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

Almudena Cánovas-Molina[1, *], Monica Montefalcone[1], Giorgio Bavestrello[1], Mauatassem Billah Masmoudi[2, 3], Anne Haguenauer[3], Pachka Hammami[3], Lamya Chaoui[2], Mohammed Hichem Kara[2] and Didier Aurelle[3,4*]

[1] DiSTAV, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università di Genova, Corso Europa 26, 16132 Genova, Italy.
[2] Laboratoire Bioressources Marines, Université d'Annaba, Badji Mokhtar, BP 230, Oued Kouba, 23008, Annaba, Algeria.
[3] Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France
[4] Aix Marseille Univ., Université de Toulon, CNRS, IRD, MIO UM 110 , 13288, Marseille, France

*Corresponding authors: Tel.: +33 486 09 06 22.
E-mail addresses: almucanovass@gmail.com (A. Cánovas-Molina), didier.aurelle@univ-amu.fr (D. Aurelle).

**Abstract**

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

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

## 1. Introduction

In a context of growing pressures affecting the marine environments, estimating connectivity among populations is essential since the scale of larval dispersal is crucial for understanding species reorganization and populations evolution after large-scale disturbances (Bellwood et al., 2004; Salm et al., 2006). Size and interconnectivity of populations are important parameters to estimate for conservation strategies, since small and isolated populations are vulnerable to inbreeding depression, limited spread of beneficial alleles (Alleaume-Benharira et al., 2006). and loss of genetic diversity, which might reduce their evolutionary potential and increase their risk of extinction (Saccheri et al., 1998). Population genetics describes connectivity by comparing allele frequencies among spatially discrete subpopulations. High levels of genetic similarity between populations can indicate gene flow over time, usually through larval dispersal, whereas significant differentiation between populations can correspond to significant and persistent barriers to larval exchange. Nevertheless, the observed level of genetic differentiation depends on the interaction between gene flow, genetic drift and mutation (depending on the time scale or molecular markers). For most benthic marine species with complex life cycles, the exchange of individuals among populations occurs primarily through the pelagic larval stage (Cowen and Sponaugle, 2009). These exchanges depend on biotic and abiotic factors including spawning outputs, larval dispersal, habitat availability, hydrodynamics and trophic interactions (Padrón and Guizien, 2015; Pineda, 2000). Evidence from direct and indirect approaches using geochemical and genetic techniques suggests that the genetic structure of marine populations, while often low, ranges from fully open to fully closed (Cowen and Sponaugle, 2009).

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

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

**Commenté [DA2]:** Made?

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

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

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

Ledoux et al., 2010), the red gorgonian *Paramuricea clavata* (Arizmendi-Mejía et al., 2015; Mokhtar-Jamaï *et al.*, 2011), the yellow gorgonian *E. cavolini* (Masmoudi et al., 2016), the only symbiotic Mediterranean gorgonian *Eunicella singularis* (Constantini et al., 2016; Pey et al., 2013) or the sponge *Crambe crambe* (Duran et al., 2004). Regarding *E. cavolini*, the first molecular study gave little information on the genetic structure of this species because of a lack of informative markers (Calderón et al., 2007). The use of microsatellite markers in *E. cavolini* allowed the identification of different genetic clusters in the Mediterranean corresponding to the main geographical regions, and with different levels of genetic diversity: the allelic richness of eastern Mediterranean populations (i.e. from Turkey) was two-fold lower than in Algeria (Masmoudi et al., 2016). The genetic structure in the region of Marseille (France) indicated a differentiation between most populations (Masmoudi et al., 2016). Although no differentiation was observed between depths (between 20 m and 40 m depth) at a given site (Masmoudi et al., 2016), significant differences in thermo-tolerance were demonstrated at the bathymetrical scale (Pivotto et al., 2015).

In the present work, we focused on the yellow gorgonian *Eunicella cavolini* (Koch, 1887), an octocoral species widely distributed in the Mediterranean, which can be observed from less than 10 m to more than 100 m depth (Sini et al., 2015). The genetic structure of this species has been studied mainly at the basin-wide Mediterranean spatial scale (Masmoudi et al., 2016). Definition of the boundaries of Marine Protected Areas (MPAs) should consider the connectivity among habitats for management and conservation of marine species (Palumbi, 2004), and this requires a more precise study of connectivity at both the regional and the local spatial scales. In this sense, NW Mediterranean offers an interesting context for genetic structure studies at different spatial scales where the yellow gorgonian forms populations from 15 m depth reaching here the maximum densities of the Mediterranean sites studied by Sini et al. (2015). The absence or low genetic differentiation between shallow and deep *E. cavolini* observed at Marseille (SE France) is of great importance considering the impact of climate change and the possibility of exchanges between depths with different thermal conditions (Pivotto et al., 2015), and it should be tested in other areas as well. Located 300 km apart in the same Ligurian Sea, Portofino (NW Italy) offers a perfect

location for the genetic study of *Eunicella cavolini* populations at regional, local and depth scale. Given the lack of information on the effects of the spatial scale on genetic structure of this species, our aims were:

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

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

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

**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...)

## 2. Materials and methods

### 2.1 Samples collection

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

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

Table 1. Information on the samples collection of *Eunicella cavolini* in Marseille (France) 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 |

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

2.2 DNA extraction and microsatellite genotyping

Total genomic DNA was extracted using QIAamp® DNA Mini Kit (Qiagen®) according to manufacturer's instructions. Individuals were genotyped at six microsatellite loci: C21, C30, C40, S14, EVER007, EVER009 (Holland et al., 2013; Molecular Ecology Resources Primer Development Consortium et al., 2010; Pivotto et al., 2015). The loci were amplified through PCR Multiplex using the Type-it® Microsatellite PCR Kit (Qiagen®). PCR amplifications were done in a final reaction volume of 10 µL containing 1 µL of DNA, 5 µL of MasterMix, 3 µL of $H_2O$ Milli-Q® and 1 µL of primer mix (with each primer at 2 µM).

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

2.3 Genetic diversity and structure

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

Linkage disequilibrium was tested among all pairs of loci at each site with a permutation test (n = 1000) using GENETIX v.4.05 (Belkhir et al., 2004). The f estimator of $F_{IS}$ (Weir and Cockerham, 1984) was computed for each population using GENETIX. Departures from panmixia were tested 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 (Ho) and Nei's
183 (1973) unbiased expected heterozygosity (He) with GENETIX. Allelic richness [Ar (g)] and
184 private allelic richness [Ap (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 Dest (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

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

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

We tested for isolation by distance (IBD) by examining the correlation between pairwise $F_{ST}/(1-F_{ST})$ 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.

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

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

## 3. Results

### 3.1 Genetic variability

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

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

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

3.2 Deviations from Hardy–Weinberg equilibrium

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

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

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

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

3.3 Population genetic structure

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

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

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

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

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

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

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

## 4. Discussion

### 4.1 Genetic diversity

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

4.2 Genetic structure at large spatial scale

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

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

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

**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.

4.3 Genetic structure at small spatial scale

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

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

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

Some of the studied sites could be more isolated because of the distance (e.g. Sarranier and 3PP cave), a particular position behind a headland (e.g. Punta Cervara), or caused by oceanographic 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.

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

**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.

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

### 4.4 Genetic structure between depths

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

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

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

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

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

**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.

## 5. Conclusion

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

## Supplementary data:

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

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

(DOC)

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

(DOC)

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

(DOC)

**S4 Table. Results of mean Dest and $F_{ST}$ estimates.**

(DOC)

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

(DOC)

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

(DOC)

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

(DOC)


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


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