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Genetic connectivity supports recovery of gorgonian populations affected by climate change

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RESEARCH ARTICLE

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Genetic connectivity supports recovery of gorgonian populations affected by climate change

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- Abstract
- 1. Climate-induced threats are increasingly affecting marine populations worldwide. In the last few decades, several gorgonian species have been affected by mass mortality events in the north-west Mediterranean, putatively linked to local sea temperature increases during heatwaves. For many benthic sessile species, recovery after disturbances depends upon larval supply shaping the connection among populations.
 - 2. In the Ligurian Sea, genetic analyses showed that some Paramuricea clavata populations recovered after mass mortality events; however, the patterns of connectivity, and the potential role of migration in supporting the recovery of the populations of P. clavata, across its distribution range within the Ligurian Sea are still unknown.
 - 3. In this study, the population genetic structure and migration patterns of P. clavata populations have been analysed across seven sites in the Ligurian Sea, some of which have been affected by mass mortality events.
 - 4. Evidence of a population bottleneck was found in most of the populations studied. Significant genetic differentiation was found among P. clavata populations, reflecting habitat fragmentation at a regional scale, except for two populations found 20 km apart. Continuing gene flow between distant populations was also revealed. Empirical data suggest that gene flow among populations may have contributed to support their recovery from mass mortality events.
 - 5. The study identified populations in the central part of the Ligurian Sea that can be strategic for the regional persistence of the species. These findings highlight that the preservation of key populations could maintain connectivity and gene flow in the metapopulation, and increase the resilience of the species.

KEYWORDS

benthos, climate change, coastal, coral, dispersal, disturbance, genetics, invertebrates

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2 WILEY 1 ↓ INTRODUCTION

Marine biodiversity on a global scale is under growing pressure from environmental alteration caused by human activities, including climate change (Hoegh-Guldberg & Bruno, 2010). Trends of increasing seawater temperature, decreasing ocean pH, and sea-level rise, along with the higher frequency and magnitude of extreme climatic events (e.g. storms and heatwaves), are threatening marine species and habitats (Doney et al., 2012). The threshold of populations and habitat resilience to climatic disturbance is still an open question in contemporary ecology and conservation biology, however (Wernberg, Russell, Thomsen, & Connell, 2014).

Severe and sudden reductions in population size (i.e. demographic 15 bottleneck) caused by mass mortality events are expected to lead to a 16 loss of genetic diversity, primarily allelic diversity, and eventually cause a genetic bottleneck (Lacy, 1987). Furthermore, within this context, genetic drift may amplify the loss of genetic variation in isolated populations, and potentially lead to inbreeding (Nei, Maruyama, & 20 Chakraborty, 1975). Nonetheless, the loss of genetic diversity caused 21 by a population collapse can be offset or reversed by migration pro-22 cesses (Crow & Kimura, 1970; Keller et al., 2001). Therefore, connectivity among populations is an asset for the recovery of the pre-24 disturbance demographic structure and genetic diversity of the population.

In marine sessile benthic species, migration between distant pop-27 ulations ensured by larval dispersal is expected to be a key aspect of 28 resilience (Almany et al., 2009). The role of migration patterns in the 29 recovery after disturbance is controversial, however: although it is 30 possible to forecast wide larval dispersal ranges with biophysical 31 modelling (Lipcius et al., 2008; Shanks, Grantham, & Carr, 2003), gene 32 flow, as assessed with molecular tools, appears to be more restricted, 33 as a result of filtering by settlement and recruitment processes 34 3<mark>Q</mark>6 (Pineda, Hare, & Sponaugle, 2007). Despite several works assuming the importance of connectivity to the persistence of sessile benthic 36 species populations, empirical evidence on how patterns of larval dis-37 persal confer resilience to populations remains rare (Bramanti, lannelli, 38 Fan, & Edmunds, 2015; Caplins et al., 2014; Gilmour, Smith, Heyward, 39 Baird, & Pratchett, 2013; Magris, Pressey, Weeks, & Ban, 2014; 40 Underwood, Smith, van Oppen, & Gilmour, 2007; Vollmer & Palumbi, 41 2007). Investigating the genetic diversity and the structure of marine 42 populations can provide estimations of their ability to withstand per-43 turbations, and of their rate of recovery (Hughes, Inouye, Johnson, 44 Underwood, & Vellend, 2008; Kovach et al., 2015; Van Oppen & $\sqrt{Q7}$ Gates, 2006). In particular, the assignment of individuals to the population of origin can provide more detailed inference on larval migration 47 patterns among populations (Waser & Strobeck, 1998). 48

During the last two decades, several mass mortality events coin-49 ciding with anomalous temperature increases (heatwaves) have 50 affected benthic sessile species along the rocky shores of the north-51 western Mediterranean Sea (Cupido, Cocito, Sgorbini, Bordone, & 52 Santangelo, 2008; but see Rivetti, Fraschetti, Lionello, Zambianchi, & 53 Boero, 2014). Among the species affected, gorgonians (Cnidaria, 54 Octocorallia) were the most damaged, with a local loss of more than 55 50% in density and biomass (Bramanti, Magagnini, De Maio, & 56 Santangelo, 2005; Cerrano et al., 2000; Coma et al., 2009; Cupido 57

et al., 2008; Garrabou et al., 2009; Santangelo et al., 2015). Gorgo-60 nians are characterized by long life span and slow growth, which along 61 with the low fecundity of small colonies delays their recovery after 62 disturbance (Linares, Coma, Garrabou, Díaz, & Zabala, 2008), even 63 when negative density-dependent control of recruitment is removed 64 (Santangelo et al., 2015). Similarly to other habitat-forming species, 65 gorgonians have an important role in promoting and stabilizing associ-66 ated assemblages, such as biogenic reefs. Therefore, the conservation 67 of these assemblages also depends on the capability of gorgonian spe-68 cies to respond to climate change and increasing human disturbance 69 (Ponti et al., 2014). 70

Among the gorgonian species affected by mass mortalities, the 71 red gorgonian Paramuricea clavata (Risso, 1826) suffered the most 72 drastic reductions in density and biomass over a broad spatial scale, 73 reaching up to 80% of colonies affected in the Gulf of Genoa in $\boxed{Q8}_{74}$ 1993 (Rivetti et al., 2014). The impact of reported mass mortality 75 events on the demographic structure and dynamics of several popula-76 tions has been assessed (Cupido et al., 2009; Santangelo et al., 2015; 77 Santangelo, Bramanti, & Iannelli, 2007). The recovery of these popula-78 tions has been attributed to local replenishment (Santangelo et al., 79 2015), overlooking the role of larval immigration from other source 80 populations, based on the assumption of low larval dispersal in P. 81 clavata (Mokhtar-Jamaï et al., 2011). Nevertheless, in the last couple Q9₈₂ of years genetic studies have revealed a mixed pattern of isolation 83 by distance and clustering on a regional scale, with connectivity occur-84 ring among neighbouring, fragmented populations (Arizmendi-Mejía Q10,5 et al., 2015; Pérez-Portela et al., 2016; Pilczynska, Cocito, Boavida, 86 Serrão, & Queiroga, 2016). In the Ligurian Sea, genetic analyses con-87 ducted on a local scale have suggested that, after mass mortality 88 events, the recovery of P. clavata populations dwelling below 20 m 89 in depth was the result of larval supply from deeper populations 90 (>60 m in depth), presumably not affected by mass mortality 91 (Pilczynska et al., 2016). Nonetheless, although decreases in mortality 92 rates with depth have been observed for this species (Linares et al., 93 2005), it has been shown that below 20 m of depth P. clavata popula-94 tions can be affected by mass mortalities (Huete-Stauffer et al., 2011). 95 In the present work we extended the geographical scale (from local to 96 regional) and the bathymetrical range (down to 40 m) of sampling in 97 order to test: (i) whether P. clavata populations dwelling between 25 98 and 40 m of depth in the Ligurian Sea show signatures of a genetic 99 bottleneck as a consequence of mass mortality events; and (ii) the 100 occurrence of gene flow among those populations. 101

To answer these questions, the genetic diversity, genetic structure, and regional migration patterns of the coastal *P. clavata* populations in the Ligurian Sea were analysed.

2 | MATERIAL AND METHODS

2.1 | Sampling

Colonies of the red gorgonian P. clavata were sampled by scuba diving at seven locations (from 25 to 40 m in depth) in the Ligurian Sea in May 2014 (Figure 1). All the samples were considered as coastal pop- F1 ulations, regardless of depth. Small (<3 cm) branch fragments from 26



FIGURE 1 Map of the seven sampling locations of Paramuricea clavata in the Ligurian Sea. Black lines represent the simulated surface currents averaged during summer (the spawning season for P. clavata in the Mediterranean Sea; Linares et al., 2008), according to Pinardi and Masetti (2000) and Rossi et al. (2014)

to 37 colonies were collected at two different sites per location (separated by distances of between 30 m and 1 km), except in Punta Mesco and Cerboli Island. At those two locations, colonies were sampled only at one site because of restricted sampling permission T1 by the Marine Protected Area authorities (Table 1). All samples were

preserved in 95% ethanol and stored at 4°C.

The sampling design of P. clavata populations includes sites in which populations of the species were affected by mass mortality events: Portofino, Punta Mesco, and Portovenere. In the Ligurian Sea, mass mortality events as a result of heatwaves were reported in 1999 and 2003. In Portovenere, the pre-disturbance density of 35 individuals/m² was reduced by approximately 70% (Cupido et al., 2008; Santangelo et al., 2015). A reduction of two-thirds of the population density was reported in Portofino (Cerrano et al., 2005), whereas in Punta Mesco, P. clavata was also heavily affected by mor-tality down to depths of 25 m (Peirano, Sgorbini, Cupido, Lombardi, & Cocito, 2009). Although to our knowledge no literature has been pub-lished on mass mortality events of P. clavata in other locations within

the Ligurian Sea, recreational divers reported observing dead gorgonian colonies in Calafuria in 1999 (L. De Nigris, pers. comm.). Moreover, other sessile invertebrates were affected by the increases in water temperature in the area of Bergeggi (Parravicini et al., 2010).

2.2 | Microsatellite genotyping

Total genomic DNA was extracted from 372 colonies using the DNA extraction kit NucleoSpin Tissue by Macherey-Nagel. All individuals were genotyped at six polymorphic microsatellite loci developed for P. clavata, as used in Mokhtar-Jamaï et al. (2011): Parcla09, Parcla10, Parcla12, Parcla14, Parcla17 (Molecular Ecology Resources Primer Development Consortium et al., 2010), and Par_d (Agell, Rius, & Pascual, 2009). Genotyping was carried out on an ABI 3130 Genetic Analyser using an internal size standard (GeneScan 600 LIZ; Applied Biosystems, Foster City CA, USA), and allele scoring was performed $\boxed{Q11}_{Q}$ using PEAK SCANNER SOFTWARE 1.0 (Applied Biosystems).

TABLE 1 Sampling locations of Paramuricea clavata in the Ligurian Sea

Location	Marine Protected Area	Site	Site ID	Latitude	Longitude	Depth (m)	Samples
Bergeggi	Area Marina Protetta Isola di Bergeggi	Bergeggi 1 Bergeggi 2	BER1 BER2	44.2416 44.2416	8.4461 8.4461	37 38	32 30
Portofino	Area Marina Protetta di Portofino	Punta del Faro Isuela	POF1 POF2	44.1753 44.1903	9.1308 9.1851	36 40	31 31
Sestri Levante	No	Punta Baffe 1 Punta Baffe 2	SES1 SES2	44.2482 44.2482	9.3963 9.3963	30 30	32 32
Punta Mesco	Area Marina Protetta delle Cinque Terre	Punta Mesco	PME	44.1247	9.6322	29	26
Portovenere	Parco Naturale Regionale di Porto Venere – Area di Tutela Marina	Secca Tinetto Parete Grotta	POV1 POV2	44.0122 44.0126	9.5104 9.5102	25 25	30 32
Calafuria	No	Gorgonie Rose 1 Gorgonie Rose 2	CAL1 CAL2	43.2812 43.2812	10.2002 10.2002	35 25	32 29
Cerboli Island	No	Cerboli Island	CER	42.8555	10.5469	30	37

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2.3 | Genetic diversity

4 Repeated multilocus genotypes were searched using GENODIVE 2.0b23 5 (Meirmans & van Tienderen, 2004). Observed and Nei's (1987) 6 unbiased expected heterozygosity (Ho and HE, respectively) were 7 estimated using GENETIX 4.05 (Belkhir, Borsa, Chikhi, Raufaste, & **Q**12 Bonhomme, 2004). Allelic richness (Ar) was estimated using FSTAT 2.9.3.2 (Goudet, 2001), whereas private allelic richness (A_p) was calcu-Q13 10 lated with a rarefaction procedure using HP-RARE software (Kalinowski, 11 2005). Linkage disequilibrium was tested for all loci using GENEPOP 4.2 12 (Rousset, 2008), as implemented for online use (http://genepop.curtin. **Q**14 edu.au). The test for Hardy-Weinberg equilibrium was computed for 14 each locus using the null hypothesis of no heterozygote deficiency, 15 and the level of significance was calculated with a Markov chain algo-16 rithm with default parameters (100 batches and 1000 iterations per 17 batch). Single and multilocus FIS values were estimated using Weir 18 and Cockerham's (1984) fixation index in GENEPOP. The presence of 19 null alleles was estimated using the expectation maximization (EM) 20 algorithm (Dempster, Laird, & Rubin, 1977) in FREENA (Chapuis & 21 Estoup, 2007).

22 Evidence of recent changes in effective population size as a result 23 of the mass mortality events that affected P. clavata populations in the 24 region during the past decade (Cupido et al., 2012) were tested using 25 BOTTLENECK 1.2.02, following the two-phase model (TPM; Piry, Luikart, 26 & Cornuet, 1999), with 95% single step mutation and a variance 27 among multiple steps of approximately 12. A Wilcoxon's test was 28 used, as suggested by Piry et al. (1999), for a data set of less than 29 20 loci. P values were corrected for multiple comparisons using false 30 discovery rate (FDR) corrections (Benjamini & Hochberg, 1995). 31

2.4 | Population genetic structure

Population genetic structure was estimated using both the global and the pairwise F_{ST} estimates of Weir and Cockerham (1984) with GENEPOP, and following the excluding null allele (ENA) method in FREENA. The significance of pairwise genetic differentiation among sites was computed with an exact test using the default parameters in GENEPOP (100 batches and 1000 iterations per batch).

For linkage disequilibrium and F_{ST} estimates, FDR corrections (Benjamini & Hochberg, 1995) were applied in order to adjust significance levels for multiple comparisons.

Isolation by distance (IBD) was tested at a regional scale 44 through correlation between pairwise $F_{ST}/(1 - F_{ST})$ values and the 45 logarithm of the geographical distance between sites, with a Mantel 46 test (Mantel, 1967), using the ISOLDE program implemented in 47 GENEPOP. Geographical distances were measured in GOOGLE EARTH 48 by using set points (determined as the centre of the area of distri-49 bution where the samples were collected), and measuring both 50 Euclidean and along-the-coast distances. All of the individuals sam-51 pled from a given site shared the same geographical coordinates. 52 The significance level of correlation between genetic differentiation 53 and geographical distance among samples was computed using 10 54 000 permutations. 55

To estimate the extent of gene diffusion, multilocus genotypes were autocorrelated within spatial distance classes using the kinship

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coefficient of Loiselle, as implemented in SPAGEDI 1.4. This revealed the distance at which the random effects of genetic drift, not gene 61 flow, are the primary determinants of genetic composition (Hardy & 62 Vekemans, 2002). Significant positive coefficients suggest that colo-63 nies within a specific distance class are more genetically related than 64 colonies randomly taken from any distance class. The first x-intercept 65 of the autocorrelogram, which corresponds to the average distance at 66 which the genetic similarity between any two quadrats drops below 67 0.0, is termed the 'genetic patch size' (Sokal & Wartenberg, 1983). 68 Spatial distance intervals for kinship comparisons were constructed 69 in such a way that the number of pairwise comparisons within each 70 distance interval was approximately constant (between 3000 and 71 8000 pairs among distance classes). A total of 14 distance classes 72 were considered: class 1, 0-14 km; class 2, 14-26 km; class 3, 26-73 31 km; class 4, 31-37 km; class 5, 37-56 km; class 6, 56-80 km; class 74 7, 80-94 km; class 8, 94-102 km; class 9, 102-117 km; class 10, 117-75 131 km; class 11, 131-156 km; class 12, 156-178 km; class 13, 178-76 214 km; and class 14, 214-250 km. The significance of the observed 77 versus expected coefficients was tested for each class using 10 000 78 random permutations of locations.

79 To evaluate the number of clusters (K) in the data set without 80 prior information regarding the geographic location of the colonies, 81 a Bayesian method implemented in STRUCTURE 2.3.4 was used 82 (Falush, Stephens, & Pritchard, 2003, 2007; Pritchard, Stephens, & 83 Donnelly, 2000). STRUCTURE was run under the admixture model, 84 choosing the assumption of correlated allele frequencies and the 85 option of recessive alleles to cope with null alleles, as suggested 86 by Falush et al. (2007). The mean and variance values of log likeli-87 hoods were estimated for the number of clusters, from K = 1 to 88 K = 12. Ten runs were performed for each K with 500 000 itera-89 tions and a burn-in period of 50 000. In order to identify the num-90 ber of clusters that best fit the data, the resulting output files were 91 then analysed based on the rate of change in the log probability of 92 data between successive K values, using the Evanno method in 93 STRUCTURE HARVESTER 0.6.94, as implemented online (http://tay-94 lor0.biology.ucla.edu/structureHarvester/\/) (Earl & von Holdt, $\boxed{Q16}_{G}$ 2011). For the chosen K, the results were averaged using CLUMPP $\boxed{Q17}_{6}$ 1.1.2 (Jakobsson & Rosenberg, 2007), and the graphical representa-97 tion was performed in DISTRUCT 1.1 (Rosenberg, 2004). A hierarchi-98 cal analysis of molecular variance (AMOVA; 1000 permutations) 99 was performed in ARLEQUIN 3.5 (Excoffier, 2005) using the clusters 100 (K = 6) defined by STRUCTURE. 101

2.5 | Migration patterns

First-generation migrants and long-term migration rates were esti-105 mated as proxies of genetic connectivity among the clusters identified 106 by STRUCTURE. First-generation migrants were detected using the 107 Bayesian computation of Rannala and Mountain (1997) (n = 10000; 108 α = 0.05) in GENECLASS 2.0 (Piry et al., 2004). Considering the possibil-109 ity of long dispersal via stepping stones, the level and direction of gene 110 flow over the last several generations among clusters was estimated 111 by performing a maximum-likelihood based analysis. MIGRATE-N 3.6 112 was used to estimate theta (Θ) and the mutation-scaled migration rate 113 M based on microsatellite data (Beerli & Felsenstein, 2001). Theta is 114

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 $_{3}$ Q18 defined as $4N_{e}\mu$ for a diploid system with nuclear microsatellite loci, where $N_{\rm e}$ is the effective population size and μ is the mutation rate per generation and site. M is defined as m/μ , representing the relative contribution of immigration and mutation to the variability brought into the population, whereas m is the fraction of new immigrants Q19 found in the population per generation. This calculation was run five times following the recommendations of Beerli (2009), and the results were averaged.

13 3 | RESULTS

3.1 | Genetic diversity

All the microsatellite loci were polymorphic. No repeated multilocus 17 genotypes were found. Allelic richness (Ar) and private allelic richness 18 (A_p) among sites were 5.96 ± 0.52 and 0.40 ± 0.15, respectively. Allelic 19 richness ranged from 4.96 for BER1 to 6.55 for POV2. The private 20 allelic richness varied from 0.08 for SES2 to 0.58 for POV1. Observed 21 $(H_{\rm O})$ and unbiased expected heterozygosity $(H_{\rm E})$ ranged from 0.49 for 22 CER to 0.71 for SES2, with a mean value of 0.59 ± 0.06 , and from 0.67 23 T2 for BER1 to 0.76 for POF1, with a mean value of 0.70 \pm 0.03 (Table 2). 24 No significant linkage disequilibrium was found among loci (P > 0.05 25 after FDR correction). Estimates of null allele frequencies varied between 0 for Parcla09, Parcla10, Parcla12, Parcla14, and Par_d, to 27 0.26 for Parcla12. 28 Multilocus estimates of F_{IS} ranged between 0.04 (BER1) and 0.33 29 (CAL1 and CER). All values were significantly different from zero

30 (P < 0.001, mean = 0.20 \pm 0.08), with the exception of BER1 (Table 2). 31 All populations showed significant levels of heterozygosity deficiency; 32 however, significant deviations from Hardy-Weinberg were not found 33 for all loci in all populations. The high frequency of null alleles found for 34 locus Parcla12 in some populations did not explain the levels of hetero-35 zygote deficiency, given that heterozygote deficiency was still signifi-36 cant when Parcla12 was excluded from the analysis. 37

Despite these populations being considered as unaffected by recent mass mortality events, recent changes in effective population

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0.65

0.63

0.61

0.64

0.59

0.71

0.56

0.59 ± 0.06

Site

BER1

BER2

POF1

POF2

SES1

SES2

PME

Mean value

-WILEY size resulting from an excess of the expected heterozygosity (a signal

of a population bottleneck) were revealed at almost all locations (BER2, POF2, SES1, POV1, and CER; P < 0.05), with the exception of Punta Mesco and Calafuria (Table S2).

3.2 | Population genetic structure

The global multilocus F_{ST} indicated strong genetic differentiation of P. clavata populations between sites (F_{ST} = 0.154; P = 0.0001). Pairwise F_{ST} values ranged from 0 to 0.181, and almost all were significantly different after FDR correction (Table 3). Pairwise F_{ST} values for sites T3 located less than 50 m apart (BER1 versus BER2, SES1 versus SES2, POV1 versus POV2, and CAL1 versus CAL2), and two sites separated by approximately 20 km (PME versus POV2), but all within a 10-m depth range, did not significantly differ. No significant differences were found between pairwise F_{ST} values and pairwise F_{ST} values corrected for null alleles (Student's t-test, P = 0.163), which suggests that the effect of null alleles was negligible.

A significant but weak pattern of isolation by distance (IBD) was detected for both measures of geographic distance: straight line $(R^2 = 0.23; P < 0.01)$ and along the coast $(R^2 = 0.21; P < 0.01)$ (Figure S1).

Six genetic clusters with different levels of admixture were identi-83 fied by the Bayesian clustering analysis (K = 6, $\Delta K = 12.26$; Figure 2; F2 84 for K = 2 to K = 5, see Figure S2): (i) BER1 and BER2 comprised the 85 Bergeggi cluster (BER), with a coefficient of population membership 86 of 90%; (ii) POF1 and POF2 formed the Portofino cluster (POF), with 87 a coefficient of population membership of 66%; (iii) SES1 and SES2 88 comprised the Sestri Levante cluster, with a coefficient of population 89 membership of 77% (SES); (iv) PME, POV1, and POV2 were grouped 90 in the Portovenere and Punta Mesco cluster (PMP), with a coefficient 91 of population membership of 60%; (v) CAL1 and CAL2 formed the $\boxed{\text{Q21}}_2$ Calafuria cluster (CAL), with a coefficient of population membership 93 of 70%; and (vi) CER made up the Cerboli cluster (CER), with a coeffi-94 cient of population membership of 80%. The results of the AMOVA 95 confirmed the clustering structure (P < 0.001; Table S1). 96

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TABLE 2 Parameters of genetic diversity for 12 sites of Paramuricea clavata at six microsatellite loci Ar Ap Fis 496 0.13 0.04 5.42 0.2 0.17 5.24 0.55 0.22 6.29 0.5 0.21 6.01 0.34 0.14 6.34 0.3 0.14 6.41 0.51 0.20 6.32 0.42 0.21 6.55 0.28 6.25 0.55 5.54 0.42 0.33 6.19 0.62 0.40 ± 0.15 0.20 ± 0.08 5.96 ± 0.52

Portovenere POV1 0.55 0.69 50 POV2 0.58 0.69 51

0.10 Calafuria CAL1 0.52 0.74 0.33 CAL2 0.54 0.68 0.27 CER 0.49 0.71 Cerboli

HE

0.67

0.73

0.66

0.76

0.68

0.70

0.70

56 Abbreviations: H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; A_r, allelic richness; A_p, private allelic richness; F_{IS}, Weir and Cockerham's (1984) estimate of Wright's (1951) fixation index, with significant values in bold (0.05 threshold after Bonferroni correction). 57

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Location

Bergeggi

Portofino

Sestri Levante

Punta Mesco



FIGURE 2 Population genetic structure of Paramuricea clavata in the Ligurian Sea, as revealed by STRUCTURE. Each individual is represented by a vertical line, where different colours indicate the individual proportion of membership to each cluster (K = 6). Key: Bergeggi (BER), 28 purple; Portofino (POF), green; Sestri Levante (SES), fuchsia; Punta Mesco and Portovenere (PMP), orange; Calafuria (CAL), blue; and Cerboli 29 (CER), pink 30

The kinship coefficient of Loiselle was positive and significant for the 0-14 km (P < 0.01, 95% CI = -0.0002 to 0.0002) and 14-26 km (P < 0.01, 95% CI = -0.002 to 0.008) distance classes 35 F3 (Figure 3). The genetic patch size was estimated to be 26 km.

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50 FIGURE 3 Autocorrelogram of Paramuricea clavata populations based on the kinship coefficient of Loiselle, estimated for multilocus 51 genotypes within 13 spatial distance classes. The spatial distance 52 classes were designated in such a way that the number of pairwise 53 comparisons within each distance interval is approximately constant: 54 class 1, 0-1 km; class 2, 2-23 km; class 3, 23-28 km; class 4, 28-40 km; 55 class 5, 40-75 km; class 6, 75-89 km; class 7, 89-104 km; class 8, 104-125 km; class 9, 125-153 km; class 10, 153-177 km; class 11, 177-229 km; class 12, 229-251 km; and class 13, 251-283 km 57

3.3 | Patterns of recent and long-term migration

Overall, migration was bidirectional among all locations. Recent migration revealed by the analysis of first-generation migrants showed that the clusters of PMP and POF were the major sources of emigrants (40.62 and 28.12%, respectively). On the other hand, the clusters of BER and CAL were identified as the smallest source of emigrants, with 3.12% in both cases (Figure 4). In terms of immigrants, the clusters F4 $_{\rm 96}$ CAL and PMP received the highest proportion (15.9 and 14.81%, respectively), with the latter receiving immigrants for almost all other clusters (except BER). On the contrary, the cluster BER received the lowest number of immigrants (3.33%), all of them coming from the neighbouring cluster POF.

Patterns of long-term migration resulting from the maximum like-102 lihood-based analysis using MIGRATE-N revealed that values of M 103 ($M = m/\mu$; migration/mutation) were > 1 for all locations, meaning that 104 the effect of migration (m) is larger than the effect of mutation (μ) 105 (Table S3). Migration rates among clusters identified by STRUCTURE 106 decreased with increasing distance between locations. Nonetheless, 107 evidence of migration among the most distant locations (>250 km) 108 was found (Table 4). The cluster that received most immigrants over T4 109 multiple generations was PMP (mean $M = 18.02 \pm 8.17$), whereas 110 the CAL cluster was identified as the one receiving the fewest immi-111 grants (mean $M = 7.78 \pm 4.76$). In terms of source of emigrants to all 112 other populations, the SES cluster had the highest value (mean 113 $M = 18.68 \pm 9.21$), and the CER cluster had the lowest (mean 114

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FIGURE 4 Pattern of first-generation migration among *Paramuricea clavata* populations in the Ligurian Sea. The pie charts represent recruitment in each cluster. The white portion indicates the proportion of self-recruits, whereas the colours represent immigrants from other locations. The bar plot indicates the contribution of emigrants (%) of each cluster: Bergeggi (BER), purple; Portofino (POF), green; Sestri Levante (SES), fuchsia; Punta Mesco and Portovenere (PMP), orange; Calafuria (CAL), blue; and Cerboli (CER), pink

TABLE 4 Long-term migration rates $M (= m/\mu; migration/mutation)$ among genetic clusters of *Paramuricea clavata* in the Ligurian Sea, as revealed by the maximum likelihood-based analysis using MIGRATE-N (M)

		Destination	Destination							
		BER	POF	SES	PMP	CAL	CER	Mean		
	BER	0	1.60	16.00	13.18	6.81	7.56	9.03 ± 5.65		
Source	POF SES PMP CAL	1.81 20.40 16.62 5.17	0 26.69 13.30 5.47	14.15 0 17.50 13.79	21.48 26.57 0 22.59	4.04 4.63 15.89 0	10.99 15.13 27.70 3.14	$\begin{array}{c} 10.49 \pm 7.92 \\ 18.68 \pm 9.21 \\ 18.20 \pm 5.53 \\ 10.03 \pm 8.12 \end{array}$		
	CER	5.49	1.62	9.84	6.29	7.50	0	6.15 ± 3.02		
	Mean	9.89 ± 8.10	9.73 ± 10.61	14.25 ± 2.88	18.02 ± 8.17	7.78 ± 4.76	12.90 ± 9.37			

 $M = 6.15 \pm 3.02$). The effective number of migrants per generation $N_{\rm em}$ (= $\Theta M/4$) was >0.22 at all sites.

Even though migration over such a large distance (>250 km) may be a rare occurrence, the level of migration (>10%) between clusters more than 60 km apart (PMP towards CAL and CER clusters), compared with the relative exchange of migrants among neighbouring clusters (<8%; e.g. POF and SES, 20 km apart), suggests that dispersal over 60 km may be more common than previously thought; however, the lowest level of migration (3.33%) was also found in one cluster (BER) located less than 60 km away from its closest neighbour (POF).

4 | DISCUSSION

Genetic evidence of population bottlenecks was found in most of the populations of *P. clavata* studied in the Ligurian Sea. Contemporary and long-term patterns of migration showed that all of the studied *P. clavata* populations are connected by larval dispersal.

4.1 | Genetic diversity and structure

The levels of genetic diversity among different *P. clavata* populations dwelling between 25 and 40 m of depth in the Ligurian Sea were similar to those found among shallow and deep populations in the same region, and in the north-west Mediterranean (Arizmendi-Mejía et al., 2015; Mokhtar-Jamaï et al., 2011; Pilczynska et al., 2016). Departures from Hardy–Weinberg equilibrium, as well as high F_{IS} values and evidence of heterozygote deficit have been previously reported in *P. clavata* populations (Mokhtar-Jamaï et al., 2011; Pérez-Portela et al., 2016; Pilczynska et al., 2016). This is a common occurrence in gorgonian species, mainly attributed to the low dispersal capabilities of the larvae (Gutiérrez-Rodríguez & Lasker, 2004; Ledoux et al., 2010).

The global F_{ST} value (F_{ST} = 0.154) was similar to what was previously reported for the species in the Ligurian Sea (Pilczynska et al.,
2016), also indicating a high level of genetic differentiation among
populations between 25 and 40 m of depth. In contrast, reported
levels of genetic differentiation (F_{ST}) among *P. clavata* populations
ranging from 30 to 55 m of depth in the Balearic Islands (global
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 F_{ST} = 0.035; Arizmendi-Mejía et al., 2015) were much lower than those found in this study. Nonetheless, non-significant F_{ST} values were found among most pairs of sites separated by 50–100 m, and generally at similar depths.

4.2 | Evidence of population bottleneck

In contrast with with Pilczynska et al. (2016), who found no evidence of a genetic bottleneck in two populations at 20–31 m of depth, a signal of a recent and drastic reduction of *P. clavata* effective population size was found in most of the populations studied (at depths of 25–40 m), with the exception of Punta Mesco and Calafuria. The findings corroborate previous studies reporting mass mortality events in populations dwelling at depths deeper than 20 m (in Sardinia, at depths of 20–40 m, Huete-Stauffer et al., 2011; in the north-western Mediterranean, at depths of 50–70 m, Pérez-Portela et al., 2016). Hence, the absence of a genetic bottleneck in Calafuria and Punta Mesco is striking.

Although very unlikely, it is possible that those populations were 21 indeed not affected by mass mortality events: in Punta Mesco, the 22 affected colonies were only reported down to 25 m of depth (Peirano 23 et al., 2009), whereas mass mortalities have not been reported in 24 Calafuria. Nonetheless, it is worth considering that high recruitment, 25 either from reproduction of local survivors or through migration, could 26 have cancelled the signature of a genetic bottleneck. Indeed, immigration rates in Calafuria and Punta Mesco were the highest in the region. Nonetheless, it is also worth mentioning the potential bias resulting 29 from the methodological limitations of reconstructing the demo-30 graphic history of populations from genetic data, which could have 31 led to underestimating the number of populations that underwent a 32 drastic decline in population size (Girod, Vitalis, Leblois, & Fréville, 33 2011; Peery et al., 2012).

A closer look at the Punta Mesco and Portovenere cluster (PMP) 35 showed that mass mortality events of similar intensity were recorded 36 in the two sites of Portovenere over 10 years ago. Also, both popula-37 tions had started recovering a few years after that, but at different 38 rates (Cupido et al., 2012). Results of the present study showed higher 39 immigration in one of the two sites within Portovenere, with the site 40 with lower immigration showing a signature of genetic bottleneck. 41 Although demographic bottlenecks may be recurrent in Portovenere, 42 migration among coastal populations may help maintain local genetic 43 diversity and offset population collapse, making the signature of a bot-44 tleneck undetectable (Aguilar, Jessup, Estes, & Garza, 2008; Spong & 45 Hellborg, 2002). Similarly, in Portofino, a mass mortality event was 46 documented in 1999, and even though the mass mortality event dra-47 matically reduced the reproductive potential of the native population, 48 after 3 years gorgonian colony densities were greater than before the 49 mortality event (Cerrano et al., 2005). The estimated age of first 50 reproduction of P. clavata is 3 years. Therefore, enhanced recruitment 51 following mortality should be attributed to immigration. The large 52 number of immigrants in Portofino supports this hypothesis. 53

Despite no reports of *P. clavata* mass mortality events in Bergeggi, Parravicini et al. (2010) reported heatwaves affecting other sessile invertebrates. The genetic bottleneck signature observed in one of the Bergeggi sites suggests that mass mortality events could have affected *P. clavata* populations in this area before 1999, and its permanence highlights the vulnerability of populations with low immigration rates.

Another potential explanation for the observed deficiency of heterozygotes among the studied populations could be the mixing of different gene pools, which would cause a Wahlund effect. This hypothesis has been proposed already for *P. clavata* populations in the Mediterranean Sea (Mokhtar-Jamaï et al., 2013), and for the Mediterranean red coral, *Corallium rubrum* (Costantini, Fauvelot, & Abbiati, 2007).

4.3 | Patterns of migration

72 The analyses of first-generation migrants and long-term migration 73 revealed a pattern of bidirectional connectivity among all P. clavata 74 populations examined. The percentage of individuals identified as 75 first-generation migrants in a population (up to 15.9%), as well as the 76 maximum dispersal distance of P. clavata found in this study (which 77 ranged from 140 km to over 200 km, based on short-term and long-78 term migration, respectively), were larger than previously reported 79 for shallower populations in the Ligurian Sea (20 km; Pilczynska et al., 80 2016), but were similar to values reported by Mokhtar-Jamaï et al. $\boxed{\text{Q22}_1}$ (2011), who found that despite strong genetic structure, some gene 82 flow could still occur over short and longer distances (up to 160 km) 83 across the Mediterranean Sea. Unlike the study of Arizmendi-Mejía 84 et al. (2015), connectivity among populations of P. clavata in the 85 Ligurian Sea was found between non-neighbouring clusters, but was 86 not homogeneus throughout the region. 87

These findings, combined with the weak correlation found between genetic and geographic distance, demonstrate, as previously proposed by Pérez-Portela et al. (2016), that other factors rather than geographical distance alone may influence the pattern of genetic structure among *P. clavata* populations. Effective migration, in fact, is the result of the filtering of dispersal potential related to larval traits (e.g. pelagic larval duration, larval buoyancy, and behaviour) by environmental pre- and post-settlement drivers, such as hydrodynamics, predation pressure, and local geomorphological features (Lortie et al., 2004).

According to these results, the most important sources of 97 migrants were the sites located in the central part of the Ligurian 98 Sea, which is consistent with the large-scale separation of currents 99 that occurs in front of Portovenere (Pinardi & Masetti, 2000). This $\fbox{\sc Q23}_{00}$ flow separation results in two hydrodynamic provinces (Rossi, Ser-101 Giacomi, López, & Hernandez-García, 2014). Although the Ligurian 102 current bordering the narrow continental shelf induces a rapid direc-103 tional drift along the coast north-west of Portovenere, the 104 detachment of meso-scale gyres south-east of Portovenere promotes $\boxed{\textbf{Q24}}_{05}$ higher regional retention with diffuse dispersal in the Gulf of La Spezia 106 and north Tyrrhenian Sea. The hydrodynamic province identified 107 north-west of Portovenere extends along the French coast to 108 Marseille, suggesting that migrants proceeding from the north-109 western Ligurian Sea (e.g. Bergeggi) should be found in that location. 110 This hydrodynamic pattern also supports the observation that the 111 population of Bergeggi receives five times less immigrants than the 112 population of Calafuria, and that a higher level of gene flow occurred 113 towards the eastern part of the Ligurian Sea. 114

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It is also worth noting that although connectivity among coastal *P. clavata* populations provided an important contribution to the recovery of populations affected by mass mortality events, it may not be the only explanation for their upturn, and migration from deeper populations should not be excluded (Pilczynska et al., 2016). In any case, the present study shows that an effective number of migrants per generation of less than 5% was not sufficient for some populations (Bergeggi and Sestri Levante) to compensate their genetic divergence resulting from the combined effects of mutation and genetic drift.

4.4 | Implications for conservation in the context of global change

According to climate change projections, the rising of ocean temperatures and increasing frequency of extreme heatwave events (de Madron et al., 2011; Meehl et al., 2000), could lead to more frequent and more severe demographic breakdowns, especially in small and isolated populations. Paramuricea clavata is an extremely vulnerable species to extreme climatic conditions, which may cause severe depletions. It is generally assumed that heatwaves affect mainly shallow populations; however, during the heatwave of September 1999, unusual temperatures were recorded down to at least 32 m in depth (Bramanti et al., 2005), and genetic data demonstrate that populations of P. clavata between 25 and 40 m in depth have also been affected by 26 drastic reductions in population size, highlighting that extreme climatic 27 events impact coastal populations down to at least 40 m in depth. 28 Nonetheless, this study provides evidence that P. clavata larvae are 29 able to move among populations up to a distance of tens of 30 kilometres, promoting population resilience and recovery from distur-31 bance through the arrival of propagules from other locations. 32

Connectivity has become an increasingly important element in 33 spatial conservation planning (Olds et al., 2016); however, only few 34 studies have actually provided empirical data on the role of connectiv-35 ity in population recovery following disturbance. Larval connectivity is 36 remarkably relevant in sessile marine invertebrates, which rely on pre-Q26 settlement processes for dispersal. The protection of populations 38 identified as sources of migrants would help the regional persistence 39 of the species. The identification of key source populations may be 40 of great value for the implementation of conservation measures and 41 regional spatial planning in the context of increasing global climate-42 related disturbance. Nonetheless, seascape genetic inference can be 43 strongly influenced by the recent demographic history of populations. 44 Demographic stability can regulate gene flow, when recruitment in 45 benthic populations is limited by a lack of space (Padrón & Guizien, 46 2016). Therefore, this study highlights the importance of integrating 17 Q27 demographic and genetic data (see Arizmendi-Mejía et al., 2015).

In the Ligurian Sea, several Marine Protected Areas have been 49 established based on the natural value of the habitats and/or adminis-50 trative requirements. This study suggests that the spatial arrangement 51 of the Ligurian Sea network of MPAs is effective in maintaining con-52 nectivity among P. clavata populations in the study area. Moreover, 53 the distance between MPAs is in the range of the dispersal distance 54 of the species. Therefore, in case of future disturbances, the network 55 of MPAs in the Ligurian Sea would support the recovery of the popu-56 lations. Nonetheless, it remains necessary to test the deep refugia 57

hypothesis, given that in the event of a disturbance affecting all shal-60 low populations, deep populations would become of utmost impor-61 tance. Taking this into consideration, it would be thus fundamental 62 to promote the creation of offshore MPAs that could be incorporated 63 into the existing MPA network, and ensure connectivity among shal-64 low and deep populations. Furthermore, the eastward direction of 65 gene flow in the study area suggests that connectivity should be fur-66 ther investigated in populations located west of Bergeggi, to provide 67 a pattern of the connectivity across national borders and foster the 68 creation of transnational MPA networks. 69

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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