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1 **Genetic insights into recolonization processes of Mediterranean octocorals**

2

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25 **Abstract**

26 Marine ecosystems are strongly impacted by the consequences of human activities, such as habitat
27 destruction or artificialization, and climate change. In the Mediterranean Sea, sessile benthic
28 species, and particularly octocorals, have been affected by mass mortality events linked with
29 positive thermal anomalies. The future survival of octocoral populations impacted by global change
30 will depend on their recolonization abilities facing local extirpation or important modification of
31 their habitat. We studied these processes in Mediterranean octocorals in two situations: the
32 colonization of artificial substrates (wrecks) by the red gorgonian *Paramuricea clavata*, and the
33 recolonization following mortality events in the yellow gorgonian *Eunicella cavolini*. With
34 microsatellite markers (seven for *P. clavata*, five for *E. cavolini*), we analyzed the genetic diversity
35 of populations on artificial substrates and their differentiation from other neighboring populations.
36 For *P. clavata* the populations on artificial substrates were not or lowly differentiated from the
37 closest populations (1.3 to 1.6 km) on natural substrates, and showed similar levels of genetic
38 diversity. Artificial substrates can then be considered as an interesting substitute for natural
39 substrates for this species. For *E. cavolini* we did not detect any variation in diversity nor
40 relatedness following recuperation after mortality events. In both cases our results suggest the input
41 from different populations in the recolonization process, which helps in maintaining the genetic
42 diversity. These results are useful for the management of these species and of associated
43 ecosystems.

44

45 **Introduction**

46 Marine biodiversity is increasingly impacted by global and local pressures such as climate change,
47 overfishing, introduction of invasive species, habitat destruction and pollution (Boudouresque et al.
48 2017; Gattuso et al. 2018). The combination of these pressures questions the possibility of evolution
49 and persistence of numerous marine species. Whereas the global impact of human activities on
50 biodiversity is still increasing, different solutions (from reduction of greenhouse gas concentrations
51 to manipulation of ecological adaptation and habitat restoration) are explored to mitigate this impact
52 in the marine realm, but with uncertainties regarding their efficiency and trade-offs (Gattuso et al.
53 2018).

54 Additionally, coastal areas face an expansion of artificial structures such as dikes, offshores
55 structures or even wrecks (Dafforn et al. 2015). The development of artificial structures can have
56 contrasting consequences on marine biodiversity such as habitat loss or indirect modifications of the
57 environment (Dafforn et al. 2015). Conversely, artificial structures can provide new habitats, and
58 act as artificial reefs and connectivity nodes. Artificial structures can increase habitat complexity in
59 seabed, complexity which has been impacted by activities such as trawling. Therefore, despite their
60 negative effects, artificial structures can be considered as potential management tools in a context of
61 global change. The future of marine biodiversity will then depend on the evolutionary trajectories of
62 populations in this changing and anthropized environment. These evolutionary trajectories include
63 the possibility of genetic adaptation, acclimatization, gene flow, recolonization and range shift (Bell
64 and Collins 2008). Global change can then be considered as a wide scale adaptive challenge for
65 many species, submitting biodiversity to important and new selective pressures (Otto 2018).

66 In this context, population genetics can be used to study genetic connectivity, the possibility of
67 sustaining gene flow among habitats, including artificial ones, and the recolonization processes
68 following disturbances. For example, a study of two scleractinian species, *Pocillopora damicornis*
69 and *Seriatopora hystrix*, has shown a recolonization pattern mainly from neighbouring populations,

70 but with occasional more distant sources (Starger et al. 2010). It is also important to consider the
71 consequences of recolonization on genetic diversity, for example through founder effects. A
72 founder effect will lead to changes in allele frequencies between source and sink populations, and to
73 a reduction in genetic diversity (Allendorf et al. 2017). Nevertheless, this is not always the case. For
74 instance, in the gastropod *Nucella lapillus*, no significant genetic effect of local extinction and
75 recolonization was detected, despite *a priori* low dispersal abilities (Colson and Hughes 2004).
76 Depending on the ecology of the species (e.g. age at sexual maturity), the strength of founder
77 effects during recolonization will vary ranging from drastic reduction of intra-population genetic
78 diversity and an increase in genetic differentiation between populations, to a lack of effect
79 (Austerlitz et al. 2000). The type of habitat (natural / artificial) can also impact the level of genetic
80 diversity, as observed in the gastropod *Patella caerulea* where populations on urban structures
81 showed less genetic diversity than populations on natural rocky habitats (Fauvelot et al. 2009).

82

83 The Mediterranean Sea, with a combination of an important species diversity and strong human
84 pressures including climate change, is a hotspot of biodiversity (Coll et al. 2010, 2012; Cramer et al.
85 2018). Mass mortality events induced by thermal anomalies have been observed in the last decades
86 in the northern Mediterranean (Garrabou et al. 2009; Verdura et al. 2019). These mortality events
87 have impacted several sessile groups of species such as sponges and octocorals. For a given species,
88 the impact was different among regions and depths (Garrabou et al. 2009). Global (e.g. warming) or
89 local (e.g. habitat destruction) pressures can lead to population decline or extinction (extirpation).
90 This combination of human pressures raises the question of the survival of octocoral populations
91 facing global change, which is linked to their adaptive potential. For example, octocoral populations
92 from different depths show in some cases different thermotolerance levels, and this diversity could
93 be important for adaptive evolution (Haguenaer et al. 2013; Ledoux et al. 2015; Pivotto et al.
94 2015). At a metapopulation level, population decline or extirpation can be compensated by
95 migration from other populations, which can have positive demographic and genetic effects (Ronce

96 2007). In this context, the aforementioned increasing availability of artificial substrates should be
97 considered, as artificial structures can allow the development of new local populations and modify
98 the connectivity network. Studying the recolonization abilities of octocorals at a genetic level, on
99 natural and artificial substrates, is therefore of paramount importance to better understand their
100 future evolution and conservation.

101 Population genetic studies of Mediterranean octocorals have regularly evidenced a significant
102 genetic differentiation at short distance, from a few tens to hundred meters. This was the case, at
103 different levels, for the red coral *Corallium rubrum* (e.g. Costantini et al. 2007; Ledoux et al. 2010a,
104 2010b; Pratlong et al. 2018), the red gorgonian *Paramuricea clavata* (Mokhtar-Jamai et al. 2011;
105 Arizmendi-Mejía et al. 2015), and the yellow gorgonian *Eunicella cavolini* (Masmoudi et al. 2016;
106 Cánovas-Molina et al. 2018). This marked genetic structure could be the result of a combination of
107 reduced mean dispersal distance (Ledoux et al. 2010a), an important genetic drift, and of priority
108 effect (Orsini et al. 2013). Studying the genetic diversity of recently colonized or recolonized sites
109 would then be interesting to better understand the processes shaping the genetic structure of these
110 species. For example a founder effect along with a priority effect could lead to a persistent genetic
111 differentiation at unexpected short distance. Genetic data on recolonization are scarce in
112 Mediterranean octocorals. Arizmendi-Mejía et al. (2015) observed that a recently established
113 population of *P. clavata* in Ibiza island (Balearic Islands, East Spain), probably originated from
114 several source populations, which can explain its relatively high levels of genetic diversity
115 compared to the other investigated populations. In the same species, a lack of marked founder effect
116 was also suggested during recolonization process following last glacial maximum (Ledoux et al.
117 2018). Focusing on *Eunicella cavolini*, Cánovas-Molina et al. (2018) suggested that colonies
118 established on artificial reefs in the area of Marseille (South-East France) could come from different
119 populations.

120 In the present study we propose to extend our knowledge regarding colonization processes in these
121 species with two case studies: the colonization of two artificial substrates (wrecks) by *P. clavata*,

122 and the recolonization of a natural substrate after a strong mortality event in *E. cavolini*. In both
123 cases we aim to estimate the contribution of neighboring populations to the newly (re)colonized
124 population. We also test if the dynamics of these colonizations are associated with founder events
125 (i.e. a reduction in genetic diversity, or a differentiation of the new populations from neighbouring
126 ones). This study sheds new light on the origin of short distance genetic differentiation in
127 Mediterranean octocorals and should improve our understanding regarding the evolution of marine
128 biodiversity in changing and artificializing seascapes.

129

130 **Material and methods**

131 Sampling and population history:

132 All colonies were sampled by scuba diving. Regarding *P. clavata*, we sampled two populations
133 established on the ships « Donator » (DON) and « Le Grec » (GRE), both ships wrecked in 1945 in
134 the same area in southeastern France. Eight additional populations on natural substrates were
135 sampled, with increasing distance from the wrecks, up to the Marseilles area for the most distant
136 sites (up to 75 km by sea; Table 1 and Fig. 1). The distance with the closest population on natural
137 substrate (SAR) is around 1.3 km for GRE and 1.6 km for DON. The sample size for *P. clavata*
138 varied between 29 and 39 colonies. For *E. cavolini*, we focused on the sampling of four populations
139 around the island of Ischia (Tyrrhenian Sea, West Italia; Table 1 and Fig. 1) which experienced
140 strong mortality events: Sant'Angelo (SAN), Secca delle Formiche (SFV), and La Nave (LAN)
141 where mortality was documented in 2002, 2003, 2005 (except in LAN) and 2009 (Gambi et al.
142 2006, 2010; Garrabou et al., 2009; Cigliano and Gambi 2007; Gambi 2014), and La Grotta Azzurra
143 at Palinuro (GAZ, Salerno; 15-25 m depth), a semi-submerged cave where a strong mortality event
144 was registered in October 2008, with 77% of dead colonies at 15 m depth (Gambi et al. 2010), and a
145 slight recovery of the population already observed in July 2011 (Gambi and Barbieri 2012). We also
146 sampled two populations that, to our knowledge, did not experience any recent mortality event: the
147 Nisida island (NISB, Gulf of Naples), and the Secchitello bank (UST, Ustica island, North Sicily)

148 where *E. cavolini* is distributed relatively deep (below 30 m depth). In the sites affected by
149 mortality events we likely sampled both young *E. cavolini* individuals and residual individuals
150 which recruited before the mortality events. These *E. cavolini* populations were sampled in June
151 and July 2013. Therefore we sampled from 4 to 11 years after mortality events for impacted
152 populations. Regarding the evolution of density and the size structure of *E. cavolini* at the site GAZ
153 after the 2008 mortality event (Gambi and Barbieri 2012), samples mainly come from colonies
154 recruited after this event. The sample size for *E. cavolini* varied between 14 and 38 colonies. For
155 both species, 3-4 cm fragments from an apical branch were sampled from each colony, and stored in
156 ethanol 95% at -20°C before DNA extraction.

157

158 Microsatellite markers:

159 DNA extraction was performed according to Mokhtar-Jamai et al. (2011), Ribout and Carpentieri
160 (2013), and Masmoudi et al. (2016) depending on the samples. The *P. clavata* colonies have been
161 genotyped with the loci Para9, Para10, Para12, Para14, Para17, Pard and Para2-37 as in Molecular
162 Ecology Resources Primer Development Consortium et al. (2010) and Mokhtar-Jamai et al. (2011).
163 Data from GAB, PTV, MTM, RIO, RIS and IMP were available from Mokhtar-Jamai et al. (2011).
164 The *E. cavolini* colonies have been genotyped with the loci S14, C21, C30, C40 and Mic56
165 following Masmoudi et al. (2016).

166

167 Genetic diversity:

168 We identified repeated multilocus genotypes (MLGs) and we computed the probability of
169 occurrence of multiple MLGs under sexual reproduction with the poppr 2.8.3 R package (Kamvar et
170 al. 2014, 2015). For all following analyses we kept one representative of each MLG. Observed and
171 expected heterozygosities were computed with the Genetix 4.05 software (Belkhir et al. 1999).
172 Allelic richness was estimated with a rarefaction procedure with the ADZE software (Szpiech et al.
173 2008), and by excluding for each species the sample with the lowest number of colonies.

174

175 Genetic structure within and among populations:

176 Departures from panmixia were tested with the Genepop 4.5.1 software with an exact test and with
177 heterozygote deficiency as an alternative hypothesis (Rousset 2008). The level of relatedness within
178 populations was computed with the estimator of Wang (2002) implemented in the COANCESTRY
179 software (Wang 2011). To compare the levels of relatedness between populations we used the
180 bootstrap approach of COANCESTRY with 1000 resamplings. The level of genetic differentiation
181 was estimated with the F_{ST} estimate of Weir and Cockerham (1984). Previous studies did not detect
182 a strong impact of null alleles for the loci analysed here in each species (Mokhtar-Jamäi et al., 2011;
183 Masmoudi et al., 2016). Nevertheless, regarding the F_{IS} values observed here and in previous
184 studies, and the missing data observed in some populations (see results), we also estimated F_{ST} with
185 a correction for null allele from the FreeNA software (Chapuis and Estoup 2007). Genic
186 differentiation between populations was tested with the exact test procedure implemented in
187 Genepop. To estimate the relative differentiation of each population, we computed the population-
188 specific F_{ST} in GESTE (Foll and Gaggiotti 2006; Gaggiotti and Foll 2010).

189 The genetic differences among individuals was visualized thanks to a Principal Component
190 Analysis (PCA; function `dudi.pca`) with the Adegenet R package (Jombart 2008). We made an
191 individual clustering analysis with the STRUCTURE software (Pritchard et al. 2000) with 10^6
192 iterations for the burn-in and 10^6 iterations for the main analysis, 10 replicates for each K, and by
193 testing $K = 1$ to $K = 6$ in the two species. We used an admixture model with correlated allele
194 frequencies. The results were visualized with the POPHELPER website (Francis 2017;
195 <http://pophelper.com/>).

196 To infer the genetic origin of populations installed on artificial substrates (DON and GRE for
197 *P. clavata*), and of new recruits after a complete recolonization (GAZ for *E. cavolini*), we used the
198 GENECLASS2 software (Piry et al. 2004) with the option to infer individual assignment, by using
199 all other populations as references. We used the Rannala and Mountain (1997) criterion, and the

200 membership probability was computed according to Paetkau et al. (2004), with 1000 simulated
201 individuals, and a type I error of 0.01.

202

203 **Results**

204 Occurrences of repeated MLGs:

205 For *P. clavata*, five pairs of repeated MLGs were observed: two in the GRE population, one in
206 MTM, one in DON, one in RIS. The corresponding probability of occurrence of repeated genotypes
207 under sexual reproduction (p_{sex}) ranged from 7.10^{-9} to 2.10^{-5} . For *E. cavolini*, three MLGs were
208 detected more than once, all in the NISB population: one was repeated nine times ($p_{sex} = 0.010$),
209 another one five times ($p_{sex} = 0.014$) and another one two times ($p_{sex} = 0.014$). The overall
210 frequency of missing genotypes was around 17% for *E. cavolini* (mainly concentrated in the SFV
211 and LAN populations), and 10% for *P. clavata* (mainly in PQL).

212

213 Genetic diversity and structure within populations:

214 When considering rarefied allelic richness for *P. clavata*, the DON and GRE wreck populations had
215 an $Ar(29)$ of 7.6 and 7.7 respectively, similar to the value observed for the neighbouring population
216 SAR (7.5; Table 1). The expected heterozygosity for DON and GRE was 0.62 and 0.65
217 respectively, whereas it was 0.61 for SAR, the lowest value found here. Regarding *E. cavolini*, the
218 rarefied allelic richness for GAZ (recolonized population; sample size $N=32$) was the second
219 highest value after the LAN population ($N=25$), with $Ar(14) = 4.9$ and 5.4 respectively. The same
220 trend was observed when considering expected heterozygosity, with values of 0.70 and 0.71 for
221 GAZ and LAN respectively. NISB and UST, for which we have no indication of mortality events,
222 showed the lowest allelic richness and expected heterozygosity values, but with small sample sizes
223 in both cases (Table 1).

224 For *P. clavata*, all samples showed a significant deviation from panmixia, with heterozygote
225 deficiencies varying from 0.06 (PTV and GRE) to 0.16 (PQL). For *E. cavolini*, three samples over

226 six showed a significant deviation from panmixia, and heterozygote deficiencies varied from 0.01
227 (SFV) to 0.20 (SAN).

228 Regarding relatedness levels for *P. clavata*, the PTV and MTM populations showed higher mean
229 relatedness than most other populations, whereas GAB, PQL and RIO tended to have lower mean
230 relatedness than other populations (Table 2). There was no clear tendency for the wreck populations
231 DON and GRE. For *E. cavolini*, NISB and SFV showed higher mean relatedness than other
232 populations.

233

234 Genetic differentiation and origin of new recruits:

235 Regarding population differentiation, the global F_{ST} estimate was 0.04 for *P. clavata*, and 0.08 for
236 *E. cavolini*. For both species, all pairwise differentiation tests were significant, apart from the
237 comparison between GRE and SAR for *P. clavata*, two sites separated by 1.3 km (Table 3). For
238 *P. clavata*, the differentiation between DON and SAR was low (uncorrected pairwise $F_{ST} = 0.02$;
239 corrected pairwise $F_{ST} = 0.03$; spatial distance: 1.6 km) but significant. For *E. cavolini* and focusing
240 on the recolonized GAZ population, the lowest differentiation was observed with the LAN
241 population ($F_{ST} = 0.08$, for corrected and uncorrected estimates; distance 140 km). The mean
242 pairwise F_{ST} for the comparison involving GAZ was 0.1 and 0.07 for the uncorrected and corrected
243 estimates respectively. Accordingly, GAZ was then slightly more differentiated or at the same level
244 of differentiation than the other populations, except UST (range 0.04 – 0.11 and 0.02 – 0.12 for the
245 uncorrected and corrected estimates respectively).

246 Local F_{ST} are presented in Table 4. For *P. clavata*, the PQL population displayed a higher local F_{ST}
247 than other populations, and the corresponding 95% confidence interval did not overlap with those of
248 other populations. For *E. cavolini*, the highest local F_{ST} was observed for NISB, but with a 95%
249 confidence interval overlapping with those of other populations. None of the (re)colonized
250 populations showed significantly different values.

251 The PCA analysis of *P. clavata* did not show any clear structure pattern, with only a few individuals

252 from IMP and RIS separated from the other ones on axes 1 and 2 (Fig. 2). For *E. cavolini*, the main
253 pattern was a distinction of GAZ and UST samples on axes 1 and 2 respectively. The individuals of
254 these two populations were genetically diverse as shown by their quite wide distribution on the
255 PCA axes.

256 The results of the individual clustering are presented in Fig. 3. For *P. clavata*, we present the results
257 for $K = 2$ to $K = 5$ since there was no informative distinction above this value. At $K = 2$, one can
258 observe a gradient between two clusters, a major one around Marseille (RIO, RIS, IMP), and a
259 minor one increasing in frequency in other populations. At $K = 3$, GAB, MTM and PTV are
260 separated from the rest. At $K = 5$, we also observe a slight distinction of two populations: MTM and
261 RIS. The wreck populations DON and GRE appear related to the SAR population. In all cases, the
262 separation among clusters was not complete. For *E. cavolini*, we present the results for $K = 2$ to $K =$
263 4, there was no informative distinction above this value. GAZ and UST are separated from other
264 populations at $K = 2$ while GAZ and UST are separated at $K = 3$. At $K = 4$ there is a distinction of
265 GAZ from other populations which are almost equally admixed among the three other clusters.
266 Nevertheless some individuals in GAZ seem more related to other populations than to the blue
267 cluster dominant in this population.

268 The results of assignment analyses are presented in supplementary Table 1. For *P. clavata*, if we
269 arbitrarily consider a threshold of 0.95 for membership probabilities, three over 33 individuals of
270 DON could be related to SAR, six to PQL, three to RIO and one to IMP (this individual also
271 showed high probability for RIO). For GRE, three over 33 individuals could be related to SAR, and
272 seven over 33 to PQL. For *E. cavolini*, eight individuals over 32 displayed high membership
273 probability for the LAN population. In both cases several individuals displayed very low
274 membership probabilities for all the tested populations suggesting that they come from non sampled
275 sites.

276

277 **Discussion**

278 *Spatial patterns of recolonization:*

279 Our data allow discussing and comparing the dispersal and recolonization abilities of two
280 Mediterranean octocoral species. For *P. clavata*, we observed a genetic proximity between the
281 samples on the two wrecks DON and GRE, and the nearest population of natural substrate SAR.
282 The differentiation was very low (and even non significant for GRE) between these two populations
283 and SAR, 1.2 km apart. The longevity of *P. clavata* may reach 50 to 100 years, but their age is
284 difficult to estimate, for example due to variation in growth rate or breakage of some branches
285 (Linares et al. 2007). The age of the wrecks is known (more than 70 years), which sets an upper
286 limit to population age, but not the age of the sampled colonies. Some colonies sampled here could
287 then correspond to the first generation of founders, but also be descendants from these founders, or
288 descendants from more recent migrants. We can consider two non-mutually exclusive explanations
289 to the observed low differentiation among wrecks and surrounding populations. First, the initial
290 recruits came from the SAR population, with no or low enough founder effect that would have
291 induced initial differentiation. Second, gene flow from SAR after colonization could have
292 contributed to the genetic homogeneity of *P. clavata* at that scale. The lack of founder effect in
293 *P. clavata* was related to the late sexual maturity observed in this species (Coma et al. 1995;
294 Ledoux et al. 2018). Indeed, during the years following the foundation of a new population, the
295 expansion of the population is mainly due to new migrants and not to local reproduction. This
296 increases the effective population size until the first reproductive event buffering the founder effect
297 (Austerlitz et al. 2000). Even if significant, the low differentiation observed with other populations
298 than SAR points to the possible participation of these populations to the newly founded ones as
299 well. This is supported by assignment analysis indicating a contribution of PQL. In their study of a
300 recently founded population of *P. clavata*, Arizmendi-Mejía et al. (2015) identified multiple source
301 populations at distances ranging from 300 m to around 1 km, which is similar to our results. The
302 larval duration of *P. clavata* has been estimated between 8 and 25 d in laboratory, which could
303 allow long distance dispersal (Linares et al. 2008). The assignment of three individuals to a

304 population from Marseille can correspond to such long distance dispersal. Similarly, Padrón et al.
305 (2018) suggested that connectivity among coastal populations of *P. clavata*, possibly at tens of
306 kilometres, had contributed to their recovery after mortality events. Nevertheless, surface brooding
307 and larval behavior (negative phototaxis), and a short swimming phase may reduce the realized
308 dispersal in this species (Linares et al. 2008). In that case, we should consider that non sampled and
309 closer population(s) could have contributed to the mixed diversity of the wreck populations.
310 For *E. cavolini*, the colonies sampled in GAZ should be mainly new recruits following the 2008
311 mortality event. These recruits could correspond to local recruitment from the few remaining
312 colonies (Gambi and Barbieri 2012). There was no significant reduction in genetic diversity at that
313 site compared to other populations. This could mean that there was enough surrounding colonies to
314 sustain population recovery; for example one can note that the mortality event was less strong at
315 25 m, and this would constitute a possible refugia. Additionally, recruits could have come from
316 different populations, as suggested by the genetic differences among individuals shown by
317 clustering and multivariate analyses. The origin of these foreign recruits in the GAZ population is
318 not clear based on our sampling. The assignment analysis suggested the possibility of LAN as
319 origin of the recruits in GAZ. LAN and GAZ are distant from around 140 km apart. This is at odd
320 with the local differentiation usually observed for this species (Cánovas-Molina et al. 2018). It
321 seems more probable that non-sampled populations, either genetically akin to LAN, or
322 corresponding to another genetic group, contributed to this signal of distant recolonization of GAZ.
323 In a preliminary study of the colonization of artificial reefs by *E. cavolini*, it was difficult to
324 precisely identify the population(s) of origin, but potential source populations could be distant by
325 two to three kilometres from these artificial structures (Cánovas-Molina et al. 2018). There is no
326 information on the larval biology of *E. cavolini* to further interpret these results. In the congeneric
327 species *E. singularis*, the settlement could be possible within 30 h (Weinberg 1979), but it would be
328 interesting to formally characterize the larval phase duration in *E. cavolini*.
329 In any case, the analysis of the colonized or recolonized populations in the two species studied here

330 clearly indicate a mixed origin of the recruits. Recolonization from mixed origins, with sporadic
331 distant input, has also been observed in hexacorals (Underwood et al. 2007; Starger et al. 2010). In
332 the case of *E. cavolini* (GAZ), this was done in a relatively short time after mortality event
333 (mortality in 2008, sampling in 2013). Interestingly, Cupido et al. (2012) have observed an increase
334 in recruitment for *P. clavata* following mortality events, possibly because a greater space
335 availability. This could both limit the erosion of genetic diversity (see below), and allow gene flow
336 from other populations. From a methodological point of view, our results underline that studying
337 recolonization in Mediterranean octocorals should not only consider neighbouring populations, but
338 increase the sampling effort to distant populations.

339

340 *Consequences on the genetic diversity of the populations:*

341 We did not observe any impact of colonization or recolonization in the population diversity
342 of both species, nor with local F_{ST} reflecting local drift. One can note that the expected
343 heterozygosities observed here for *E. cavolini* are higher than those previously reported by
344 Cánovas-Molina et al. (2018), and in the range of values obtained for populations from western
345 Mediterranean by Masmoudi et al. (2016), but in both cases with a microsatellite panel partly
346 different from the one used here.

347 As already mentioned, in the case of *E. cavolini* most other populations analysed here suffered from
348 mortality events which may have reduced their genetic diversity as well. Regarding *P. clavata*, the
349 levels of diversity observed here are in the lower range of those observed by Mokhtar-Jamai et al.
350 (2011) or Pérez-Portela et al. (2016), again with a partially different panel of microsatellite markers.
351 In the case of this species, depth has been shown to be positively correlated with genetic diversity
352 (Pilczynska et al. 2019). Even if the sampling scheme does not allow a precise study of this
353 question, a similar tendency was observed here with a mean expected heterozygosity of 0.67 for the
354 depth range 20 to 34 m, and 0.69 for 40-41 m. In both species we observed significant heterozygote
355 deficits in most populations. This is in line with previous observations of departures from panmixia

356 in Mediterranean gorgonians (e.g. Ledoux et al., 2010b; Mokhtar-Jamaï et al., 2011). These
357 departures have been explained by a combination of inbreeding and null alleles, and this does not
358 seem linked with patterns of recolonization here.

359 The retention of genetic diversity and the lack of change in relatedness following recolonization can
360 be the consequence of the mixed origin of the recruits. In *E. cavolini*, we did not observe any
361 genetic effect of mortality events either. Nevertheless detecting such effects of mortality might
362 require the comparison of more populations, and cohorts within populations, with more information
363 on the date and impact of mortalities. In *P. clavata*, Pilczynska et al. (2016) did not detect any
364 reduction in genetic diversity following mortality event as well. One can note that to have a genetic
365 impact, any demographic shrinkage should be sustained over several generations (Allendorf 1986),
366 whereas we consider here only a very low generation number. Additionally, partial mortality of
367 colonies, long generation time (Lippé et al. 2006), late sexual maturity (Ledoux et al. 2018), and an
368 increase in recruitment (Cupido et al. 2012) can buffer the genetic impact of bottlenecks or
369 demographic accidents on populations. As shown here, in a metapopulation context, exchanges
370 from more or less distant populations will also limit the genetic consequences of mortality events.
371 These conclusions might be different on a longer term. The low survivorship success of the early
372 stages, despite the high investment in the production of gametes in *P. clavata* (Coma et al. 1995),
373 suggests that the species may have a low capacity to recover during large or frequent disturbances
374 (Linares et al. 2008). More generally, if climate change induces strong and recurrent scale mortality
375 events, this might lead to a reduction of larval recruitment of different species. This has been
376 observed in scleractinians of the Great Barrier Reef after mass bleaching event (Hughes et al. 2019).
377 In a context of population fragmentation, and anthropization of coastal areas, it is important to
378 consider the role of artificial structures as potential substrates for settlement and to enhance
379 biodiversity. In the Bay of Marseille, after 11 years of immersion, artificial reefs are already
380 colonized by several octocoral species, such as *E. cavolini*, *E. singularis* and *E. verrucosa*
381 (Guillemain et al., unpublished data). Our results show that the colonization of wrecks did not lead

382 to a reduction of genetic diversity at a given range depth for *P. clavata*. Similarly, Ordóñez et al.
383 (2013) did not observe any significant difference in genetic diversity between populations of the
384 ascidian *Microcosmus squamiger* on artificial and natural substrates. Conversely a lower genetic
385 diversity was observed for the limpet *Patella caerulea* on artificial structures compared to natural
386 rocky substrates (Fauvelot et al. 2009). Such different results are probably linked to different
387 population dynamics, and to different interaction with the substrates according to the species. It will
388 then have different consequences on the role of these new substrates and associated populations in
389 the connectivity and genetic diversity of populations. In the case of octocorals, our results indicate
390 that artificial substrates provide good opportunities for the development of new populations, or the
391 extension of existing ones. Artificial substrates situated at a few kilometres from natural populations
392 can possibly be colonized and allow the development of new populations. It will be interesting to
393 extend connectivity studies in this context to test if these populations could have other impacts, such
394 as for example act as stepping stone to allow gene flow between previously isolated areas.

395

396 *Factors driving short distance differentiation in Mediterranean octocorals*

397 Genetic differentiation at short distance has been repeatedly observed in Mediterranean octocorals
398 (e.g. Costantini et al. 2007; Ledoux et al. 2010a; Mokhtar-Jamai et al. 2011; Cánovas-Molina et al.
399 2018). Several factors could contribute to such patterns. First a short mean dispersal distance has
400 been inferred from the analysis of local genetic structure, and from the observation of related
401 individuals at a very short distance in the red coral *C. rubrum* (Ledoux et al. 2010a). This may seem
402 at odd with the long larval survival (up to 42 days) estimated for this species in aquarium
403 (Martínez-Quintana et al. 2015). Also, the observation of a short-distance differentiation does not
404 preclude for the presence of sporadic more distant exchanges allowing gene flow at a larger
405 metapopulation scale. Such discrepancy between the spatial scale of genetic structure and the
406 duration of larval phase has been observed for example in the fish *Elacatinus lori* (D'Aloia et al.
407 2015), calling to more detailed study of factors driving connectivity. Second, genetic drift is the

408 other important driver of genetic structure, and analyses of local genetic structure pointed to a
409 relatively low effective size in the red coral *C. rubrum* (Ledoux et al. 2010a). Finally, priority effect
410 at the genetic level can increase genetic differentiation, when the arrival of new recruits is limited
411 by the presence of already installed individuals (isolation by colonization; Orsini et al. 2013). Our
412 results, and previous ones (Arizmendi-Mejía et al. 2015), suggest that in a metapopulation context,
413 colonization or recolonization may not be limited by dispersal. Available habitat, either artificial or
414 natural, following mortality, seem to be easily recolonized, at least in a metapopulation context. A
415 temporal survey over several generations, or indirectly the comparison of different cohorts, would
416 allow estimating the strength of isolation following the founding of new populations.

417

418 **Perspectives:**

419 Our results show the possibility of successful (in terms of genetic diversity) colonization or
420 recolonization in Mediterranean gorgonians, including on artificial substrates. These data will be
421 useful for the spatial design of similar studies in these species. The use of a higher number of
422 markers such as SNPs could increase the power of assignment analyses as well (e.g. Glover et al.
423 2010; Benestan et al. 2015). Finally, it would be interesting to study the adaptation of these species
424 to new substrates with population genomics approaches (Manel et al. 2016). As this may be difficult
425 in a context of strong genetic structure (Pratlong et al. 2018), such results based on microsatellite
426 loci in different species will be useful to choose the best sampling scheme.

427

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446
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450
451 **Data availability:**

452 All genotypes have been deposited in the Zenodo database under reference:

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454

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- 660

661 **Figure legends:**

662 **Figure 1:** sampling locations for *Paramuricea clavata* and *Eunicella cavolini*. Asterisks indicate
663 populations issued from colonization (wrecks for *P. clavata*) or recolonization after mortality event
664 (*E. cavolini*). Maps were produced with SimpleMappr (Shorthouse, 2010).

665

666 **Figure 2 :** plots of PCA results. A) *Paramuricea clavata* with axis 1 (2.7 % of variance) horizontal
667 and axis 2 (2.4 % of variance) vertical; B) *P. clavata* with axis 1 horizontal and axis 3 (2.2 % of
668 variance) vertical. C) *Eunicella cavolini* with axis 1 (4.7 % of variance) horizontal and axis 2 (4.4 %
669 of variance) vertical; D) *E. cavolini* with axis 1 horizontal and axis 3 (3.9 % of variance) vertical.

670

671 **Figure 3:** results for the individual clustering analysis with STRUCTURE for A) *Paramuricea*
672 *clavata* and B) *Eunicella cavolini*.

673

1 **Table 1, samples description:** codes of sampling sites, GPS coordinates, depth, sample size
2 without repeated MLGs (N), mean number of alleles per locus rarefied for g individuals ($Ar(g)$; $g =$
3 29 for *Paramuricea clavata*, except for PQL with $g = 12$; and 14 for *Eunicella cavolini*, except UST
4 with $g = 10$), observed heterozygosity (H_O) and expected heterozygosity (H_E), multilocus F_{IS} (values
5 in bold correspond to significant test of panmixia after FDR correction at a 0.05 level).
6

site	code	GPS	depth (m)	year	N	$Ar(g)$	H_O	H_E	F_{IS}
<i>Paramuricea clavata</i>									
Pointe du Vaisseau	PTV	42°59'42.9"N 6°24'24.2"E	20-25	2008	29	7.2	0.59	0.63	0.06
Donator wreck	DON	42°59'35.9"N 6°16'26.1"E	34	2011	33	7.6	0.58	0.62	0.08
Le Grec wreck	GRE	42°59'37.0"N 6°16'42.0"E	34	2011	33	7.7	0.61	0.65	0.06
Sec de Sarraniers	SAR	42°59'16.3"N 6°17'30.2"E	40	2011	35	7.5	0.57	0.61	0.08
Gabinière	GAB	42°59'21.5"N 6°23'49.2"E	22-25	2008	32	7.8	0.63	0.69	0.09
Les Mèdes	PQL	43°01'43.0"N 6°14'28.0"E	31	2009	29	4.6	0.61	0.73	0.16
Montrémian	MTM	43°01'07.2"N 6°21'46.0"E	20-25	2008	29	6.1	0.57	0.64	0.12
Riou Marseille	RIO	43°10'21.66"N 5°23'25.16"E	20-25	2008	35	8.5	0.60	0.71	0.15
Riou Marseille	RIS	43°10'21.66"N 5°23'25.16"E	40	2008	30	8.0	0.66	0.73	0.10
Impériales Marseille	IMP	43°10'22.79"N 5°23'35.39"E	41	2007	39	8.3	0.63	0.72	0.12
<i>Eunicella cavolini</i>									
Nisida Island (Napoli)	NISB	40°47'22.8"N 14°09'42.0"E	20	2013	16	3.8	0.54	0.58	0.08
Secca Formiche di Vivara (Ischia)	SFV	40°44'20.45"N 13°58'45.08"E	15	2013	38	4.5	0.64	0.65	0.01
Sant'Angelo (Ischia)	SAN	40°41'30.58"N 13°53'37.76"E	18-20	2013	31	4.6	0.53	0.65	0.20
La Nave (Ischia)	LAN	40°42'25.35"N 13°51'12.73"E	20-25	2013	25	5.4	0.59	0.71	0.17
Grotta Azzura (Palinuro, Salerno)	GAZ	40°1'53.01"N 15°16'9.01"E	15	2013	32	4.9	0.66	0.70	0.06
Ustica Island Secchitello (Palermo)	UST	38°41'25.54"N 13°10'25.35"E	35-38	2013	14	4.0	0.61	0.63	0.04

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10 **Table 2, comparisons of relatedness levels:** comparison of within-population relatedness levels
 11 based on the estimator of Wang (2002), A) for *Paramuricea clavata*, and B) for *Eunicella cavolini*.
 12 Each comparison corresponds to the observed difference in mean relatedness between population in
 13 column and population in row. Blue and red values indicate significantly positive and negative
 14 differences respectively. Asterisk indicate extreme values based on 1000 bootstraps, with the
 15 corresponding percentiles: * 5 % ** 2.5 % *** 1 %.

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17 A) *P. clavata*

	PTV	DON	GRE	SAR	GAB	PQL	MTM	RIO	RIS
PTV									
DON	0.083***								
GRE	0.074***	-0.0092							
SAR	0.065***	-0.018	-0.0094						
GAB	0.11***	0.031***	0.032***	0.041***					
PQL	0.12***	0.036***	0.045***	0.054***	0.013				
MTM	-0.019	-0.1***	-0.093***	-0.084***	-0.13***	-0.14***			
RIO	0.14***	0.053***	0.062***	0.071***	0.03***	0.017	0.16***		
RIS	0.076***	-0.0073	0.0019	0.011	-0.03***	-0.043***	0.095***	-0.06***	
IMP	0.11***	0.029***	0.038***	0.047***	0.0056	-0.0073	0.13***	-0.024***	0.036***

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19 B) *E. cavolini*

	NISB	SFV	SAN	LAN	GAZ	UST
NISB						
SFV	0.14***					
SAN	0.21***	0.071***				
LAN	0.3***	0.15***	0.081***			
GAZ	0.21***	0.073***	0.0026	-0.079***		
UST	0.2***	0.2***	-0.0061	-0.087**	-0.0086	

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Table 3: pairwise F_{ST} values for A) *Paramuricea clavata*, and B) *Eunicella cavolini*. Below diagonal: standard F_{ST} estimates (Weir and Cockerham 1984); above diagonal: F_{ST} estimates from FreeNA. Values in bold correspond to significant genic differentiation after FDR correction at a 0.05 level.

A)	DON	GRE	SAR	GAB	MTM	PTV	PQL	RIO	RIS	IMP
DON	-	0.02	0.03	0.05	0.05	0.05	0.08	0.01	0.04	0.03
GRE	0.01	-	0.01	0.03	0.05	0.06	0.06	0.02	0.05	0.03
SAR	0.02	0.00	-	0.03	0.05	0.05	0.10	0.03	0.06	0.04
GAB	0.04	0.04	0.04	-	0.04	0.05	0.10	0.03	0.07	0.04
MTM	0.05	0.06	0.07	0.04	-	0.04	0.13	0.04	0.08	0.06
PTV	0.03	0.05	0.05	0.04	0.05	-	0.13	0.04	0.07	0.06
PQL	0.04	0.04	0.06	0.05	0.10	0.06	-	0.08	0.06	0.06
RIO	0.01	0.02	0.04	0.03	0.04	0.03	0.04	-	0.03	0.01
RIS	0.05	0.06	0.07	0.07	0.08	0.06	0.06	0.02	-	0.03
IMP	0.03	0.04	0.04	0.03	0.06	0.05	0.05	0.01	0.03	-

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B)	GAZ	LAN	NISB	SAN	SFV	UST
GAZ	-	0.08	0.08	0.07	0.06	0.05
LAN	0.08	-	0.12	0.07	0.07	0.11
NISB	0.11	0.05	-	0.03	0.05	0.07
SAN	0.10	0.04	0.04	-	0.02	0.07
SFV	0.09	0.04	0.06	0.04	-	0.07
UST	0.10	0.13	0.12	0.11	0.12	-

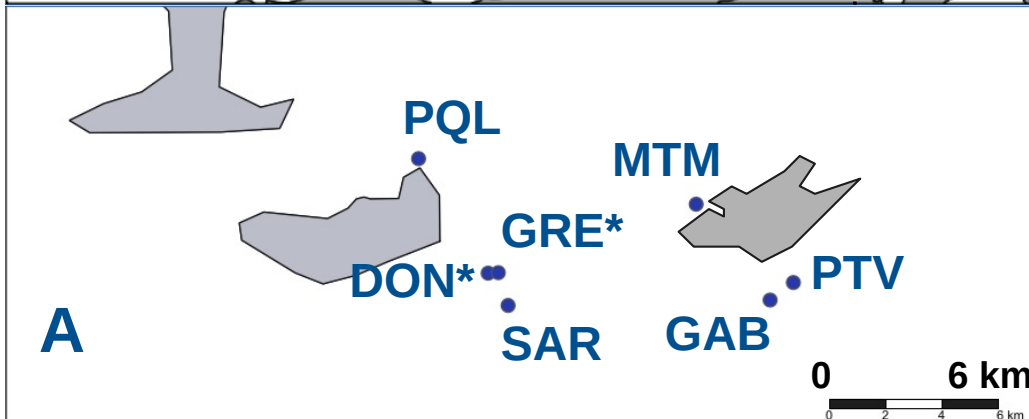
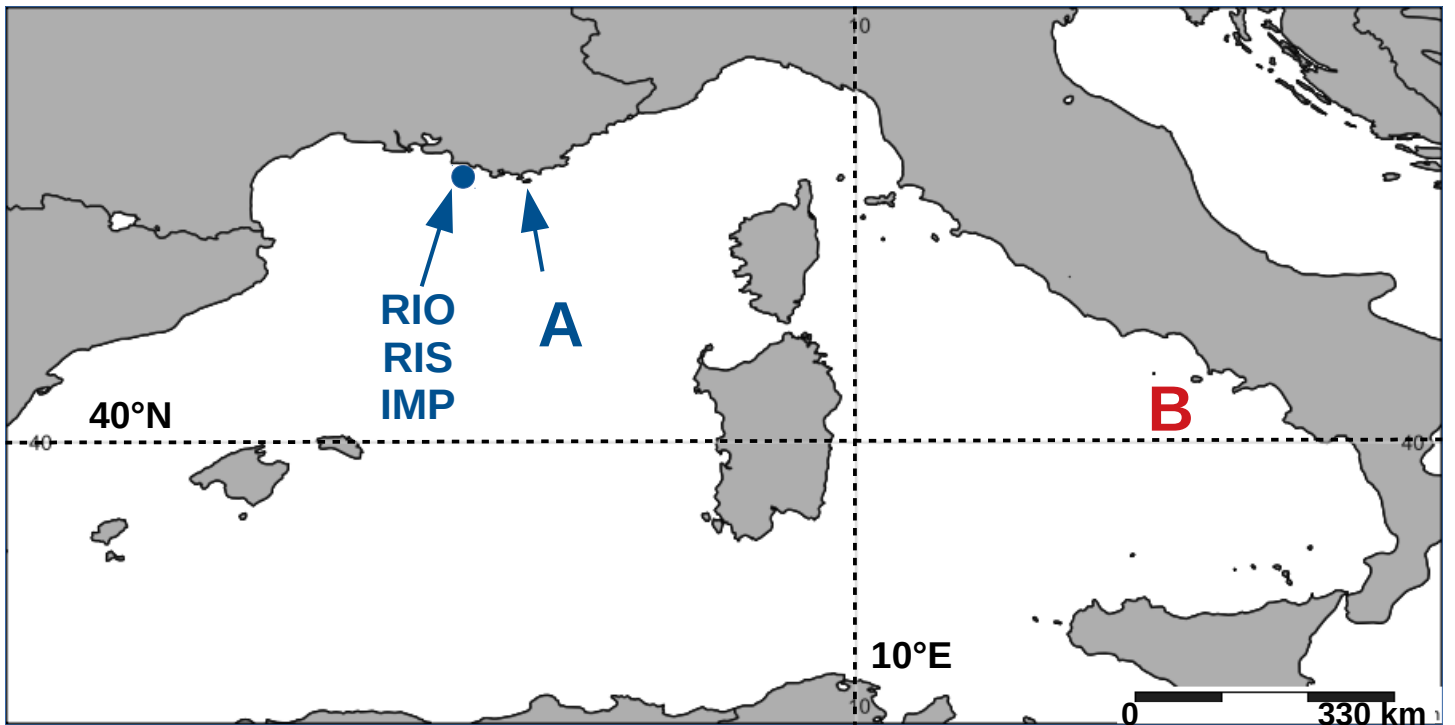
31

32 **Table 4:** estimates of local F_{ST} with 95% highest probability density interval (HPDI).

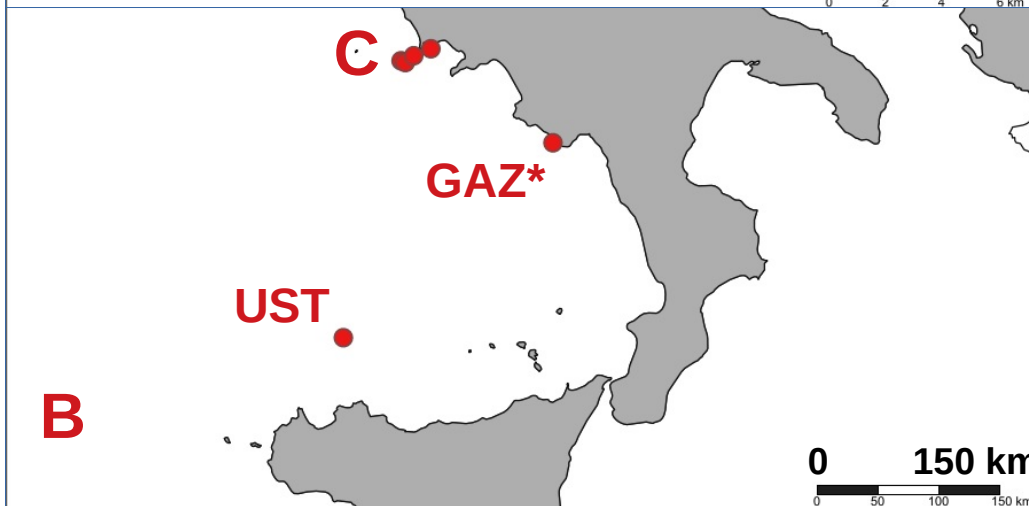
33

Population	mean	95% HPDI
<i>P. clavata</i>		
DON	0.08	0.0556 ; 0.109
GRE	0.08	0.0545 ; 0.107
SAR	0.07	0.0471 ; 0.0950
GAB	0.05	0.0327 ; 0.0768
MTM	0.12	0.0779 ; 0.162
PTV	0.08	0.0514 ; 0.109
PQL	0.23	0.165 ; 0.295
RIO	0.03	0.0205 ; 0.0498
RIS	0.07	0.0489 ; 0.0960
IMP	0.05	0.0327 ; 0.0671
<i>E. cavolini</i>		
GAZ	0.15	0.0940 ; 0.208
LAN	0.18	0.117 ; 0.238
NISB	0.30	0.188 ; 0.427
SAN	0.17	0.107 ; 0.235
SFV	0.22	0.142 ; 0.302
UST	0.24	0.144 ; 0.332

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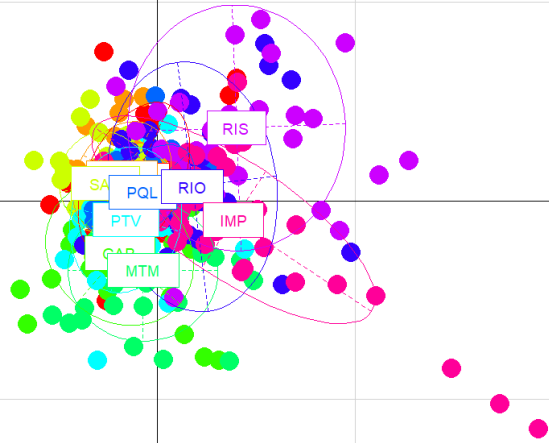
P. clavata



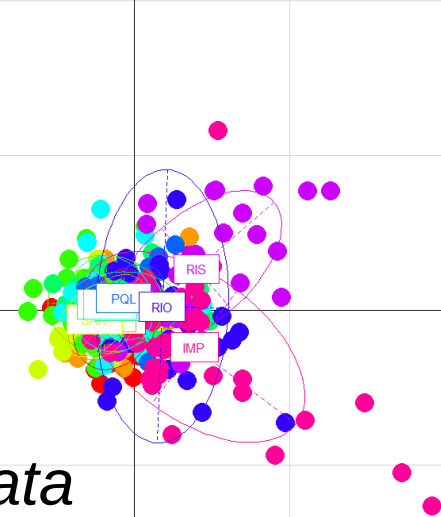
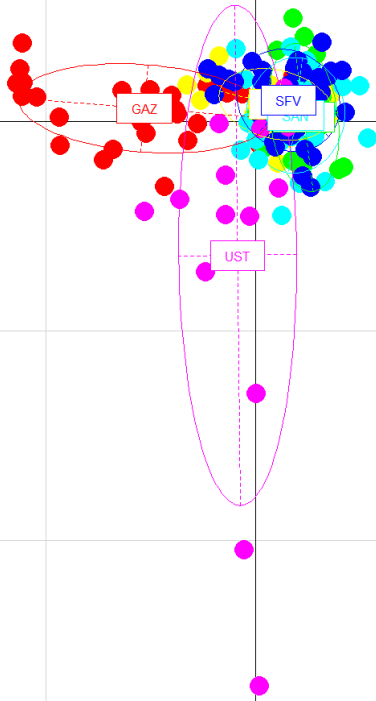
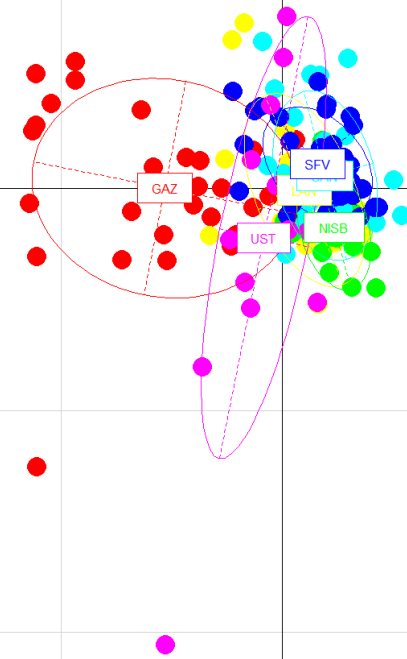
E. cavolini



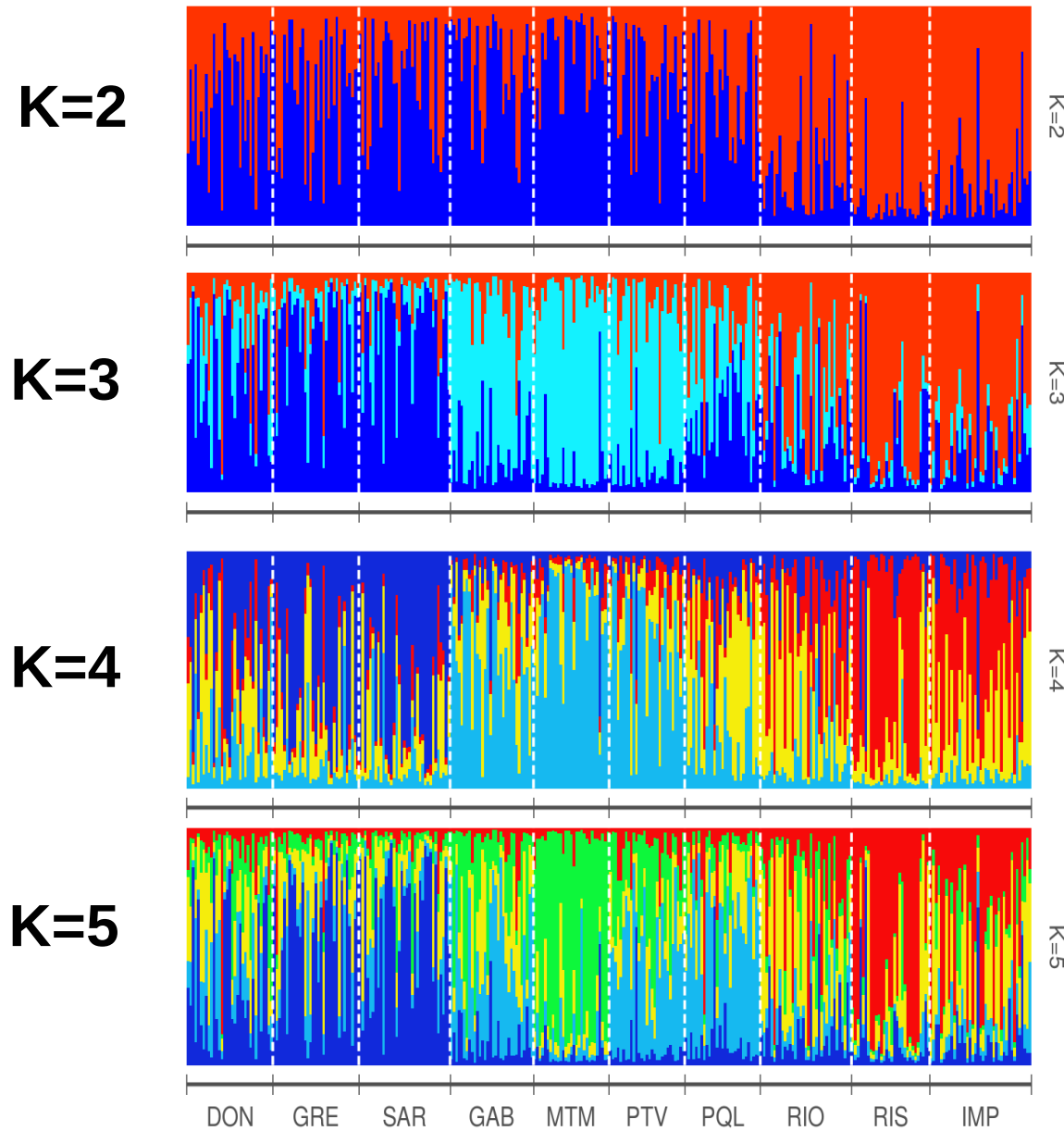
E. cavolini

A*P. clavata*

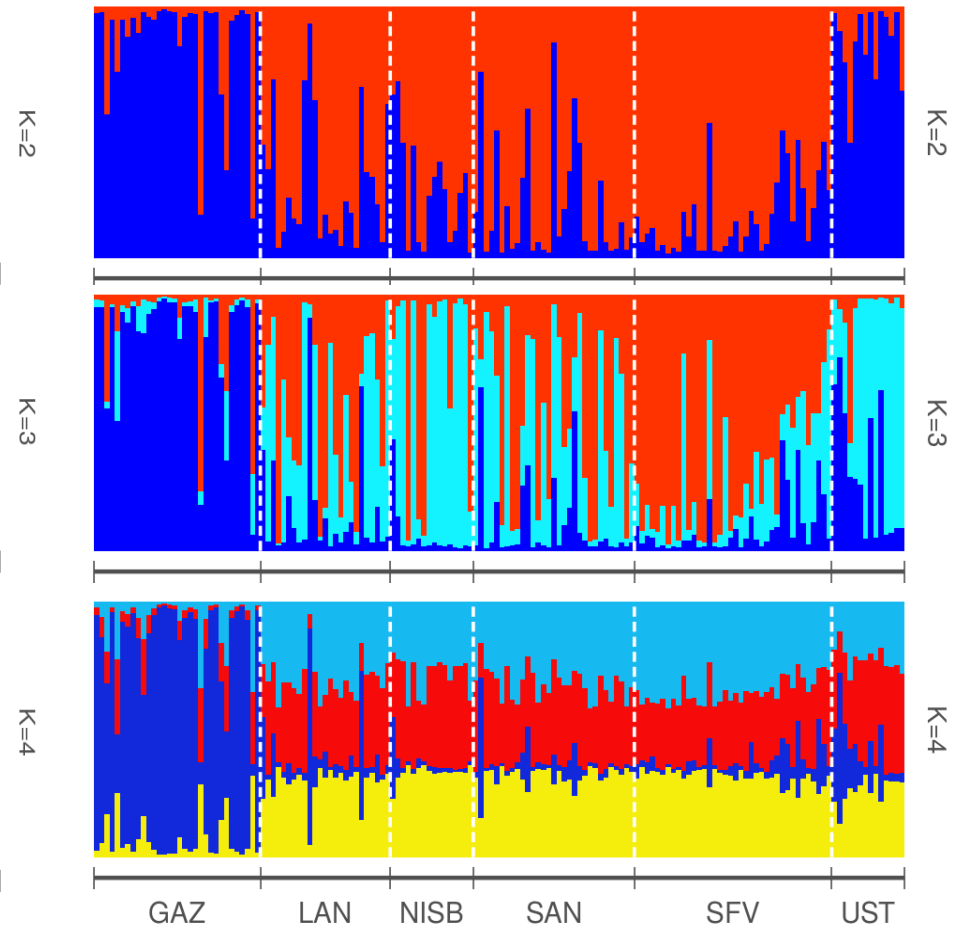
d = 5

B*P. clavata***C***E. cavolini***D***E. cavolini*

A) *P. clavata*



B) *E. cavolini*



Genetic insights into recolonization processes of Mediterranean octocorals

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Supplementary Table 1: results of the assignment of individuals to other populations. For the three focal populations DON, GRE and GAZ, we indicate the probability of each individual to belong to each of the other analyzed population. Bold values correspond to probabilities superior or equal to 0.95. The mean of individual probabilities is given for each potential source population.

a) *Paramuricea clavata*

	SAR	GAB	MTM	PTV	PQL	RIO	RIS	IMP
DON1	0.097	0.009	0.000	0.011	0.421	0.014	0.255	0.106
DON2	0.498	0.620	0.206	0.116	0.175	0.742	0.086	0.067
DON3	0.455	0.022	0.002	0.000	0.123	0.396	0.285	0.083
DON4	0.323	0.686	0.068	0.160	0.707	0.457	0.303	0.108
DON5	0.365	0.083	0.000	0.004	0.008	0.042	0.425	0.078
DON6	0.123	0.320	0.002	0.061	0.081	0.163	0.309	0.376
DON7	0.012	0.002	0.000	0.006	0.664	0.014	0.016	0.058
DON8	0.375	0.221	0.059	0.155	0.124	0.226	0.695	0.108
DON9	0.008	0.010	0.000	0.007	0.577	0.039	0.057	0.051
DON10	0.176	0.498	0.061	0.060	0.681	0.614	0.325	0.222
DON11	0.973	0.335	0.050	0.166	0.161	0.462	0.639	0.227
DON12	0.552	0.072	0.014	0.097	0.993	0.906	0.301	0.676
DON13	0.056	0.049	0.002	0.022	0.364	0.005	0.000	0.012
DON14	0.078	0.003	0.008	0.058	0.828	0.227	0.913	0.764
DON15	0.433	0.022	0.055	0.033	0.537	0.019	0.334	0.057
DON16	0.313	0.003	0.000	0.001	0.767	0.012	0.055	0.006
DON17	0.922	0.658	0.045	0.005	0.944	0.827	0.641	0.515
DON18	0.818	0.269	0.487	0.819	0.988	0.999	0.752	0.928
DON19	0.377	0.006	0.061	0.027	0.902	0.272	0.063	0.137
DON20	0.475	0.116	0.069	0.364	1.000	0.909	0.131	0.510
DON21	0.534	0.346	0.010	0.049	0.647	0.399	0.446	0.298
DON22	0.932	0.425	0.272	0.066	0.690	0.983	0.265	0.598
DON23	0.552	0.107	0.014	0.073	1.000	0.323	0.506	0.604
DON24	0.077	0.010	0.001	0.007	0.040	0.034	0.032	0.178
DON25	0.420	0.290	0.086	0.183	0.893	0.990	0.440	0.974
DON26	0.466	0.050	0.105	0.333	0.649	0.560	0.051	0.168
DON27	0.006	0.002	0.000	0.020	0.907	0.031	0.561	0.096
DON28	0.954	0.260	0.059	0.002	0.594	0.770	0.462	0.785
DON29	0.723	0.117	0.221	0.403	0.600	0.827	0.405	0.665
DON30	0.328	0.009	0.000	0.005	0.672	0.027	0.069	0.049

DON31	0.836	0.178	0.001	0.043	1.000	0.605	0.464	0.836
DON32	0.049	0.056	0.021	0.021	0.092	0.313	0.239	0.343
DON33	0.962	0.899	0.876	0.536	0.970	0.918	0.655	0.714
mean	0.432	0.205	0.087	0.119	0.600	0.428	0.339	0.345
GRE1	0.707	0.208	0.000	0.011	0.761	0.165	0.302	0.167
GRE2	0.491	0.144	0.005	0.091	0.965	0.136	0.610	0.667
GRE3	0.138	0.146	0.011	0.002	0.660	0.218	0.102	0.007
GRE4	0.091	0.004	0.000	0.000	0.002	0.014	0.079	0.001
GRE5	0.781	0.157	0.116	0.001	0.426	0.821	0.025	0.181
GRE6	0.663	0.287	0.023	0.048	0.999	0.262	0.491	0.924
GRE7	0.619	0.005	0.002	0.001	0.862	0.027	0.051	0.103
GRE8	0.953	0.125	0.121	0.105	0.691	0.567	0.138	0.167
GRE9	0.687	0.090	0.001	0.004	0.413	0.524	0.324	0.431
GRE10	0.373	0.018	0.002	0.003	0.101	0.052	0.090	0.025
GRE11	0.928	0.541	0.036	0.152	0.427	0.504	0.323	0.252
GRE12	0.739	0.063	0.002	0.011	0.668	0.024	0.105	0.194
GRE13	0.054	0.005	0.000	0.017	0.008	0.322	0.029	0.047
GRE14	0.022	0.027	0.000	0.093	0.660	0.061	0.038	0.088
GRE15	0.015	0.001	0.000	0.000	0.240	0.001	0.007	0.022
GRE16	0.540	0.058	0.008	0.012	0.843	0.384	0.528	0.481
GRE17	0.834	0.042	0.024	0.024	0.281	0.299	0.241	0.166
GRE18	0.183	0.003	0.000	0.016	0.705	0.101	0.126	0.076
GRE19	0.765	0.011	0.015	0.123	0.011	0.007	0.087	0.102
GRE20	0.132	0.015	0.002	0.086	0.065	0.348	0.378	0.237
GRE21	0.950	0.918	0.053	0.029	0.980	0.511	0.619	0.711
GRE22	0.906	0.001	0.006	0.003	0.958	0.080	0.454	0.457
GRE23	0.769	0.010	0.003	0.000	0.086	0.163	0.038	0.034
GRE24	0.511	0.033	0.039	0.347	0.746	0.207	0.134	0.323
GRE25	0.997	0.021	0.002	0.004	0.000	0.020	0.067	0.000
GRE26	0.096	0.012	0.001	0.000	0.229	0.443	0.003	0.132
GRE27	0.608	0.001	0.002	0.002	0.019	0.154	0.015	0.033
GRE28	0.552	0.501	0.096	0.874	0.991	0.322	0.316	0.870
GRE29	0.240	0.010	0.001	0.006	0.417	0.147	0.390	0.018
GRE30	0.888	0.083	0.148	0.328	0.957	0.793	0.523	0.919
GRE31	0.188	0.027	0.044	0.159	0.565	0.265	0.028	0.075
GRE32	0.490	0.028	0.021	0.093	0.512	0.656	0.053	0.041
GRE33	0.898	0.176	0.059	0.010	0.951	0.869	0.505	0.591
mean	0.540	0.114	0.026	0.080	0.521	0.287	0.219	0.259

b) *Eunicella cavolini*

	LAN	NISB	SAN	SFV	UST
GAZ1	0.635	0.000	0.010	0.011	0.017
GAZ2	0.015	0.000	0.000	0.000	0.003
GAZ3	0.999	0.001	0.371	0.260	0.004
GAZ4	0.606	0.000	0.000	0.027	0.000
GAZ5	1.000	0.138	0.390	0.420	0.167
GAZ6	0.921	0.000	0.013	0.016	0.061
GAZ7	0.994	0.456	0.480	0.605	0.016
GAZ8	0.834	0.000	0.079	0.283	0.005
GAZ9	0.927	0.061	0.337	0.181	0.013
GAZ10	0.017	0.000	0.000	0.000	0.002
GAZ11	0.665	0.009	0.028	0.059	0.095
GAZ12	0.968	0.000	0.017	0.382	0.010
GAZ13	0.376	0.000	0.000	0.000	0.000
GAZ14	0.504	0.000	0.000	0.000	0.000
GAZ15	0.467	0.000	0.001	0.000	0.003
GAZ16	0.554	0.000	0.010	0.018	0.039
GAZ17	0.979	0.005	0.701	0.287	0.173
GAZ18	0.932	0.000	0.018	0.043	0.021
GAZ19	0.450	0.000	0.000	0.005	0.000
GAZ20	0.131	0.000	0.003	0.001	0.005
GAZ21	0.965	0.000	0.091	0.557	0.010
GAZ22	0.507	0.000	0.000	0.006	0.145
GAZ23	0.116	0.000	0.000	0.000	0.000
GAZ24	0.253	0.000	0.002	0.001	0.000
GAZ25	0.988	0.001	0.050	0.782	0.013
GAZ26	0.306	0.000	0.015	0.153	0.000
GAZ27	0.976	0.001	0.109	0.128	0.038
GAZ28	0.820	0.063	0.041	0.150	0.058
GAZ29	0.303	0.000	0.000	0.000	0.000
GAZ30	0.807	0.000	0.050	0.114	0.008
GAZ31	0.746	0.023	0.329	0.370	0.005
GAZ32	0.707	0.000	0.001	0.001	0.001
mean	0.640	0.024	0.098	0.152	0.029