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Almudena Cánovas-Molina, Monica Montefalcone, Giorgio Bavestrello, Mauatassem Billah Masmoudi, Anne Haguenaer, Pachka Hammami, Lamya Chaoui, Mohammed Hichem Kara, D. Aurelle

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1 **From depth to regional spatial genetic differentiation of *Eunicella cavolini* in the NW**

2 **Mediterranean**

3 Almudena Cánovas-Molina<sup>1, \*</sup>, Monica Montefalcone<sup>1</sup>, Giorgio Bavestrello<sup>1</sup>, Mauatassem Billah  
4 Masmoudi<sup>2, 3</sup>, Anne Haguenaue<sup>3</sup>, Pachka Hammami<sup>3</sup>, Lamy Chaoui<sup>2</sup>, Mohammed Hichem Kara<sup>2</sup>  
5 and Didier Aurelle<sup>3, 4\*</sup>

6

7 <sup>1</sup> DiSTAV, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università di Genova,  
8 Corso Europa 26, 16132 Genova, Italy.

9 <sup>2</sup> Laboratoire Bioressources Marines, Université d'Annaba, Badji Mokhtar, BP 230, Oued Kouba,  
10 23008, Annaba, Algeria.

11 <sup>3</sup> Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France

12 <sup>4</sup> Aix Marseille Univ., Université de Toulon, CNRS, IRD, MIO UM 110 , 13288, Marseille, France

13

14 \*Corresponding authors: Tel.: +33 486 09 06 22.

15 E-mail addresses: almucanovass@gmail.com (A. Cánovas-Molina), didier.aurelle@univ-amu.fr  
16 (D. Aurelle).

17 **Abstract**

18 Connectivity studies in the marine realm are of great importance to understand the evolutionary  
19 potential of populations in a context of growing pressures on the marine environment. Here, we  
20 investigated the effect of the local, regional and depths spatial scale on population genetic structure  
21 of the yellow gorgonian *Eunicella cavolini*, one of the most common octocoral species of the  
22 Mediterranean hard-bottom communities. This species, along with other sessile metazoans typical  
23 of coralligenous ecosystems, plays an important role in supporting biodiversity, but is also  
24 impacted by direct and indirect consequences of human activities, such as physical destruction or  
25 mortality events due to thermal anomalies. Samples were taken from 15 sites located in the  
26 Ligurian Sea (NW Mediterranean) in two adjacent regions 100s kilometres apart, i.e. from the areas  
27 of Marseille (France) and Portofino (Genoa, Italy), and were analyzed using six microsatellite loci.  
28 A pattern of isolation by distance was observed at the regional as well as the local scales. Although  
29 *E. cavolini* showed less genetic structure than other Mediterranean octocorallian species, we  
30 observed a significant genetic differentiation between populations few kilometres apart. A low  
31 genetic differentiation was also observed between shallow and deep populations. The occurrence of  
32 genetically differentiated populations of *E. cavolini* at the scale of kilometres has important  
33 consequences for the management of this species and of the associated communities.

34

35 **Key-words:** *Eunicella cavolini*, gorgonian, connectivity, spatial scale, Mediterranean, genetic  
36 structure.

37 **1. Introduction**

38 In a context of growing pressures affecting the marine environments, estimating connectivity  
39 among populations is essential since the scale of larval dispersal is crucial for understanding  
40 species reorganization and populations evolution after large-scale disturbances (Bellwood et al.,  
41 2004; Salm et al., 2006). Size and interconnectivity of populations are important parameters to  
42 estimate for conservation strategies, since small and isolated populations are vulnerable to  
43 inbreeding depression, limited spread of beneficial alleles (Alleaume-Benharira et al., 2006). and  
44 loss of genetic diversity, which might reduce their evolutionary potential and increase their risk of  
45 extinction (Saccheri et al., 1998). Population genetics describes connectivity by comparing allele  
46 frequencies among spatially discrete subpopulations. High levels of genetic similarity between  
47 populations can indicate gene flow over time, usually through larval dispersal, whereas significant  
48 differentiation between populations can correspond to significant and persistent barriers to larval  
49 exchange. Nevertheless, the observed level of genetic differentiation depends on the interaction  
50 between gene flow, genetic drift and mutation (depending on the time scale or molecular markers).

Commenté [DA1]: c'est un essai, à voir si c'est mieux?

51 For most benthic marine species with complex life cycles, the exchange of individuals among  
52 populations occurs primarily through the pelagic larval stage (Cowen and Sponaugle, 2009). These  
53 exchanges depend on biotic and abiotic factors including spawning outputs, larval dispersal, habitat  
54 availability, hydrodynamics and trophic interactions (Padrón and Guizien, 2015; Pineda, 2000).  
55 Evidence from direct and indirect approaches using geochemical and genetic techniques suggests  
56 that the genetic structure of marine populations, while often low, ranges from fully open to fully  
57 closed (Cowen and Sponaugle, 2009).

58 Understanding the scale of population connectivity is fundamental for the development of suitable  
59 conservation strategies and for the design of marine reserves (Almany et al., 2009; Palumbi, 2004;  
60 Shanks et al., 2003). Decisions **predicated** on the assumption that larvae disperse at long distance  
61 may lead to erroneous actions if this assumption is wrong (Cowen et al., 2000). Studies regarding  
62 connectivity conducted in the Mediterranean Sea showed the existence of different patterns of  
63 genetic differentiation, ranging from complete panmixia to an east–west or north–south genetic

Commenté [DA2]: Made?

64 partitioning. These findings warn against any generalization based on the presently available  
65 information and strongly indicate the need to obtain genetic connectivity data from a wider array of  
66 species, including keystone species such as habitat formers as well as rare or threatened species  
67 (Paterno et al., 2017).

68 Among the benthic sessile invertebrates of the Mediterranean, gorgonian octocorals play an  
69 important role, contributing to the enhancement of the overall structural complexity, biomass, and  
70 species diversity of habitats by their three-dimensional growth pattern (Ballesteros, 2006; Gili and  
71 Ros, 1985; True, 1970). Furthermore, organisms living in habitats characterized by the presence of  
72 gorgonian corals may experience a sort of buffer zone where environmental alterations occur more  
73 slowly and within narrower ranges with respect to the surrounding 'unforested' environment (Ponti  
74 et al., 2014).

75 The slow population dynamics of gorgonians makes them susceptible to a wide range of direct or  
76 indirect anthropogenic stressors: mechanical damage (mainly caused by fishing and unregulated  
77 recreational diving), pollution and mucilaginous algal aggregates (Bavestrello et al., 1997, Coma et  
78 al., 2004; Mistri and Ceccherelli, 1996), biological invasions (Cebrian et al., 2012), bacterial  
79 pathogens and mass mortality events linked to thermal anomalies (Cerrano et al., 2000; Garrabou et  
80 al., 2009). For example, during the 2003 mortality event, the yellow gorgonian *Eunicella cavolini*  
81 was affected from the coasts of Provence in south-east France to the gulf of Naples in south Italy,  
82 with a percentage of affected colonies reaching 50.8% (Garrabou et al., 2009). This species, along  
83 with other anthozoans, is also impacted by other human pressures such as, for example, the side-  
84 effects of fisheries (Cánovas-Molina et al., 2016a; Topçu and Öztürk, 2015). Considering these  
85 threats and the need to extend the knowledge on population dynamics and connectivity for  
86 conservation and ecosystem-based management purposes, several studies have been performed on  
87 the genetic structure of Mediterranean species from the coralligenous assemblages, most of them  
88 on octocorals. These previous genetic studies revealed strong population genetic structure  
89 potentially because of short-distance larval dispersal for some coralligenous species such as the red  
90 coral *Corallium rubrum* (Abbiati et al., 2009; Boavida et al., 2016; Costantini et al., 2007a, b;

91 Ledoux et al., 2010), the red gorgonian *Paramuricea clavata* (Arizmendi-Mejía et al., 2015;  
92 Mokhtar-Jamaï *et al.*, 2011), the yellow gorgonian *E. cavolini* (Masmoudi et al., 2016), the only  
93 symbiotic Mediterranean gorgonian *Eunicella singularis* (Constantini et al., 2016; Pey et al., 2013)  
94 or the sponge *Crambe crambe* (Duran et al., 2004). Regarding *E. cavolini*, the first molecular study  
95 gave little information on the genetic structure of this species because of a lack of informative  
96 markers (Calderón et al., 2007). The use of microsatellite markers in *E. cavolini* allowed the  
97 identification of different genetic clusters in the Mediterranean corresponding to the main  
98 geographical regions, and with different levels of genetic diversity: the allelic richness of eastern  
99 Mediterranean populations (i.e. from Turkey) was two-fold lower than in Algeria (Masmoudi et al.,  
100 2016). The genetic structure in the region of Marseille (France) indicated a differentiation between  
101 most populations (Masmoudi et al., 2016). Although no differentiation was observed between  
102 depths (between 20 m and 40 m depth) at a given site (Masmoudi et al., 2016), significant  
103 differences in thermo-tolerance were demonstrated at the bathymetrical scale (Pivotto et al., 2015).  
104 In the present work, we focused on the yellow gorgonian *Eunicella cavolini* (Koch, 1887), an  
105 octocoral species widely distributed in the Mediterranean, which can be observed from less than 10  
106 m to more than 100 m depth (Sini et al., 2015). The genetic structure of this species has been  
107 studied mainly at the basin-wide Mediterranean spatial scale (Masmoudi et al., 2016). Definition of  
108 the boundaries of Marine Protected Areas (MPAs) should consider the connectivity among habitats  
109 for management and conservation of marine species (Palumbi, 2004), and this requires a more  
110 precise study of connectivity at both the regional and the local spatial scales. In this sense, NW  
111 Mediterranean offers an interesting context for genetic structure studies at different spatial scales  
112 where the yellow gorgonian forms populations from 15 m depth reaching here the maximum  
113 densities of the Mediterranean sites studied by Sini et al. (2015). The absence or low genetic  
114 differentiation between shallow and deep *E. cavolini* observed at Marseille (SE France) is of great  
115 importance considering the impact of climate change and the possibility of exchanges between  
116 depths with different thermal conditions (Pivotto et al., 2015), and it should be tested in other areas  
117 as well. Located 300 km apart in the same Ligurian Sea, Portofino (NW Italy) offers a perfect

118 location for the genetic study of *Eunicella cavolini* populations at regional, local and depth scale.

119 Given the lack of information on the effects of the spatial scale on genetic structure of this species,  
120 our aims were:

121 i) to examine the genetic differentiation of *E. cavolini* at the regional scale in the Ligurian  
122 Sea, by focusing on the differences between two adjacent regions 300s kilometres apart,  
123 i.e. Marseille in SE France and Portofino in NW Italy, where a number of MPAs have  
124 already been established;

125 ii) to analyze the genetic differentiation at the local scale 10s kilometres apart, i.e. within  
126 the areas of Marseille and Portofino, in order to acquire insights into the dispersal abilities  
127 of this species at small scale;

128 iii) to investigate the vertical connectivity between populations by including samples from  
129 different depths. We tested whether the absence of a vertical genetic differentiation  
130 previously observed in Marseille can be extended in other areas of the Ligurian Sea.

131

## 132 **2. Materials and methods**

### 133 2.1 Samples collection

134 Colonies of *Eunicella cavolini* were sampled by scuba divers removing a 3 cm branch (usually  
135 terminal) using sea-snips (n=628 colonies in total; see Tab. 1). In order to have colonies separated  
136 by few and hundreds of km and at different depths, samples were collected few kilometres apart at  
137 five sites in the MPA of Portofino (Genoa, NW Italy) and at ten sites in the area around Marseille  
138 (SE France) (Fig. 1). Samples around Marseille were taken at two depths (20 and 40 m) in Riou,  
139 Veyron and Méjean sites and at one depth in the other seven sites. Nine colonies were sampled on  
140 artificial reefs (REE), which were installed in 2007-2008. Samples at Portofino were collected at  
141 two depths (20 m and 40 m), except for Punta Cervara (PAR), where *E. cavolini* only occurs at 13-  
142 18 m depth. Geographical distances between sites in the Marseille area ranged from 2 km to 100  
143 km whereas in the Portofino area they ranged from 1 km to 6 km (Fig. 1). Distances between  
144 Marseille and Portofino ranged from 381 km to 274 km. Sample size was even in all sites, around

**Commenté [DA3]:** Suggestion: "In the area of Marseille, a new Marine Protected Area has recently been designed. It is then important to study its potential connectivity with other populations and MPAs along the coast." (we can also indicate that there are almost no other populations on the west side...)

145 30 colonies per site were taken. Once collected, samples were preserved in 95% ethanol at -20°C  
 146 until DNA extraction. Permits for sampling in the MPA of Portofino were obtained from the local  
 147 competent authority of the *Area Marina Protetta Portofino*. All sampling activities were performed  
 148 in accordance with the Italian and French laws.

149

150 Table 1. Information on the samples collection of *Eunicella cavolini* in Marseille (France)  
 151 and Portofino (Italy). Sampling dates are indicated (day/ month/ year).

Area	Site	Label	Depth (m)	Latitude	Longitude	Sample size (n)	Date
Portofino	Punta Cervara-Paraggi	PAR	14	44° 18.653'N	9° 12.785'E	28	8/05/2015
Portofino	Punta Faro shallow	PFS	20-23	44° 17.910'N	9° 13.156'E	22	12/05/2015
Portofino	Punta Faro deep	PFD	36-40	44° 17.910'N	9° 13.156'E	15	12/05/2015
Portofino	Casa del Sindaco shallow	CSS	19-21	44° 18.182'N	9° 12.147'E	33	6/05/2015
Portofino	Casa del Sindaco deep	CSD	38-40	44° 18.182'N	9° 12.147'E	26	6/05/2015
Portofino	Secca Gonzatti shallow	SGS	18-20	44° 18.547'N	9° 10.686'E	29	28/04/2015
Portofino	Secca Gonzatti deep	SGD	39-42	44° 18.547'N	9° 10.686'E	31	28/04/2015
Portofino	Punta Torretta shallow	PTS	15	44° 18.713'N	9° 09.979'E	30	22/04/2015
Portofino	Punta Torretta deep	PTD	35	44° 18.713'N	9° 09.979'E	31	22/04/2015
Marseille	Sec de Sarraniers	SAR	40	42° 59.272'N	6° 17.030'E	33	7/11/2011
Marseille	Cave 3PP	3PP	15	43° 09.795'N	5° 36.000'E	30	17/02/2012
Marseille	Riou shallow	RIS	20	43° 10.360'N	5° 23.420'E	32	15/03/2012
Marseille	Riou deep	RID	40	43° 10.360'N	5° 23.420'E	31	15/03/2012
Marseille	Pouard on Île Plane	POU	15-25	43° 11.340'N	5° 23.130'E	30	14/02/2012
Marseille	Veyron shallow	VES	20	43° 12.414'N	5° 15.176'E	30	24/11/2011
Marseille	Veyron deep	VED	40	43° 12.414'N	5° 15.176'E	30	24/11/2011
Marseille	Somlit	SOM	58	43° 14.050'N	5° 17.050'E	30	28/11/2011
Marseille	Saména	SAM	10	43° 13.780'N	5° 20.880'E	34	16/01/2012
Marseille	Cap Cavau	CAV	25	43° 15.630'N	5° 17.390'E	29	1/02/2012
Marseille	Méjean shallow	MJS	18-20	43° 19.700'N	5° 13.480'E	33	16/03/2012
Marseille	Méjean deep	MEJ	30-40	43° 19.700'N	5° 13.480'E	31	24/02/2012
Marseille	Artificial reef	REE	15-20	43° 15.702'N	5° 20.398'E	10	7/05/2015

152



153 **Fig. 1.** Geographical location of the 15 sites in the Ligurian Sea where populations of *Eunicella*  
154 *cavolini* were sampled and analyzed in the present study. A: ten sampling sites in the area of  
155 Marseille (SE France); B: five sampling sites in the area of Portofino (NW Italy). Refer to Tab. 1  
156 for the labels of each site.

157

## 158 2.2 DNA extraction and microsatellite genotyping

159 Total genomic DNA was extracted using QIAamp® DNA Mini Kit (Qiagen®) according to  
160 manufacturer's instructions. Individuals were genotyped at six microsatellite loci: C21, C30, C40,  
161 S14, EVER007, EVER009 (Holland et al., 2013; Molecular Ecology Resources Primer  
162 Development Consortium et al., 2010; Pivotto et al., 2015). The loci were amplified through PCR  
163 Multiplex using the Type-it® Microsatellite PCR Kit (Qiagen®). PCR amplifications were done in a  
164 final reaction volume of 10 µL containing 1 µL of DNA, 5 µL of MasterMix, 3 µL of H<sub>2</sub>O Milli-  
165 Q® and 1 µL of primer mix (with each primer at 2 µM).

166 Electrophoresis was carried out at the IMBE laboratory on an ABI 3130 genetic analyzer and at the  
167 Genotyping and Sequencing facility at Montpellier (Plateforme Génotypage - Séquençage,  
168 Université de Montpellier) on an ABI 3500xL Genetic Analyzer (Applied Biosystems), using  
169 GeneScan LIZ 600 (Applied Biosystems) as the internal size standard. Four reference individuals  
170 were included in each migration for cross-validation. Electropherograms were analysed with  
171 GeneMapper® version 4.0 (Applied Biosystems).

172

## 173 2.3 Genetic diversity and structure

174 We used MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004) to check for scoring errors  
175 owing to stutters, large allele dropout and to estimate null allele frequencies. We used GENCLONE  
176 2.0 (Arnaud-Haond and Belkhir, 2007) to test for the occurrence of repeated multilocus genotypes  
177 (MLGs). Repeated MLGs were omitted from the following analyses.

178 Linkage disequilibrium was tested among all pairs of loci at each site with a permutation test ( $n =$   
179 1000) using GENETIX v.4.05 (Belkhir et al., 2004). The  $f$  estimator of  $F_{IS}$  (Weir and Cockerham,  
180 1984) was computed for each population using GENETIX. Departures from panmixia were tested  
181 with an exact implemented in GENEPOP 4.5.1 with default parameters (Rousset, 2008).

182 We analysed the genetic diversity for each population by computing observed ( $H_o$ ) and Nei's  
183 (1973) unbiased expected heterozygosity ( $H_e$ ) with GENETIX. Allelic richness [ $A_r$  (g)] and  
184 private allelic richness [ $A_p$  (g)] were estimated with a rarefaction procedure using the HP-RARE  
185 software (Kalinowski, 2005) with the minimum number of genes set to 18. FreeNA was used to  
186 estimate  $F_{ST}$  for each pair of populations using the ENA correction described in Chapuis and  
187 Estoup (2007), to provide more precise estimates of  $F_{ST}$  considering the presence of null alleles. As  
188 a complementary measure of genetic differentiation, we computed the estimator of actual  
189 differentiation  $D_{est}$  (Jost, 2008) with the online Software for the Measurement of Genetic Diversity  
190 (Crawford, 2010; SMOGD 1.2.5). Exact tests of genic differentiation were performed with  
191 GENEPOP with default parameters.

192 We estimated contemporary connectivity among populations with BAYESASS 3.0 (Wilson and  
193 Rannala, 2003). This software was used to assess the proportions of individuals assigned to their  
194 hypothesized population and to the other populations. As the S14 locus showed strong departures  
195 from panmixia, we performed this analysis without this locus. The current implementation of  
196 BAYESASS is limited to 20 populations. We therefore performed two analyses: one for Portofino  
197 with 9 populations, and another for Marseille with 13 populations. The added benefit of this  
198 method over a standard genetic distance method is the ability to estimate the direction of gene flow.  
199 To ensure consistent and accurate estimates, we varied seed numbers and explored the number of  
200 runs needed (i.e. burn-in) to be discarded before data collection. The burn-in length was set after  
201 log-likelihood values peaked. We also varied seed numbers and mixing parameters to get  
202 acceptance rates between 40% and 60% (Wilson and Rannala, 2003). Delta values of  $m = 0.05$ ,  $P =$   
203  $0.15$ , and  $F = 0.3$  yielded an average number of changes in the accepted range. For each analysis,  
204 we performed five independent (i.e. with different seeds) replicate runs (their average value was  
205 then used) of the algorithm for 500 000 iterations, and a burn-in of 200 000 generations was  
206 determined an appropriate number of runs for convergence and stabilization of posterior  
207 probabilities with a sampling frequency of 2000. As a complementary analysis to BAYESASS, we  
208 used the GENECLASS2 software for the assignment of individuals (Piry et al., 2004). We used the

209 criterion of Rannala and Mountain (1997), and the computation of the probability according to  
210 Paetkau et al. (2004) with 1000 simulated individuals, and a type I error of 0.01. The colonies  
211 sampled on artificial reefs in Marseille (REE) were also analyzed separately with GENECLASS2:  
212 we used the other populations from Marseille as reference samples to study the potential origin of  
213 colonies on the reefs. Considering the strong departure from panmixia observed for the S14 locus,  
214 we did not use it with GENECLASS2.

215 The Bayesian method implemented in STRUCTURE v.2.2 (Falush et al., 2003, 2007; Pritchard et  
216 al., 2000) was used to evaluate the number of genetic clusters (K) in our data set from individual  
217 genotypes without prior information on their geographical locations. Again, as the S14 locus  
218 showed strong departures from panmixia, we repeated the analysis without this locus.  
219 STRUCTURE was launched using the admixture model, with correlated allele frequencies among  
220 clusters and the recessive allele option to cope with null alleles (Falush et al., 2007). Ten  
221 independent runs were performed for each K using 500000 iterations and a burn-in period of  
222 200000. Following the recommendations of Jakobsson et al. (2008) and Rosenberg et al. (2002) for  
223 cases of large datasets, we first studied the results of STRUCTURE at several small values of K  
224 during a first round of analysis, and then we looked for additional substructure on partitioned  
225 datasets during a second round. For the first round, the whole dataset (622 individuals) was used. K  
226 was set to vary between 1 and 6. As the analysis of the whole dataset indicated the presence of two  
227 clusters corresponding to the two sampling regions, we performed a second analysis by separating  
228 the Marseille sites from the Portofino sites. The choice of the best K values was based on the result  
229 of STRUCTURE HARVESTER (Earl and von Holdt, 2012) with the Evanno's criterion (Evanno et  
230 al., 2005), and we retained the values which seemed the most informative, i.e. those with the lowest  
231 levels of individual admixture: we therefore discussed K = 2 and 3 for the global analysis and K =  
232 2 for the analysis within regions. The merging of the different runs and the visualization of the  
233 results were done with the CLUMPAK software (Kopelman et al., 2015).

234 We tested for isolation by distance (IBD) by examining the correlation between pairwise  $F_{ST}/(1-$   
235  $F_{ST})$  values and the logarithm of the geographical distances [Ln(d)] between populations (Rousset,

**Commenté [DA4]:** Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, 14(8), 2611-2620.

236 1997) with IBD Web Software (IBDWS 3.16; Jensen et al., 2005). Geographical distances were  
237 measured using GOOGLE EARTH 6.2.2.6613 (available at [https:// www.google.fr/intl/fr/earth/](https://www.google.fr/intl/fr/earth/)),  
238 considering the minimum distances by sea between sites. IBD was assessed independently within  
239 Marseille and Portofino, as well as across the entire Liguro-Provençal area in the Ligurian Sea. The  
240 significance of the correlation between the two distance matrices was tested using a Mantel test ( $n$   
241 = 1000) in IBD Web Software.

242 Whenever necessary, corrections for multiple tests were performed with the False Discovery Rate  
243 (FDR) method of Benjamini and Hochberg (1995).

244

### 245 **3. Results**

#### 246 3.1 Genetic variability

247 All loci were polymorphic in all populations except for locus EVER007 in PFD and C30 in PTD.  
248 The total number of alleles ranged between 4 for EVER007 and 16 for S14, with a mean of 10.5  
249 alleles per locus. Observed heterozygosity varied from 0 for EVER007 in PFD and C30 in PTD to  
250 0.86 for EVER009 in PAR and PFD. Expected heterozygosity ranged from 0 for EVER007 in PFD  
251 and C30 in PTD to 0.86 for S14 in VED. MICRO-CHECKER suggested the presence of null  
252 alleles for S14, as indicated by the general excess of homozygotes for most allele size classes.  
253 Global significant linkage disequilibrium (LD) between the loci C21 and S14 was detected  
254 considering all samples only ( $P < 0.05$ ). When considering each population separately, significant  
255 LD among pairs of loci was observed in nine populations, but these were not the same pairs of loci  
256 in the different populations. After FDR correction, significant LD was only detected in VES  
257 between loci EVER009 - C30. Seven cases of duplicated MLGs were observed (Table S1). In two  
258 cases, the repeated MLGs were observed in different populations (PTD vs SGD, MJS vs CAV).  
259 Two repeated MLGs were observed on artificial reefs and four MLGs were observed in the CSS  
260 population.

261 At the population level, expected heterozygosity ranged from 0.58 for VED to 0.46 for SAM. Ar  
262 (18) values ranged from 3.41 in PAR to 4.25 in MEJ. Ap (18) values were scattered from 0 in RIS

263 and REE to 0.38 in PFS (Tab. 2). No difference of expected heterozygosity was found between the  
 264 areas of Portofino ( $He = 0.52 \pm 0.02$ ) and Marseille ( $He = 0.51 \pm 0.02$ ).

265

266 3.2 Deviations from Hardy–Weinberg equilibrium

267 Significant multilocus heterozygote deficiencies were observed in ten populations: PFS, CSS, CSD,  
 268 SGS, SGD, PTS, SAR, 3PP, POU and VED (Tab. S2). When not considering the locus S14,  
 269 significant heterozygote deficiencies was observed only in VED and 3PP (Tab. S2). For EVER007,  
 270 EVER009 and C21, the null hypothesis of panmixia was rejected for two populations. For C30 and  
 271 C40, the null hypothesis of panmixia was not rejected for any population. Multilocus  $F_{IS}$  values ( $f$ )  
 272 ranged between -0.03 for RID and 0.27 for 3PP, with a mean value equal to 0.10. Considering each  
 273 locus separately,  $f$  values ranged from -0.34 for C40 in SOM to 0.78 for S14 in 3PP (Tab. S2).

274

275 Table 2. Measures of genetic diversity for 22 populations of *Eunicella cavolini* based on six  
 276 microsatellites loci.  $H_o$ : observed heterozygosity;  $H_e$ : unbiased expected heterozygosity;  $f$ : Weir  
 277 and Cockerham (1984) estimator of multilocus  $F_{IS}$ ;  $Ar(18)$  and  $Ap(18)$ : allelic and private allelic  
 278 richness, respectively, with rarefaction for a corresponding sample size of 18. Standard deviations  
 279 over loci are in brackets. Values in bold are significant at the 0.05 level after FDR correction.

280

	<b>H<sub>o</sub></b>	<b>H<sub>e</sub></b>	<b>f</b>	<b>Ar(18)</b>	<b>Ap(18)</b>
<b>PAR</b>	0.51 (0.26)	0.53 (0.24)	0.04	3.41 (1.59)	0.07 (0.18)
<b>PFS</b>	0.48 (0.22)	0.55 (0.27)	<b>0.16</b>	4.16 (1.90)	0.38 (0.55)
<b>PFD</b>	0.52 (0.31)	0.54 (0.31)	0.06	3.66 (1.96)	0.01 (0.02)
<b>CSS</b>	0.47 (0.11)	0.55 (0.24)	<b>0.15</b>	4.10 (2.14)	0.03 (0.08)
<b>CSD</b>	0.45 (0.24)	0.52 (0.29)	<b>0.14</b>	4.06 (2.19)	0.06 (0.15)
<b>SGS</b>	0.42 (0.24)	0.49 (0.30)	<b>0.17</b>	4.06 (2.19)	0.03 (0.05)
<b>SGD</b>	0.44 (0.31)	0.50 (0.35)	<b>0.13</b>	3.95 (2.18)	0.07 (0.13)
<b>PTS</b>	0.42 (0.29)	0.49 (0.33)	<b>0.16</b>	3.78 (2.11)	0.01 (0.02)
<b>PTD</b>	0.46 (0.29)	0.47 (0.31)	0.05	3.63 (2.01)	0.22 (0.25)
<b>SAR</b>	0.42 (0.20)	0.47 (0.25)	<b>0.13</b>	3.96 (2.08)	0.03 (0.08)
<b>3PP</b>	0.37 (0.27)	0.50 (0.31)	<b>0.27</b>	3.63 (1.56)	0.13 (0.14)
<b>RIS</b>	0.50 (0.28)	0.53 (0.32)	0.09	3.96 (2.08)	0 (0)
<b>RID</b>	0.55 (0.32)	0.52 (0.31)	-0.03	3.86 (1.80)	0.06 (0.12)
<b>POU</b>	0.42 (0.30)	0.51 (0.35)	<b>0.22</b>	3.82 (2.01)	0.05 (0.11)
<b>VES</b>	0.48 (0.30)	0.50 (0.32)	0.05	3.78 (2.12)	0.03 (0.05)
<b>VED</b>	0.52 (0.21)	0.58 (0.20)	<b>0.13</b>	4.16 (2.16)	0.11 (0.13)

<b>SOM</b>	0.46 (0.18)	0.49 (0.24)	0.07	3.65 (1.64)	0.05 (0.12)
<b>SAM</b>	0.43 (0.26)	0.46 (0.27)	0.08	3.62 (1.83)	0.07 (0.18)
<b>CAV</b>	0.48 (0.18)	0.51 (0.24)	0.08	4.22 (1.68)	0.17 (0.18)
<b>MJS</b>	0.51 (0.33)	0.49 (0.33)	-0.02	3.99 (2.18)	0.14 (0.23)
<b>MEJ</b>	0.48 (0.31)	0.50 (0.34)	0.06	4.25 (2.36)	0.13 (0.31)
<b>REE</b>	0.50 (0.29)	0.51 (0.25)	0.06	3.60 (1.55)	0 (0)
<b>Mean Value</b>	0.47 (0.04)	0.51 (0.03)	0.10	3.88 (0.24)	0.08 (0.09)

281

### 282 3.3 Population genetic structure

283 The mean global  $F_{ST}$  was 0.07 ( $\pm$  0.04), the mean FreeNA corrected  $F_{ST}$  was 0.07 ( $\pm$  0.03), and the  
284 mean  $D_{est}$  was 0.17 ( $\pm$  0.08). Pairwise multilocus  $F_{ST}$  between all populations ranged from 0.002  
285 between MJS and MEJ to 0.166 between PAR and SAM (Tab. S3). Pairwise FreeNA corrected  $F_{ST}$   
286 ranged from 0.01 (PFS vs PFD, CSS vs CSD, CSD vs SGD, RIS vs RID, VES vs SOM, MEJ vs  
287 SAM) to 0.17 (PAR vs SAM). Pairwise  $D_{est}$  ranged from 0 (VED vs VES) to 0.38 (CSD vs POU)  
288 (Tab. S4). After the FDR correction, all pairwise exact tests of genic differentiation were  
289 significant apart from CSS vs CSD and RIS vs RID (two comparisons between depths; Tab. S3).  
290 Apart from depth, significant comparisons were found with a minimum distance of 0.99 km in  
291 Portofino and 2.95 km in Marseille. When locus S14 was omitted, two additional differentiation  
292 tests were non-significant: VES vs SOM and PFS vs PFD.

293 The correlation between  $F_{ST} / (1 - F_{ST})$  and  $\text{Ln}(dist)$  was significant using geographical distances  
294 ( $r_{\text{Ln}(dist)} = 0.596$ ;  $P < 0.001$ ), confirming the occurrence of an IBD model of gene flow (Fig. 2).  
295 Within areas, correlations were also significant for the Portofino ( $r_{\text{Ln}(dist)} = 0.625$ ;  $p < 0.001$ ) and  
296 Marseille areas ( $r_{\text{Ln}(dist)} = 0.436$ ;  $p < 0.001$ ) (Fig. 2B and 2C). The IBD slope was similar in the two  
297 areas (0.019 in Portofino and 0.015 in Marseille). When using  $F_{ST}$  corrected by FreeNA, IBD was  
298 significant at the global level ( $p < 0.001$ ), within Marseille ( $p = 0.04$ ) and within Portofino ( $p =$   
299 0.002). Again, the IBD slope was similar in the two areas (0.032 at Portofino and 0.035 at  
300 Marseille; data not shown).

301

302 **Fig. 2.** Isolation by distance (IBD) pattern for all populations (A), taking into account the minimum  
303 geographical distances between populations. Separate analyses of IBD for Portofino populations  
304 (B) and Marseille populations (C). The graphs show the linear regression of the genetic distance  
305 measured as  $F_{ST}/(1 - F_{ST})$  over logarithms of the geographical distance (m).

306

307 The results of the genetic clustering using STRUCTURE indicated that the highest Delta (K) value  
308 for the whole dataset was 2 (data not shown). With  $K = 2$ , the clustering showed a clear distinction  
309 between Marseille and Portofino, and  $K = 3$  did not provide additional information (Fig. 3). The  
310 addition of locus S14 to the analysis gave a very similar result (data not shown). Each of these two  
311 regional clusters was then subjected to a second round of STRUCTURE. The highest Delta (K)  
312 value was 3 at Marseille and 4 at Portofino. Nevertheless, inside each region, the results did not  
313 indicate the presence of any additional cluster: in both cases, all individuals were equally shared  
314 among clusters for all K (data not shown).

315

316 **Fig. 3.** Population structure as inferred by the STRUCTURE analysis. Each individual is  
317 represented by a vertical line partitioned into K-coloured segments that represent the individual's  
318 membership fraction in K clusters. Each population is delineated by black vertical lines and named  
319 as in Table 1.

320

321 Migration analysis using the BAYESASS analysis indicated similar levels of self-recruitment in the  
322 two regions: the proportion of individuals derived from the source population varied between 0.69  
323 (PFD) and 0.80 (PAR) at Portofino, and between 0.68 (CAV) and 0.80 (POU) at Marseille (Tab.  
324 S5). The estimates of migration rates between populations were generally low and with quite large  
325 standard errors. The assignment analysis of GENECLASS2 indicated that the probability of  
326 assignment (averaged over all individuals of the population) was higher for populations from their  
327 region of origin than for another region: the intra-region assignment probability was 0.31 ( $\pm 0.10$ )  
328 for Portofino and 0.32 ( $\pm 0.10$ ) for Marseille (Table S6). The within-region highest assignment  
329 probabilities corresponded to the population where individuals were sampled: these retention  
330 probabilities were very similar among populations and regions: 0.46 ( $\pm 0.02$ ) at Portofino and 0.45  
331 ( $\pm 0.03$ ) at Marseille. The assignment probability of Portofino individuals to Marseille populations

332 was 0.11 ( $\pm$  0.07) and 0.16 ( $\pm$  0.07) in the other direction. The assignment probabilities of *E.*  
333 *cavolini* colonies sampled on artificial reefs near Marseille did not allow the identification of a  
334 single main origin, and for a given colony the probabilities were often similar among different  
335 candidate populations (Table S7). Only the colony 14B displayed a much higher probability of  
336 origin for one population (Saména) than for the other populations. The colonies 15J, 15M, 15O and  
337 15P displayed low assignment probabilities ( $<$  0.10) for all the populations tested here.

338

#### 339 **4. Discussion**

##### 340 4.1 Genetic diversity

341 The analysis of six microsatellite loci evidenced low genetic diversity for *Eunicella cavolini* (He by  
342 population over loci = 0.51) when compared with other Mediterranean octocorals: *Corallium*  
343 *rubrum* studied in the same area (He by population over loci = 0.77; Ledoux et al., 2010) and  
344 *Paramuricea clavata* (He by populations over loci = 0.74; Mokhtar-Jamaï et al., 2011). There  
345 could be several possible and non-mutually exclusive reasons. i) the microsatellites used here were  
346 less variable because of their sequence or origin: S14 and EVER007, and 009 correspond to cross-  
347 amplification from *E. singularis* and *E. verrucosa* respectively; ii) the species is less variable than  
348 other octocoral species, likely due to the impact of past bottlenecks; iii) a geographical effect: the  
349 comparison of populations from Marseille with other Mediterranean areas indicated that in the NW  
350 Mediterranean, the genetic diversity of *E. cavolini* is lower than in the SW Mediterranean  
351 populations, but higher than in the E Mediterranean (Masmoudi et al., 2016). Nevertheless, even in  
352 the area of the highest genetic diversity for *E. cavolini*, the expected heterozygosity was somewhat  
353 lower than in the two others Mediterranean octocorals ( $H_e$  = 0.68 in Algeria; Masmoudi et al.,  
354 2016). A comparative study of the demographic history of these species with similar markers (such  
355 as Single Nucleotide Polymorphisms, SNPs) could help in understanding the origin of these  
356 differences.

357



358 4.2 Genetic structure at large spatial scale

359 A pattern of isolation by distance was observed at the regional spatial scale with significant genetic  
360 structuring found between the two regions. Values of  $F_{ST}$  for the Liguro-Provençal geographical  
361 area ranged between 0.002 and 0.166, with a mean value of 0.09. If we make comparisons with  
362 other octocorals,  $F_{ST}$  for the Liguro-Provençal area was between 0.07 and 0.14 with a mean value  
363 of 0.09 for *P. clavata* (Mokhtar-Jamaï et al., 2011), and between 0.09 and 0.2 with a mean value of  
364 0.13 for *C. rubrum* (Ledoux et al., 2010). Such comparisons between species can be impacted by  
365 different levels of heterozygosities among markers, which influences the highest  $F_{ST}$  values (Jost et  
366 al. 2018). As previously mentioned,  $H_{exp}$  is lower in *E. cavolini* based on our markers, than in *C.*  
367 *rubrum* and *P. clavata*. Despite this,  $F_{ST}$  is lower for *E. cavolini* than for the other species. It thus  
368 shows that genetic differentiation within the Liguro-Provençal area is higher for *C. rubrum* than for  
369 *E. cavolini* and *P. clavata*. Such differences in the levels of genetic differentiation between species  
370 could be due to differences in dispersal abilities or in local effective sizes. Although all brooders  
371 are more likely to exhibit restricted gene flow (Carlou et al., 1999), *C. rubrum* has a mean dispersal  
372 ability of 10s of meters or less (Constantini et al., 2007a), whereas for *P. clavata* it would be around  
373 1 km (Mokhtar-Jamaï et al., 2011) and for *E. cavolini* our results suggest a mean dispersal ability of  
374 less than 1 km. Nevertheless this may be at odd with experimental data on larval phase duration in  
375 the red coral (Martinez-Quintana et al., 2015), and this does not preclude the possibility of  
376 exchanges at higher distances (Ledoux et al., 2010b), even if their frequency remains unknown.  
377 The structure pattern revealed by STRUCTURE showed a distinction between Portofino and  
378 Marseille. This pattern is also supported by the higher values of  $F_{ST}$  between areas (average  $F_{ST}$ =  
379 0.092) than within areas (average  $F_{ST}$ = 0.063 for Portofino and average  $F_{ST}$ = 0.069 for Marseille),  
380 and by the marked differences in assignment probabilities between regions compared to within  
381 regions. These results indicate a limited gene flow between the two regions for the yellow  
382 gorgonian, which could be linked to intrinsic limits to dispersal at such distances (the two regions  
383 are 273 km apart), and/or to the effect of a barrier to gene flow. The genetic analyses of yellow  
384 gorgonian populations in other intermediate positions between Marseille and Portofino, could help

385 in testing whether there is a genetic barrier between the two regions, or if the observed differences  
386 are induced by sampling along a geographical gradient of genetic differences (i.e. IBD, Aurelle and  
387 Ledoux, 2013). The asymmetry of assignment probabilities between the two regions suggests a  
388 higher gene flow from east to west that is consistent with the main direction of currents in this area  
389 (Berline et al., 2014). Nevertheless, the confidence intervals are overlapping and more precise  
390 estimates would be useful here to test this hypothesis.

391 Regarding the larval biology of *E. cavolini*, very little information is available, but it may  
392 contribute to the observed genetic structure. *Eunicella cavolini* is also a brooding species, which  
393 implies more reduced larval dispersal abilities. Such low larval dispersal is observed in other  
394 coralligenous species, where the studies available to date showed significant differentiation from a  
395 few kilometres or tens of cm (Abbiati et al., 2009; Calderon et al., 2007; Goffredo et al., 2004;  
396 Ledoux et al., 2010). In contrast to these cases, another Mediterranean benthic species, the sea  
397 urchin *Paracentrotus lividus*, displayed a much larger genetic structure (Paterno et al., 2017). It  
398 would therefore be interesting to study on more species the ecological or evolutionary factors  
399 leading to the selection of different dispersal strategies in these species. Indeed multispecific  
400 approaches can reveal common barriers to gene flow and identify cold and hot spots of genetic  
401 diversity (Cahill et al., 2017).

402

#### 403 4.3 Genetic structure at small spatial scale

404 A pattern of isolation by distance was observed also at the local spatial scale with significant  
405 genetic structuring found within the two regions. The populations from each area, Marseille and  
406 Portofino, correspond to a single genetic cluster according to STRUCTURE. The additional genetic  
407 clusters identified within regions were not informative and corresponded to the limits of inference  
408 of this method. Within regions, most populations appeared genetically differentiated except for  
409 comparisons between depths (see below). Significant genetic differentiation was found between  
410 sites few kilometres apart. The pattern of differentiation within regions did not correspond to  
411 different clusters, but to the isolation by distance of differentiated populations. The use of  $F_{ST}$  and

**Commenté [DA5]:** Cahill, A. E., De Jode, A., Dubois, S., Bouzaza, Z., Aurelle, D., Boissin, E., ... & Merigot, B. (2017). A multispecies approach reveals hot spots and cold spots of diversity and connectivity in invertebrate species with contrasting dispersal modes. *Molecular ecology*, 26(23), 6563-6577.

412 Dest gives different and complementary information on genetic differences between populations  
413 (Jost et al., 2018). Here at different scales,  $D_{EST}$  estimates where higher than  $F_{ST}$ :  $D_{EST}$  then  
414 indicates an important allelic differentiation between populations, higher than the differentiation  
415 estimated from fixation index alone (Jost et al., 2018). At a local scale, and on the basis of  $D_{EST}$ ,  
416 one can identify populations with particularly high differentiation: such as Pouard in Marseille, or  
417 Punta Faro Deep in Portofino. These sites did not correspond to particularly distant populations,  
418 and the origin of these differences remains to be studied (see below). More generally, in the short  
419 term, the interaction between gene flow and genetic drift precludes the homogenization of genetic  
420 diversity at the metapopulation scale.

421 The BAYESASS software was used to estimate contemporary connectivity among sites. The  
422 inferred non-migration rates fell mostly within two classes (around 0.67 and 1). Such values  
423 correspond to the bounds of the prior distribution and could indicate a bias in the inference  
424 (Meirmans, 2014), despite the use of five independent runs. The Sarranier, 3PP cave and Punta  
425 Cervara populations displayed higher non-migration rates (above 0.9), which would agree with  
426 their relative geographical isolation, but this was also observed for a few *a priori* less isolated  
427 populations such as Somlit and Saména. The GENECLASS2 software was then used to obtain  
428 another estimate of contemporary migration. With this method, the low probabilities of assignment  
429 to the population of origin could be the result of a quite low within region genetic structure. As a  
430 comparison, much higher levels of self-recruitment were inferred with this method for *P. clavata*,  
431 including for some sites studied here around Marseille (Mokhtar-Jamaï et al., 2011). This confirms  
432 that the dispersal abilities of *E. cavolini* are higher than those of *P. clavata*. Within a given region,  
433 sporadic gene flow could take place and contribute to limiting the observed differentiation  
434 compared to other octocoral species. In this case, the use of additional genetic markers could be  
435 tested to obtain higher probabilities of assignment.

436 Some of the studied sites could be more isolated because of the distance (e.g. Sarranier and 3PP  
437 cave), a particular position behind a headland (e.g. Punta Cervara), or caused by oceanographic  
438 features, which still remain to be investigated (Pouard, Punta Faro Deep). Population genetic

**Commenté [DA6]:** Jost, L., Archer, F., Flanagan, S., Gaggiotti, O., Hoban, S., & Latch, E. (2018). Differentiation measures for conservation genetics. *Evolutionary Applications*.

439 structure is a pattern shaped by the interaction between physical drives (e.g. currents, barriers) and  
440 biological processes, such as larval biology and demography (e.g. fluctuations in population  
441 density due to habitat fragmentation and recruitment; Padrón and Guizien, 2015). Different levels  
442 of genetic drift could induce contrasted levels of genetic structure, either because of different  
443 current population sizes or different demographic histories. Regarding the colonization of artificial  
444 reefs near Marseille, several populations could correspond to potential sources. The very low  
445 assignment probabilities for four colonies indicate that non-sampled populations may have  
446 contributed to this new population. This possibility of connection with different populations may  
447 seem at odds with the observed differentiation for most populations. Nevertheless, gene flow in  
448 populations might be limited by established colonies (e.g. through space limitation), which is not  
449 the case in new populations. Recolonization might then not be limited in an area with enough  
450 surrounding populations. As similar results was obtained on *P. clavata* by Arizmendi-Meja et al.  
451 (2015), who observed that a recently founded population may correspond to several source  
452 populations.

453 A study combining more markers (such as SNPs, Paterno et al., 2017) and integrating the putative  
454 larval characteristics with local hydrodynamics, could help to better understand the observed  
455 structure and to estimate connectivity patterns (e.g. Thibaut et al., 2016). In addition, the possibility  
456 of hybridization between *E. cavolini* and the other species of the genus *Eunicella* (i.e. *E. singularis*  
457 and *E. verrucosa*) occurring in the same area (Aurelle et al., 2018) could also shape the observed  
458 genetic structure and diversity, depending on the density of the different species and on the  
459 frequency of genetic incompatibilities. These processes are only beginning to be considered in this  
460 context, but would require an in-depth study.

461

#### 462 4.4 Genetic structure between depths

463 Over seven comparisons between depths, only in two sites we found no genetic difference with  
464 depths. This is partly contradictory with the lack of genetic differentiation observed between depths  
465 in the Marseille area (Masmoudi et al., 2016), where seven loci (six loci in the present paper) and a

**Commenté [DA7]:** Arizmendi-Mejía, R., Linares, C., Garrabou, J., Antunes, A., Ballesteros, E., Cebrian, E., ... & Ledoux, J. B. (2015). Combining genetic and demographic data for the conservation of a Mediterranean marine habitat-forming species. *PLoS One*, 10(3), e0119585.

466 permutation test (exact test in the present paper) were used. These differences suggest that the  
467 differentiation between depths is quite low, but near significance depending on sampling effort or  
468 drift effects, and that the exact test is more powerful than the permutation test. Two others  
469 Mediterranean octocoral species, *Paramuricea clavata* (Mokhtar-Jamaï et al., 2011) and *Corallium*  
470 *rubrum* (Constantini et al., 2011; Ledoux et al., 2010) showed a generalized genetic differentiation  
471 with depths in the area of Marseille. Intrinsic biological differences (e.g. spawning date compared  
472 to water stratification or larval properties) interacting with hydrodynamics might explain the  
473 differences observed between these two species and *E. cavolini*. For the congeneric species *E.*  
474 *singularis*, a strong restriction to gene flow was observed between 30 m and 40 m depth (Constantini  
475 et al., 2016). We did not observe this for *E. cavolini*, but enlarging this study over a wider  
476 bathymetrical range might be interesting.

477 The pattern of genetic structure along the depth gradient can be compared with the pattern of  
478 adaptation of *E. cavolini*. Environmental variables, such as light and temperature, vary along the  
479 depth gradient and can induce local adaptation (Webster et al., 2017). Thermo-tolerance  
480 experiments showed that the response to thermal stress is very different between depths in *E.*  
481 *cavolini*, with a lower thermo-tolerance in deep individuals (Pivotto et al., 2015). Such differential  
482 phenotypic buffering is observed in a context of low and non-significant genetic differentiation for  
483 a studied site (e.g. in the site of Riou, and see also Pivotto et al., 2015). For *E. cavolini*, the  
484 adaptation to the different thermal regimes could correspond to acclimatization or to a combination  
485 of genetic and non-genetic effects. In addition, the reduced number of loci analysed here does not  
486 allow us to reject the possibility of genetic adaptation determined by other genomic regions. More  
487 generally, *E. cavolini* can be found in a wide range of light conditions (Cánovas Molina et al.,  
488 2016b), which could point to the general physiological plasticity of the species.

489 The role of deep environments as possible refugia from disturbances for shallow marine species  
490 (i.e. the deep refugia hypothesis) has been widely recognized (Bongaerts et al., 2010); however, the  
491 genetic differentiation of *E. cavolini* with depth, although low, questions the possibility for deep  
492 populations to act as a regular source of new larvae in the event of mass mortality of shallow

493 colonies due to thermal anomalies (Cerrano et al., 2000; Garrabou et al., 2009). Moreover the  
494 possible adaptation of octocorals to different depths (Ledoux et al., 2015), including in *E. cavolini*  
495 (Pivotto et al., 2015), could influence the evolution of populations along this depth gradient.

496

## 497 **5. Conclusion**

498 Our results confirm the strong genetic structure observed in the Mediterranean octocorals studied  
499 so far, which contrasts with what has been observed for other anthozoan species (e.g. *Alcyonium*  
500 *digitatum* in the northeast Atlantic; Holland et al., 2017). The genetic structure in *Eunicella*  
501 *cavolini* is lower than in *Paramuricea clavata* and *Corallium rubrum*, the other two typical benthic  
502 species occurring in coralligenous assemblages of the Mediterranean Sea. The high differentiation  
503 observed at the large spatial scale of 100s of kilometres, i.e. between the two regions, argues for the  
504 importance of local management of such benthic species. For example, a network of connected  
505 MPAs focusing on this species, as well as on other species of the coralligenous assemblages with  
506 similar dispersal abilities, should ideally consider these spatially restricted dispersal abilities. The  
507 weaker but significant genetic structure observed in *E. cavolini* at the small spatial scale of  
508 kilometres, i.e. among sites within the same region, suggests that gene flow can be important in the  
509 short-term evolution of populations. The identification of sites with important genetic  
510 differentiation could be taken into account in conservation. Regarding the response to climate  
511 change, the observed diversity in thermo-tolerance levels is correlated with the genetic structure  
512 estimated with microsatellites.

513

## 514 **Supplementary data:**

515 Supplementary material is available at the online version of the manuscript.

### 516 **S1 Table. Results of the test of occurrence.**

517 (DOC)

### 518 **S2 Table. Multilocus and monolocus values of $F_{IS}$ estimator.**

519 (DOC)

**Commenté [DA8]:** Ledoux, J. B., Aurelle, D., Bensoussan, N., Marschal, C., Féral, J. P., & Garrabou, J. (2015). Potential for adaptive evolution at species range margins: contrasting interactions between red coral populations and their environment in a changing ocean. *Ecology and evolution*, 5(6), 1178-1192.

520 **S3 Table. Pairwise  $F_{ST}$  values.**

521 (DOC)

522 **S4 Table. Results of mean  $D_{est}$  and  $F_{ST}$  estimates.**

523 (DOC)

524 **S5 Table. Results of the Bayesian assessment of migration proportions.**

525 (DOC)

526 **S6 Table. Assignment probabilities with GENECLASS2.**

527 (DOC)

528 **S7 Table. Assignment probabilities with GENECLASS2 for colonies from artificial reefs.**

529 (DOC)

530

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540

541 I state in behalf of all co-authors, that no conflict of interest exists in this work.

542

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