-55 0.035 yes</td>
<td>Arizmendi-Mejia et al., 2015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paramuricea clavata</td>
<td>Whole Mediterranean Sea</td>
<td>Pcla-09, Pcla-12, Pcla-10, Pcla-14, Pcla-17, Par_d</td>
<td>0.2 20 0.140 no</td>
<td>Mokhtar-Jamai et al., 2013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paramuricea clavata</td>
<td>Whole Mediterranean Sea</td>
<td>Pcla-09, Parcla-10, Pcla-12, Pcla-14, Pcla-17, Par_d</td>
<td>3000 10-40 0.116 yes</td>
<td>Mokhtar-Jamai et al., 2011</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<sub>ST</sub>, 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.

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B/2.1/1073 (‘Towards Ecosystem Conservation and Sustainable Artisanal Fisheries in
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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.