

Ecological and genomic characterization of a remarkable natural heritage: a mesophotic 'giant' Paramuricea clavata forest

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1 1 Running page head: Characterisation of Mesophotic gorgonian populations 2 Ecological and genomic characterization of a remarkable natural heritage: a mesophotic 3 4 'giant' Paramuricea clavata forest 5 Sartoretto Stéphane^{1*}, Ledoux Jean-Baptiste², Gueret Elise³, Guillemain Dorian⁴, Ravel 6 Christophe¹, Moirand Laurine⁵, Aurelle Didier^{5,6} 7 8 ¹ Ifremer, LITTORAL, F-83500 La Seyne-sur-Mer, France 9 10 ² CIIMAR/CIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal. 11 ³ MGX-Montpellier GenomiX, Univ. Montpellier, CNRS, INSERM, Montpellier France 12 ⁴ OSU Institut Pythéas, CNRS, IRD, Aix Marseille University, Université de Toulon, Mar-13 seille, France 14 ⁵ Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO, Marseille, France 15 ⁶ Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire natu-16 relle, CNRS, Sorbonne Université, EPHE, Université des Antilles, CP 26, 75005 Paris, 17 18 France. 19 20 *Corresponding author. stephane.sartoretto@ifremer.fr 21 22 ABSTRACT: 23 Paramuricea clavata is an ecosystem engineer, structuring hard bottom communities in

the Mediterranean Sea, from 10 to 200m depth. For more than two decades, shallow populations of *P. clavata* have been impacted by marine heatwaves, resulting in massive mortality events. Recently, a new dense population characterised by the tallest colonies ever

27 recorded (up to 1.80m) in the Mediterranean Sea was discovered between 50 and 60m depth. Here, we analyzed the size and genetic structure of this "deep giant population" and 28 29 conducted a reciprocal transplant experiment with a shallow population inhabiting the 30 same area to test for local adaptation. The experiment showed no significant difference in 31 the survival rate despite the high temperatures registered in the shallow area. Neverthe-32 less, we observed a significant differentiation by depth in this area, with low estimates of 33 gene flow. The particular local oceanographic conditions could lead to a relative isolation of the "deep giant colony" population. In conclusion, the reduced connectivity of this newly 34 35 discovered giant populations with shallower ones, question its potential role as a climate 36 refuge. Additionally, this population constitutes a unique natural heritage site that should 37 be effectively protected from physical impacts and other direct consequences of anthro-38 pogenic activities.

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40 Key Words: *Paramuricea clavata*, Mediterranean Sea, Climate refugee, Environmental
41 adaptation; transplant experiment; RAD-Seq.

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43 1. INTRODUCTION

Gorgonians are present in the world's ocean, from the surface to the bathyal zones. These 44 anthozoans are ecosystem engineers, structuring their habitats (Boudouresque et al. 45 46 2016). Among the gorgonian species present in the Mediterranean Sea, the populations of 47 red gorgonian Paramuricea clavata (Risso, 1826) constitute noteworthy submarine landscapes (Carpine & Grasshoff 1975, Grasshoff 1992, Linares 2006). This Mediterranean 48 49 endemic species, living from 10m to 200m depth (Boavida et al. 2016; Coelho et al. 2023; 50 Fourt et al. 2017), has a long life span (decades) and a slow growth rate (mean values: 51 from 1.8 to 3 cm/yr in colony height) (Mistri & Cecherelli, 1994; Coma et al. 1998; Coma et 52 al., 2001; Cupido et al., 2012). Paramuricea clavata is a gonochoric species with low re-

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53 cruitment rates (Coma et al. 1995, 2001). Asexual reproduction is negligible and the maintenance of the population is based on the recruitment of sexually produced offspring 54 (Coma et al. 1995, Pilczinska et al. 2017). A late first reproductive age depending to 55 56 Mediterranean region (3 to 13 years old) characterizes this species (Coma et al. 1995; Cupido et al., 2012). Paramuricea clavata is a suspension feeder, feeding on a large range of 57 58 particles and this species constitutes a major interface for the transfer of carbon between 59 pelagic and benthic components (Coma et al. 1994, Gili & Coma 1998, Linares et al. 2007). The presence of *P. clavata* enhances the hard bottom biodiversity and reduces the 60 61 sedimentation impacting benthic habitats (Ponti et al. 2016, Valisano et al. 2015).

62 Paramuricea clavata is an emblematic species of the coralligenous habitat and its populations are of major interest for recreational scuba divers (Coma et al. 2004, Linarès 2006). 63 64 They can be impacted by different physical pressures (anchoring, SCUBA diving, fishing) 65 nets) (Ballesteros 2006, Betti et al. 2020). In addition, shallow populations until 40 to 50 meters depth have been affected by several mass mortality events linked to marine heat-66 waves in recent decades (Perez et al. 2000, Garrabou et al. 2022). These thermal anom-67 alies, which occur in late summer and autumn periods, are characterized by long periods 68 of high seawater temperatures (Romano et al. 2000, Crisci et al. 2011, Pairaud et al. 69 70 2014). The consequences are a partial or complete necrosis of living tissues (coenenchyme and polyps) surrounding the skeleton, which can result in the death of gorgoni-71 72 ans (Garrabou et al. 2009, 2021). Unfortunately, P. clavata has overall low resilience capacity (Garrabou et al. 2001, Cerrano et al. 2005, Linarès et al. 2005; Gomez-Gras et al., 73 74 2022) even if locally this species seems to recover faster (Cupido et al., 2009; Ruffaldi 75 Santori et al., 2021). Therefore, recurrent marine heat waves are a major threat for the 76 sustainability of shallow gorgonian populations in the Mediterranean Sea (Pairaud et al. 77 2014).

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78 Therefore, the study of the genetic diversity of P. clavata populations and of their thermotolerance is crucial in order to assess: (i) how unimpacted populations can contribute to 79 the resilience of populations affected by mortality events through genetic connectivity and 80 81 (ii) whether or not the newly established populations are adapted to the local thermal 82 regime, and could cope with warming. These guestions started to be addressed focusing 83 on three Mediterranean gorgonians affected by thermal anomalies: the red coral (Corallium rubrum) (Ledoux et al. 2015), the white gorgonian (Eunicella singularis) (Pey et al. 84 2013) and the red gorgonian (Paramuricea clavata) (Arizmendi-Meija et al. 2015a). Re-85 garding P. clavata, the genetic structure was studied in different regions and depths 86 87 (Mokhtar-Jamaï et al. 2011, Padrón 2015, Padrón & Guizen 2016, Arizmendi-Meija et al. 88 2015b, Pérez-Portela et al. 2016, Ledoux et al. 2018). It has been shown that significant 89 genetic structure exists at short distances (a few hundred meters) and, occasionally, differ-90 ences between depths can be observed within the same site (Mokhtar-Jamaï et al. 2011, Pilczynska et al. 2019). However, the limited number of genetic markers used in these 91 92 studies did not provide a clear understanding of the adaptation of this species to local envi-93 ronmental conditions (but see Crisci et al. 2017). On the other hand, shallow populations 94 of P. clavata (between 20 and 40m depth) have been more extensively studied than 95 deeper ones, which remain poorly understood, apart from the study of Pérez-Portela et al. 96 (2016).

In the last decade, new populations of *P. clavata* have been discovered between 50 and 60 m depth in the Natura 2000 site "Côte Bleue marine" (north of Marseille) (Astruch et al. 2011). These populations settle on rocky outcrops sparsely covering 2300 hectares ("Catchoffs" site). This site is influenced by the flow of the Rhône River and remarkable seasonal upwelling (Fraysse 2014). By their exceptional size (up to 1.80m high) and density, the gorgonians that form the Catchoffs site stand out from the colonies usually observed (see Results). Such gigantic morphologies could correspond to a phenotypic plas-

104 ticity linked to environmental conditions and/or particular genetic characteristics. Finally, unlike the shallow populations, these deep populations do not appear to be affected by 105 marine heat waves. In this context, the main objective here is to test whether these deep 106 107 populations of gigantic P. clavata constitute potential climatic refuge populations in the face of current global warming. Could they contribute to the resilience of neighboring and 108 109 shallower populations impacted by mass mortalities? To answer this guestion, the environmental conditions and size structure of P. clavata populations in the Catchoffs site were 110 characterized and compared to a shallower population from the same geographical area. 111 Next, we analyzed the differentiation between this deep population and neighboring shal-112 113 lower populations at the genomic level using restriction site associated DNA sequencing (RAD-Seg). Finally, the local adaptation signals of the populations to their local environ-114 115 ment (especially to the thermal regime) were tested using experimental (reciprocal in situ transplantation) and genomic (RAD-Seq) approaches. 116

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118 2. MATERIAL AND METHODS

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120 2.1. Studied area

Two populations of *P. clavata* were considered along the "Côte Bleue", north of Marseille 121 (France), near the Rhône Delta. The first one (Catchoffs - COF) is located two nautical 122 miles south of Carry-le-Rouet (Fig. 1). This population is included in a large Natura 2000 123 site (Leleu et al. 2014). This site is characterized by rocky outcrops emerging from the 124 125 sediment on a surface covering 2300 ha. Giant colonies of P. clavata (between 1.5 and 126 1.8m high) can be observed on these outcrops (Astruch et al. 2011). Other gorgonian species also occur at this site: Eunicella cavolini (Koch, 1887), Eunicella singularis (Esper, 127 128 1791), Eunicella verrucosa (Pallas, 1766), Leptogorgia sarmentosa (Esper, 1791), and 129 *Corallium rubrum* (Linnaeus, 1758). The marine habitat is characterized by a high turbidity

130 of water and by sandy-muddy sediments around rocky outcrops due to the proximity of the Rhône Delta and the impact of associated river plume (Fraysse 2014). In this Natura 2000 131 site, two stations were selected at: (i) 60 m (COF-station A) and (ii) 58 m depth (COF-sta-132 133 tion B) (Fig. 1a; Table 1). A third station was studied at 30 m depth (Yeux de chat - ODC), 4,500 m away from COF, it is an inshore site characterized by a rocky peak colonized by a 134 dense population of P. clavata with some large isolated colonies of Eunicella cavolini. As 135 for the previous COF sites, the ODC station is strongly impacted by the Rhône River (Fig. 136 1a; Table 1). 137

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139 2.2. Characterization of marine environment and gorgonian populations

We monitored the temperature and current regime of both habitats (ODC station and COF-140 station A) continuously for 13 months between the 1st of July 2019 and the 31st of July 141 2020. Temperature was recorded every 30 minutes with a HOBO water temp pro V2 sen-142 sor (@Prosensor). A Tilt Current Meter Model 1 (TCM-1) (@Lowell Instruments) was used 143 144 to characterize the current regime (speed and orientation of current) among the giant 145 colony populations (60 m depth) (Fig. S1). Measurements were also made every 30 minutes. We also characterized the density and size structure of the *P. clavata* populations 146 147 studied (COF-site A, B and ODC station) (Table 2). According to Linares et al. (2008), we used 20 open quadrats (50x50 cm) per station (total surface: 5m²) (Fig. S2). In each 148 guadrat, the number of colonies was counted and their height was measured with centime-149 150 ter precision. In gorgonians, the characterization of the demographic structure of a population is challenging. Indeed, the annual growth rate decreases with the age of the consid-151 152 ered colonies (Coma et al., 1998; Sartoretto & Francour, 2012; Viladrich et al., 2016). In P. clavata, annual growth rates values were almost exclusively estimated on small and young 153 154 colonies (e.g. Mistri & Cecherelli, 1994; Coma et al., 1998; Cupido et al., 2012). In this 155 context and considering the maximal size of P. clavata observed in Catchoff site (180cm high), we only focused on the characterization of the size structure of the populations. Following Linares et al. (2008), we defined 16 size classes, each corresponding to a 10 cm
range.

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160 2.4. Experimental design

161 To test the potential for local adaptation of *P. clavata* populations according to depth, we performed a reciprocal transplant experiment between the two localities (COF-station A 162 and ODC station) from 1st July 2019 to 3rd June 2020. The transplant experiment included 163 one control (colonies transplanted at their native depth) and one transplant (colonies trans-164 165 planted at the foreign depth) in both locations. Sixty 10 cm long apical tips (two per colony) were randomly sampled with scuba diving from each population. For each population, 30 166 167 fragments were attached to experimental plates (Fig. S2) and installed at their original depth (control transplants). Plates with the remaining 30 fragments were installed at a for-168 eign depth (reciprocal transplants; i.e. 30 m to 60 m and 60 m to 30 m). Each sampled 169 170 colony was genotyped as explained below. Photographic monitoring of each transplant 171 was carried out every two months depending on meteorological conditions.

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173 2.3. Genomic analysis

We aimed to characterize the genetic make-up of the COF deep site and to involve these results in a wider context. Therefore, we sampled *P. clavata* colonies in different areas: Northern Marseille Bay (COF station A and ODC), Calanques (Grand Congloue (GOC) and Impérial (IMP) stations) and Porquerolles (Sec du Petit Langoustier (SPL) and Cap d'Armes (CAA) stations), at increasing distance from COF (from 5 km up to 94 km) (Fig. 1b). In each area we sampled sites corresponding to different depths: around 30 m deep (hereafter "shallow"), and below 50-55 m ("deep"). Details of the sampling sites are pre-

181 sented in Table 2. It should be noted that we analyzed more individuals from the Northern
182 Bay of Marseille (including the deep COF site) which was our main study area.

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186 2.3.1. Library preparation and sequencing

DNA from the samples was extracted using the Macherey-Nagel NucleoSpin DNA RapidL-187 yse extraction kit. After DNA quantification, the extracted DNA samples were sent to the 188 MGX platform (https://www.mgx.cnrs.fr/) for RAD library preparation and sequencing. We 189 190 included in the batch of individuals four replicates with two DNA extractions from the same colony. Library preparation followed the protocol of Baird et al. (2008) with the Pstl restric-191 192 tion enzyme. The library was sequenced on an Illumina NovaSeg 6000. We obtained a mean of 6,426,012 raw sequences per sample (minimum: 618,725; maximum: 9,181,838; 193 194 Fig. S3).

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196 2.3.2. Analysis of RAD sequencing data

197 We checked the quality of sequences with FastQC (Andrews 2019). In a preliminary analy-198 sis we included an individual of the congeneric species *Paramuricea placomus* (Linnaeus, 1758) from Tjärnö (Sweden). A first Principal Component Analysis on this dataset led to 199 the main separation of *P. placomus* and *P. clavata* (Fig. S4). As this analysis did not lead 200 201 us to suspect the presence of different cryptic lineages among the P. clavata samples, we 202 removed the *P. placomus* sample for further analyses. This allowed us to increase the 203 number of SNP loci (from 45,251 to 65,726 SNPs before further filtering), and to improve the characterization of the genetic structure within P. clavata. At that stage we also re-204 205 moved a P. clavata individual with a very low number of reads (618,725). We then re-206 moved potential contaminant sequences with Kraken 2 (Wood et al. 2019) with the stan207 dard database including references from archae, bacteria, human, plasmid, and viral sequences. The sequences identified by Kraken 2 were filtered with KrakenTools (Lu 2020). 208 The assembly of RAD loci was done with ipyrad 0.9.79 on these filtered sequences (Eaton 209 210 & Overcast, 2020). We used the genome of *P. clavata* as a reference (Ledoux et al. 2020). We then performed a first PCA which showed the main separation of two samples of COF 211 212 with high rates of heterozygous loci. The inbreeding coefficient F computed with VCFTOOLS 0.1.15 (Danecek et al. 2011) for these two samples was -0.24 and -0.08, 213 compared to a mean value of 0.13 and a minimum of -0.03 for other samples. We re-214 moved these two samples for further analyses. We used the output of ipyrad to build a ma-215 216 trix of pairwise p-distances among samples with VCF2Dis 1.45 (https://github.com/BGIshenzhen/VCF2Dis.git). We then compared the pairwise distance observed among differ-217 218 ent samples with those observed among sequencing replicates. This last analysis led us to identify four sample pairs which showed the same distance than the distance observed be-219 tween replicates (Table S1). For each pair, we removed the sample with the lowest num-220 221 ber of reads, resulting in a total of 82 individuals. We then filtered SNPs with the following 222 filters: two alleles, a maximum of 25% of missing data, in Hardy-Weinberg equilibrium, not in linkage disequilibrium, and one SNP per locus. Filtering on the basis of Hardy-Weinberg 223 224 equilibrium is useful to remove problematic markers, such as paralogous loci for example. and previous studies based on microsatellites did not suspect any asexual reproduction in 225 this species (Mokhtar-Jamai et al., 2011; 2013). The filter based on linkage disequilibrium 226 aims at retaining only independent loci. The filtering was performed with vcftools 0.1.16 227 and the GBS SNP filter 1.17 pipeline (Alexander 2020, Danecek et al. 2011). 228

We used the GENEPOP R package (Rousset 2008) to compute gene diversity within individuals (1-Qintra; corresponding to observed heterozygosity) and among individuals within sampling sites (1-Qinter; corresponding to expected heterozygosity), and *F* statistics (Weir & Cockerham 1984). We tested the pairwise differentiation among sampling sites with

1,000 permutations with Arlequin 3.5 (Excoffier & Lischer 2010). We analysed the genetic
structure among samples with a Principal Component Analysis (Jombart et al. 2010), and
a clustering analysis followed by a Discriminant Analysis of Principal Components (DAPC;
Jombart et al. 2010), with the adegenet 2.1.5 R package (Jombart & Ahmed 2011). We
tested for the presence of loci potentially involved in local adaptation with three approaches: Arlequin (Excoffier et al. 2009, Excoffier & Lischer 2010), OUTFLANK (Whitlock

239 & Lotterhos, 2015), and PCADAPT (Luu et al. 2017).

We performed demographic inferences to estimate effective size and gene flow among 240 populations in COF and ODC populations for which we obtained the highest sample sizes. 241 242 We used the dataset obtained from the assembly of these two populations only. We used fastsimcoal2 for demographic inferences (Excoffier et al. 2013, 2021), under a model of di-243 244 vergence with gene flow (Fig. S5). The site frequency spectrum (SFS) was computed with easySFS (https://github.com/isaacovercast/easySFS). The inference was first performed 245 on the basis of 100 replicated analyses of this initial dataset, and the run with the highest 246 247 estimated likelihood was retained to estimate demographic parameters. We performed 50 non-parametric bootstraps of the original dataset: each bootstrapped dataset was ana-248 249 lysed with 10 replicates and we retained the best run to get a confidence interval of the es-250 timated parameters.

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252 **3. RESULTS**

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254 3.1. Characterization of the marine environment

255 **3.1.1. Temperature**

At the ODC station (-30 m), the range of values of seawater temperature values extended from 23.2°C during autumn 2019 to 13.1°C during winter 2019-2020. In summer and autumn, temperature trend showed successions of warm water peaks (21-23°C at 30 m

259 depth). These peaks were observed during short time periods (less than a week) in July and August (Fig. 2). Between September and November 2019, two longer periods (two 260 and three weeks) were observed with temperatures reaching 20°C and 23°C. In the COF 261 262 station (-60 m), temperature values ranged from 22.54°C in autumn 2019 to 13.40°C in winter 2019-2020. During the summer, the same sequence of temperature increases as at 263 the ODC station was observed with lower maximum values (16-17°C). During the autumn, 264 the warm water periods were also the same as at the ODC station with the same maxi-265 mum values (20°C to 23°C) but of a shorter duration (two weeks) (Fig. 2). 266

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268 3.1.2. Current regime

The current regime in the COF station is characterized by low speed values (< 0.09 m.s⁻¹). During spring and summer, we observed a maximal period of time with a current speed of less than 0.03 to 0.04 m.s⁻¹. (Fig. 3A). During autumn and winter, the current speed values are more unstable, ranging from 0.01 to 0.09 m.s⁻¹. The current orientation shows a unimodal pattern in spring and summer with a predominance of the southeast direction (bearing: 220°). In autumn and winter, this pattern is bimodal with: (i) southeast predominance (bearing: 230°) and (ii) to a lesser extent northeast predominance (bearing: 50°) (Fig. 3B).

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3.2. Size structure

The shallow population studied (ODC station, -30m) is characterized by a density of 26 ± 7.3 colonies.m⁻². The size structure of this population shows a unimodal distribution of size classes. The small (10 to 20 cm) and average sized gorgonians (20 to 50 cm) represent 67 % of the total (Fig. 4). The tallest colonies (50-110 cm) represent 23 % with only 1 % of the colonies belonging to the class size 110-120 cm.

283 Compared to the ODC station, the COF-station A (-60m) shows a lower value for density 284 with 18.05 \pm 7.29 colonies.m⁻². The gorgonian population is characterized by a bimodal

285 size structure with a predominance of average sized colonies (20 to 50 cm) representing 50 % of total and high colonies (100-130 cm), representing 23 % of the colonies. Tall 286 colonies (130 to 150 cm) and giant (>150 cm with a maximum of 180 cm) represent re-287 288 spectively 9 % and 6 % of the total (Fig. 4). In the COF-station B (-58m), the density value is 23.6 \pm 7.4 colonies.m⁻², with small (10-20 cm) and average sized colonies (20 to 50 cm) 289 representing 17% and 39% of the total number of colonies. Tall colonies (130-150 cm) and 290 giant (>150 cm with a maximum of 160 cm) represent 13 % and 2 % of the observed 291 292 colonies.

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294 3.3. Transplantation experiments and genomic analysis

295 3.3.1. Monitoring of transplants

296 At 60 m depth (COF-Station A), 24 fragments from the control transplant (60 to 60 m) died 297 one month after their installation. Ten months later, all fragments were dead. Regarding the reciprocal transplants from ODC (30 to 60 m), the survival rate was higher than for the 298 299 control transplant with 11 dead fragments 5 months after the beginning of the experiment. 300 One year later, 14 fragments were dead (47 %) and the remaining ones showed between 25 % and 75 % of tissue necrosis. At 30 m depth (ODC station), after four months the sur-301 302 vival rate reached 96.67 % for the control transplants (30 to 30 m), and 83.33 % for the reciprocal transplants from COF (60 to 30 m). After 12 months, these values respectively 303 reached 60 % and 40 % (Fig. 5). At the end of the experiment, no significant differences 304 305 (Wilconson Rank Sum Test, p =1) in final necrosis levels was observed between control transplants and reciprocal transplants coming from COF. The majority of colonies pre-306 307 sented a mean necrosis level lower or equal to 50 %. In June 2020 (end of experiment), 90 % of COF transplants and 92 % ODC transplants presented fertile polyps. Finally, the kep-308 309 toparasitic annelid Haplosyllis chamaeleon (Pola et al. 2020) was observed on 28.6 % of 310 the reciprocal transplants from COF and 22 % of the control transplants (ODC).

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312 3.3.1. Genetic diversity and genetic structure

After the different filtering steps, statistical analyses were performed with 82 individuals 313 314 and 49,215 SNPs. The number of loci retained at each stage of the analysis is shown in Tables S2 and S3. The levels of diversity were roughly similar between samples, with 1-315 316 Qintra around 0.06 and 1- Qinter around 0.07 (see Table 2). We did not observe any regular increase or decrease of genetic diversity along depth. The F_{IS} estimates indicated a sig-317 nificant heterozygous deficit for all populations, varying from 0.08 for SPL to 0.13 for CAA. 318 When computed over all sites and loci, the observed F statistics were 0.094 for F_{IS} , 0.044 319 320 for F_{ST} , and 0.135 for F_{IT} . The pairwise F_{ST} averaged over all loci, are presented in Table 3. The highest F_{ST} value (0.053) was observed for the comparison between the two sites in 321 322 the northern bay of Marseille, Catchoffs (COF) and Yeux de Chat (ODC), and between COF and GCO (different depths, Marseille North and Calangues). The mean F_{ST} value for 323 the comparisons between depths within a given area was 0.032. Between areas, the mean 324 325 F_{ST} value was 0.038, 0.044 and 0.030 considering all sites, shallow sites and deep sites, 326 respectively. All pairwise differentiation tests were significant according to the permutation 327 tests (highest p-value: 0.014 for the CAA/IMP comparison).

328 The result of the PCA analysis on all loci is presented in Fig. 6A. The first axis mainly separated populations COF and ODC (2.1 % of variance), while the second axis was driven 329 by a few individuals from SPL (2 % of variance). The DAPC analysis did not indicate a 330 clear cut-off for the choice of the number K of clusters (Fig. S6). The clustering at K= 2 331 separated ODC from other populations, and the clustering at K= 3 further separated COF 332 333 from ODC and other populations (Fig. S7). The clustering at K= 4 separated COF, ODC, the two Calangues populations (GCO and IMP), and the two Porquerolles populations 334 335 (SPL and CAA; Fig. 7) with high membership probabilities (near 1 in all cases). One can

336 note that one individual from Porquerolles was assigned to the Calanques cluster, and one

individual from Calanques was assigned to the Porquerolles cluster.

338 We identified 267 potentially selected outlier loci with PCADAPT, 25 with Arlequin and 339 none with OUTFLANK. None of the outliers has been identified with both methods simulta-340 neously.

We performed a separate PCA analysis with the outlier loci identified by each method. With PCADAPT, the first axis of this last PCA separated a small group of individuals from various populations, whereas the second axis mainly separated ODC from other populations (Fig. 6B). The third axis separated the COF population, and the fourth axis mainly separated the two Porquerolles populations (CAA and SPL; Fig. 6C). With the Arlequin outliers, there was no clear signal of differentiation in the PCA (results not shown).

The results of demographic inferences are presented in Table S4. Estimates of effective size indicate a much higher population size for the 30 m population (ODC) compared to the 58 m population (COF), with non-overlapping distributions based on bootstraps. Gene flow estimates indicate very low levels of connectivity between these two populations, but gene flow from ODC to COF appears higher than in the reverse direction (0.0041 compared to less than 0.0001; non-overlapping distributions).

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354 4. DISCUSSION

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356 4.1. Size structure

At the two stations of COF site, mean values for colony height of *Paramuricea clavata* reached 71.08 \pm 47.9 cm (site A) and 57.39 \pm 39.1 cm (site B), respectively. These values are higher than those reported in the ODC station (32.44 \pm 22.54 cm), and, more generally, higher than those reported from the same depth in the northwestern Mediterranean basin (Linares et al. 2008, Pérez-Portela et al. 2016). Surprisingly, the presence of tall

362 (1.25-1.50 m) and giant colonies (>1.50 m) is associated with density values close to those noted in shallower sites with smaller colonies (Harmelin & Garrabou 2005, Linarès 2008, 363 Ibora et al. 2022). By referring to the growth rate value estimated by Mistri & Ceccherelli 364 365 (1994), Cerrano et al. (2005) and Cupido et al. (2012) on small colonies (2.7 to 3.7 cm.yr⁻ ¹), the highest *P. clavata* (1.8 m) could be 50 to 70 years old. Nevertheless, the smallest 366 367 (and youngest) gorgonians usually show hight values of annual growth rate (Coma et al., 1998; Viladrich et al., 2016). Based on the growth rate value calculated by Coma et al. 368 (1998) on higher colonies (from 3 to 55cm), they could be around 100 years, old even 369 though the authors underline the great variability of the growth rate within and between the 370 371 colonies of *P. clavate*. This age is well above the upper limit of this gorgonian's previously estimated life span (50 years). Nevertheless, this astonishing size could be also due to a 372 373 growth rate boosted by environmental conditions. Indeed, the Catchoff study area is characterized by a low current and the proximity of the Rhône River induces high input of or-374 ganic matter with a high productivity (chlorophyll a) potentially beneficial to the growth of 375 376 colonies (Cocito et al. 1997, Linares 2008, Fraysse 2014). Further studies are needed to formally estimate the age of the highest colonies of *P. clavata*. To date, it should be noted 377 378 that these giant deep-sea populations of *P. clavata* are not only unique but also constitute 379 a natural heritage in the north-western Mediterranean basin deserving special attention in terms of conservation and management. 380

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382 4.2. Transplant experimentation

At 30 m, the survival rate of transplants was similar to the percentage obtained in other experiments with different methods and gorgonian species (Weinberg 1979, Fava et al. 2010, Villechanoux et al. 2022). The presence of the kleptoparasite *Haplosyllis chamaeleon* after one year did not cause injuries or any malformation on transplants as usually observed on colonies of *P. clavata* (Pola et al. 2020). A majority of fertile polyps at

388 the end of the experiment was a sign of a good adaptation of reciprocal and control transplants to the 30 m habitat. On the contrary, the high mortality rate at 60 m was surprising. 389 The factors explaining this mortality are probably of different origins such as handling dur-390 391 ing experimental set up or environmental conditions. At 60 m, the high level of sediments falling on the experimental disposal could strongly impact transplants (Cocito et al. 1995, 392 393 Rowley 2014). Identifying their relative impacts on the mortality rates is challenging, yet the high level of sediments on experimental disposal observed at 60 but not at 30 m may 394 be a prominent factor. 395

396

397 4.3. Genetic originality of the deep population of Paramuricea clavata

With nearly 50,000 SNPs genotyped, our results confirm the interest of RAD-sequencing 398 399 for population genetics in non-model species. This number of loci is comparable to results with the same restriction enzyme by Quattrini et al. (2022) on different Paramuricea 400 species (almost 30,000 SNPs), and by Pratlong et al. (2021) on C. rubrum with more dis-401 402 tant populations (more than 25,000 SNPs). Within P. clavata, we observed significant dif-403 ferentiation between sampling sites. When considering our three replicate comparisons between depths, clustering analysis indicated grouping first by sampling area and then by 404 405 depth. This is consistent with the results obtained with microsatellites by Pérez-Portela et al. (2016) focusing on populations around 50-60 m depth, and by Mokhtar-Jamaï et al. 406 (2011) for shallower populations (mainly above 40 m). There is then a local differentiation 407 along depth, rather than the presence of deep and shallow lineages, which would be 408 409 shared among sites. The highest F_{ST} value (0.053) was observed for the comparison be-410 tween the deep population of giant colonies and the nearest population at around 30 m depth (30 m). This differentiation also corresponded to the first axis of the PCA. It can be 411 noted that with microsatellite loci, Mokthar-Jamal et al. (2011) also observed a clear differ-412 413 entiation with a shallow population from the Côte Bleue where the ODC site is located. The

414 particular oceanographic conditions of the Côte Bleue (Fraysse 2014) could lead to a relative isolation of this area explaining our results. The deep population of giant colonies 415 (COF) therefore corresponds to a unique population well differentiated from shallower pop-416 417 ulations from the same area, as well as from the populations of the Southern Calanques area. The results of demographic inferences support this distinction with very low gene 418 flow between COF and ODC, but higher from COF and ODC. Indeed, these populations 419 are 4,500 m apart and currents are very low (<0.1 m.s⁻¹), limiting the dispersal of planula 420 with a short life span before their transformation into new colonies (around 48h, see 421 Linares et al. 2008, Coma et al. 1995). This may explain the relatively high F_{ST} observed 422 423 between these two spatially close sites. The observed F_{ST} between COF and ODC is also similar to the F_{ST} estimated between *P. clavata* populations below 50 m and above 30 m 424 425 with microsatellites by Perez-Portela et al. (2016; F_{ST} 0.049-0.066). The F_{ST} observed here is nevertheless lower than most pairwise F_{ST} observed for *C. rubrum* in the Marseille area 426 using RAD-sequencing (variation between 0.03 and 0.10; Pratlong et al. 2021): this would 427 428 indicate either lower gene flow or higher drift for C. rubrum compared to P. clavata. For P. 429 clavata, with respect to effective population size, the estimated value with RAD-Seq, is 430 much higher for the shallow ODC population compared to the deep COF population. This 431 result is in contradiction with the analyses of Pilczynska et al. (2019), who observed an increase of genetic diversity with depth, a pattern not observed here. To take demographic 432 inference analyses further, it would be necessary to test the impact of additional connectiv-433 434 ity with other neighboring populations, and of variations in census population size on the 435 inferred patterns of effective population size.

436

437 **4.4. Local adaptation to depth**

438 The *P. clavata* populations from COF and ODC occur under contrasting ecological condi-439 tions, including different thermal regimes with higher and more variable temperatures in

440 ODC compared to COF. Nevertheless, it can be noted that the deep population in COF was exposed to temperatures above 22°C during our survey. We could not formally test 441 the hypothesis of local adaptation in our reciprocal transplant experiment due to the loss of 442 443 the samples at the COF site. Indeed, a full test of local adaptation would require a comparison of fitness in both habitats (Kawecki & Ebert 2004, Ledoux et al. 2015). Nevertheless, 444 the treatment at 30 m depth including reciprocal transplantation (from COF) and control 445 446 transplantation (ODC) can be considered as a common garden experiment. Interestingly, we did not observe any significant difference in necrosis rates between colonies from COF 447 and ODC, despite the high temperatures in this shallower habitat (especially in July-Au-448 449 gust 2019). This result would indicate a lack of local adaptation, which is at odds with the differences in tolerance capacity towards warm water, observed between 20 and 40 m 450 451 depth in C. rubrum (Oriol Torrent 2007, Ledoux et al. 2015) and E. cavolini (Pivotto et al. 2015). In an ex-situ common garden, Crisci et al. (2017) observed no difference in thermo-452 tolerance between P. clavata colonies of different depths (20 m vs 40 m) from the Mar-453 454 seille area (Calanques). The same lack of phenotypic differences was observed in Corsica 455 and Catalonia when comparing gorgonians populations along a depth gradient. This result could be the consequence of genetic drift blurring the result of local selection (Crisci et al. 456 457 2017). In line with this apparent lack of local adaptation at the phenotypic level, the search for outlier loci has identified loci that confirm the geographical separation of populations, 458 but without a clear link to environmental variables and local adaptation. Such a RAD-se-459 460 quencing based approach may miss locally selected alleles (e.g., due to reduced genome 461 representation), or may be limited especially when local adaptation is based on a combina-462 tion of loci with small effect (Hoban et al. 2016).

463

464 4.4. Conservation aspects

465 The results of demographic inferences indicate that the deep population of giant P. clavata is isolated and unique with a reduced effective size compared to the shallower popula-466 tions. Our results, and previous ones on such deep populations (Pérez-Portela et al. 2016) 467 468 do not support the hypothesis of deep refugia for *P. clavata*, at least for the studied areas. This also strengthens the need to protect such a deep population which is original, both 469 470 from a genetic and demographic point of view. This need is reinforced by the major impact of anchoring and use of fishnets on the giant populations of P. clavata in a Marine Pro-471 tected Area, with up to 100 boats per day (Leleu et al. 2014, Charbonnel et al. 2020, 472 PMCB 2022) (Fig. S8). In this, the Marine Park of the 'Côte Bleue' (manager of the Natura 473 474 2000 site) has proposed a new technical disposal to the recreational fishermen in order to prevent the impact of anchoring (Fig. S8). Despite the fact that the deeper populations are 475 476 less impacted by warming (Cupido et al. 2008), proper management of this human activity is strongly needed to help preserve these populations (Betti et al. 2020). Indeed, gor-477 gonian forests enhance α and β diversity, and their loss replaces a structurally complex 478 479 habitat dominated by long-lived species with a simplified habitat (turf forming species) with 480 a loss of biodiversity (Verdura et al. 2019, Piazzi et al. 2021).

481

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Table 1. Studied sites with tasks performed. TM: temperature monitoring, CM: current
monitoring, TRE: Transplant experimentation, DS: Density of colonies and size structure,
G: Genomic analysis.

Area	Station	Geographical coordinates	Depth	Task
Marseille north	Catchoff – Station A	43°17.640 N/05 11.151 E	60m	TM, CM, DS, TRE, G
Marseille north	Catchoff – Station B	43°17.840 N/05 11.009 E	58m	DS
Marseille north	Yeux de Chat	43°11.640 N/05°11.151 E	30m	TM, CM, DS, TRE, G
Calanques	Grand Congloue	43°10.582 N/05°24.185 E	33m	G
Calanques	Impérial du large	43°10.177 N/05°23.694 E	54m	G
Porquerolles	Sec Petit Langoustier	42°59.895 N/06°09.612 E	30m	G
Porquerolles	Cap d'Arme	42°58.692 N/06°12.250 E	54m	G

Table 2. Characteristics of the sampling sites for the genetic analyses. N initial, N all and N Marseille North indicate the initial number of samples, the number of samples retained for the complete analysis and the number of samples for the analysis focused on the two sites in the North of the Bay of Marseille respectively. The last columns present the estimates of gene diversity within (1-Qintra) and among individuals (1-Qinter), and of F_{IS} computed with all samples and averaged over 49 215 loci. The lines with a grey background indicate the populations around 30 m depth.

Station	Code	Sampling date	N initial	N all	N Marseille North	1-Qintra	1-Qinter	F _{IS}
Vouw do abot	000	01/07/10	22	07	07	0.000	0.066	0.001
reux de chat	ODC	01/07/19	32	21	21	0.060	0.066	0.091
Catchoff - Station A	COF	01/07/19	32	28	28	0.061	0.067	0.087
Grand Congloue	GCO	30/04/19	7	6	-	0.058	0.064	0.099
Impérial du large	IMP	30/04/19	7	6	-	0.059	0.067	0.115
Sec Petit Langoustier	SPL	22/05/19	8	8	_	0.063	0.068	0.080
Cap d'Arme	CAA	22/05/19	8	7	-	0.058	0.066	0.127

- 790 Table 3. Pairwise F_{ST} for the whole dataset averaged over all loci. Grey areas highlight the comparisons between depths in the same
- area. ODC: Yeux de chat; COF: Catchoff Station A; GCO: Grand Congloue; IMP: Imperial du large, SPL: Sec Petit Langoustier; CAA:
- 792 Cap d'Arme.

	ODC	COF	GCO	IMP	SPL	CAA
ODC	-					
COF	0.053	-				
GCO	0.046	0.053	-			
IMP	0.039	0.030	0.024	-		
SPL	0.044	0.041	0.043	0.027	-	
CAA	0.050	0.039	0.023	0.020	0.020	-

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Fig. 1. Location of studied *Paramuricea clavata* populations. A: Catchoff-station A, Yeux de Chat (temperature and current monitoring, transplant experiment, density and size structure) and Catchoff-station B (density and size structure). B: Additionnal sites selected for the genomic analysis of *Paramuricea clavata* populations.

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 815 Fig. 3. Characterisation of the courantology in Catchoff-Station A (High: bearing in degree,





colonies.m⁻²



Fig. 4. Characterisation of *Paramuricea clavata* populations: size structure (up) and density of colonies (down).

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Yeux de Chat
Catchoffs (site A)
Catchoffs (site B)





- 827 Fig. 5. Survival rate of *Paramuricea clavata* transplants after one year of experiment. COF:
- 828 Catchoff-Station A; ODC: Yeux de Chat station.





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Fig. 6. Plots of individual colonies with the PCA analysis. The numbers in brackets indicate the percentages of inertia for the corresponding axes. A) PCA on all 49 215 loci; B) PCA on the 267 outlier loci identified with pcadapt; C) PCA on the 267 outlier loci identified with pcadapt. COF: Catchoff – Station A; GCO: Grand Congloue; IMP: Impérial du large; SPL: Sec du Petit Langoustier; CAA: Cap d'Arme.



Fig. 7. Barplot of the results of the DPAC analysis for K= 4 clusters. ODC: Yeux de Chat;
COF: Catchoff – Station A; GCO: Grand Congloue; IMP: Impérial du large; SPL: Sec du
petit langoustier; CAA: Cap d'Arme.

1 SUPPLEMENTARY TABLES

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Table S1. Pairs of sequencing replicates (with grey background) and of samples separated by *p* distance similar or lower than distances observed for replicates (on the basis of 51 439 RAD loci obtained with ipyrad; see table S3). The read numbers correspond to the number of reads after the Kraken analysis. For each sample pair, we retained the sample with the highest number of reads for further analyses. See main text for details. PCCOF: Catchoff – Station A; PCODF: Yeux de chat; PCIMP: Impérial du large; PCGCO: Grand Congloue.

Sample 1	Reads	Sample 2	Reads	p distance
PCCOF58-17	7708166	PCCOF58-17-2	6,380,908	0.018
PCCOF58-8	6733912	PCCOF58-8-2	9,117,315	0.022
PCODC30-25	5057861	PCODC30-25-2	4,444,312	0.027
PCODC30-9	6613213	PCODC30-9-2	4,153,968	0.022
PCODC30-21-2	4365254	PCODC30-5	6,556,789	0.020
PCODC30-18	9148556	PCODC30-20-2	5,220,776	0.018
PCIMP54-18	6264317	PCIMP54-13	5,090,699	0.020
PCGCO33-8	6548623	PCGCO33-4	6,513,661	0.025

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- 12 Table S2. Number of loci retained at each filtering step for all populations, with the soft-
- ¹³ ware used at each step. The final dataset included 49,215 SNPs for 82 samples.

	retained loci	software
total_prefiltered_loci	109,1960	ipyrad
filtered_by_rm_duplicates	109,1960	ipyrad
filtered_by_max_indels	109,1960	ipyrad
filtered_by_max_SNPs	109,1081	ipyrad
filtered_by_max_shared_het	108,7331	ipyrad
filtered_by_min_sample	51,439	ipyrad
max 75 % of missing data, 2 alleles per SNP, no LD, one SNP per locus	49,215	vcftools and GBS_SNP_filter

Table S3. Number of loci retained at each filtering step for the analysis of the two sites in the North of the Bay of Marseille, with the software used at each step. The last two lines correspond to the dataset Mars_1 and Mars_2 respectively, with 55 samples in both cases.

	retained loci	software
total_prefiltered_loci	757,308	ipyrad
filtered_by_rm_duplicates	757,308	ipyrad
filtered_by_max_indels	757,308	ipyrad
filtered_by_max_SNPs	756,695	ipyrad
filtered_by_max_shared_het	751,168	ipyrad
filtered_by_min_sample	62,313	ipyrad
max 75% missing data per locus, 2 alleles per SNP, no LD, one SNP per locus	49,215	vcftools and GBS_SNP_filter
no missing data per locus, 2 alleles perSNP, no LD, one SNP per locus	7,653	Vcftools and GBS_SNP_filter

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Table S4. Demographic inferences for the ODC (Yeux de Chat) and COF (Catchoffs - Sta-tion A) populations. The model used for inferences is presented in Figure S2. The esti-mated parameters are effective sizes N COF and N ODC for COF and ODC respectively, the gene flows M ODC COF (from ODC to COF in forward time) and M COF ODC (from COF to ODC), and the divergence time (TDIV in generations). The first line presents the value estimated for the best run (over 100 replicates) for the analysis of observed data. The following lines indicate the statistics describing the distribution of the parameters over 50 non-parametric bootstraps: median, standard deviation, minimum and maximum val-ues. The computation was performed with a mutation rate of 2.9 x 10⁻⁸ / site / generation as estimated in Acropora corals by Mao et al. (2018).

	N_COF	N_ODC	M_ODC_COF	M_COF_ODC	TDIV
Best run	5,378	1,247,133	0.0041	< 0.0001	1987
Median	12,507	1,710,848	0.0018	< 0.0001	5004
Standard deviation	5,879	714,299	0.0053	< 0.0001	2161
Minimum	954	183,719	0.0010	< 0.0001	384
Maximun	20,015	2,651,810	0.0251	< 0.0001	7815



- Fig. S1. Temperature sensor (left) and current meter (right) used to characterize the environment in the Catchoff site (Annex).



- 45 Fig. S2. Open quadrat used to characterize the size structure (50x50cm) (A) and Experi-
- 46 ment design for the transplantation of *Paramuricea clavata* colonies (samples of colonies
- 47 are fixed on a plastic plate and numbered) (B).





Fig. S3. Distribution of the number of raw reads per individuals obtained with RAD-sequencing.



- 54 Fig. S4. Results of the preliminary PCA including *P. clavata* and *P. placomus* (one individ-
- ⁵⁵ ual). The analysis is based on 45 251 SNPs.



Fig. S5. Model of evolution used for demographic inferences with fastsimcoal2. This is a
model of divergence with gene flow. T_DIV indicates the time of divergence,
M_ODC_COF and M_COF_ODC the gene flow (forward in time) between the two populations, N_COF and N_ODC the effective size of populations COF and ODC respectively.



Fig. S6. Evolution of the BIC parameter according to the number of clusters for the DAPCanalysis.



- ⁷⁶ large; SPL: Sec du petit langoustier; CAA: Cap d'Arme.



94 Fig. S8. Impact of anchoring on giant *Paramuricea clavata* populations. A: Giant colony of Paramuricea clavata (h: 170cm) ripped appart by an anchor belonging to a small fishing 95 boat; B: Giant colony of P. clavata (h: 152cm) brought to the Marine Park of the 'Côte 96 Bleue' by a recreational fisherman; C: Group of fishing boats anchored on gorgonian popu-97 lation (15th November 2016); D: Mooring droped with a sliding buoy on the Catchoff site 98 by a recreational boat. When the fisherman uses his boat to pull it, the buoy stop the an-99 100 chor at the surface. E: User instructions for the blocker used for a mooring with a sliding buoy (disposal implemented by the Marine Park of the 'Côte Bleue' to prevent the damage 101 on the giant gorgonians). What is a blocker ? this is a reinforced wooden plate which can 102 block the sliding buoy in order to keep straight the rope and the chain. How to use it ? the 103

blocker can be installed by a person. In a first step, the mooring with the sliding buoy is droped until the anchor touch the bottom. In the next step, the mooring is pulled. When the anchor begins to take off from the bottom, the blocker is fixed on the rope keeping away the chain from the rocky substrate.