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Genome-wide SNPs data provides new insights into the population structure of the Atlantic-Mediterranean gold coral *Savalia savaglia* (Zoantharia: Parazoanthidae)

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52 **Abstract**

53 *Savalia savaglia* is an Atlantic-Mediterranean zoantharian species with a patchy geographic and
54 bathymetric distribution. Due to its longevity, *S. savaglia* may form large-sized colonies which play
55 a crucial role in the ecosystem as habitat formers. Despite its ecological importance, little is known
56 about the population structure and intraspecific genetic diversity of this species. Using ddRAD-Seq
57 genotyping, we obtained genome-wide single nucleotide polymorphisms (SNPs) from 50 *S.*
58 *savaglia* individuals collected at different depths (8 – 60 m) and localities across the Mediterranean
59 Sea (Marseille, Sardinia, Puglia and Montenegro) and eastern Atlantic (Portugal). Our molecular
60 observations were discussed with the reproductive behaviour of the species to understand the
61 observed patterns of connectivity and gene flow. These results highlight the presence of three main
62 genetic clusters (Marseille; Sardinia; and Montenegro + Portugal + Puglia), with some of the
63 Mediterranean individuals being genetically closer to the Atlantic population rather than to other
64 Mediterranean populations. The strong linkage recorded across loci and the detection of clonal
65 individuals in the shallow populations suggest that asexual reproduction seems to be the dominant
66 reproductive strategy among the *S. savaglia* populations sampled at lower depths. Our work
67 highlights the potential of genome-wide SNP data to study the reproductive behaviour in species
68 such as *S. savaglia* that are difficult to investigate in the field. The genetic connectivity data
69 obtained in this study can be used in the future to better guide the development of effective
70 management and conservation plans.

71

72

73 **1. Introduction**

74 Over the last few decades, many ecological studies have been published where molecular results
75 were used for marine conservation purposes [1-3]. For example, genetic data have been successfully
76 deployed for marine biodiversity conservation in the context of spatial planning [4-6], and to predict
77 evolutionary responses of marine species to human-induced impacts [7] and climate change [8,9].
78 Genomic studies can be used to identify geographic regions that may serve as key population
79 connectivity areas [10-12]. Such genetic connectivity data can be of great importance when

80 studying sessile organisms with a patchy distribution, such as mesophotic anthozoan corals, which
81 have been documented as vulnerable when facing human-induced environmental changes [13,12].
82 *Savalia savaglia* (Bertoloni, 1819), commonly known as the gold coral, is a long-living Atlantic-
83 Mediterranean zoantharian (Anthozoa: Hexacorallia: Zoantharia) species most commonly found in
84 the mesophotic twilight zone. *Savalia savaglia* is unusual among zoantharians, being one of the few
85 parasitic species that grows over other anthozoans (e.g., antipatharians and holaxonians) until it
86 causes their death through necrosis [14], after which it produces its own hard layered proteinaceous
87 skeleton [15,16]. Since its description, *S. savaglia* has been considered a rare species with a
88 localized distribution, mostly in deep-sea waters [17]. However, over the last few decades, its
89 geographic and bathymetric distribution appear to be wider than previously thought [18,19]. The
90 geographic distribution of *S. savaglia* within the Mediterranean Sea ranges from the Gibraltar Strait
91 [20] to the Marmara Sea [21,22], yet the bathymetric distribution at shallow depths (< 50 m) is
92 mainly limited to the Boka Kotorska Bay in Montenegro. In the eastern Atlantic, colonies have been
93 found in Galicia [23], Portugal [24-26], the Canary Islands [27,28], and Cape [28].
94 *Savalia savaglia* typically occurs at depths ranging from 8 m to 90 m [29]. However, deeper records
95 of > 500 m depth have also been reported off Sicily [30] and off the Balearic Islands [31]. Colonies
96 in the mesophotic zone increase habitat complexity, providing substrata for other organisms [29]. In
97 addition, high densities (as observed in Boka Kotorska Bay, Montenegro) make this species an
98 important substrate for benthic communities, therefore contributing to an increase of faunal biomass
99 [29]. However, *S. savaglia*, together with other branching anthozoans that host epibionts, is
100 threatened by human activities such as artisanal and recreational fishing, coastal pollution, and boat
101 anchoring, and it is thus currently listed within the Annex II of the Barcelona Convention for the
102 Protection of the Marine Environment and the Coastal Region of the Mediterranean. Additionally, *S.*
103 *savaglia* has been considered a ‘near threatened’ species since recently by the International Union
104 for Conservation of Nature (IUCN) [32].

105 Given the ecological importance and vulnerability of *S. savaglia*, it is of crucial importance to
106 quantify the magnitude, patterns, and trajectories of changes in the population structure and genetic
107 diversity of this species. Data on genetic connectivity can provide significant insight into the
108 dynamics of population maintenance and replenishment following environmental perturbations, and
109 can be used as a proxy for population resilience [33]. However, population genetic studies on *S.*
110 *savaglia* have not yet been performed, most likely due to a lack of species-specific molecular
111 markers with high resolutive power at the intraspecific level, a common problem in anthozoans
112 [34,35]. Among Zoantharia, population genetic analyses have until now been limited to only a few
113 taxa [36,37]. The majority of the studies published on Atlantic-Mediterranean zoantharians have
114 focused on molecular phylogenetic analyses, using either partial mitochondrial and nuclear genes
115 [38,39], or complete mitochondrial genomes [40,41].

116 The use of Single Nucleotide Polymorphisms (SNPs) has greatly increased over the last decade, and
117 restriction site-associated DNA sequencing (RAD-Seq) methods [42] are particularly useful to study
118 non-model organisms for which preliminary genetic information is not available. The large number
119 of obtained SNPs using RAD-Seq allows to resolve population structuring and genetic diversity
120 patterns with much higher resolutions compared to those of traditional PCR markers [43-45].
121 However, the application of high-throughput sequencing technologies (including RAD-Seq) to
122 population-level studies on zoantharians is lacking [46], although Terzin et al. (unpublished) have
123 recently used the 2bRAD genotyping method to investigate population connectivity patterns of the
124 common zoantharian *Parazoanthus axinellae* (Schmidt, 1862) in the Mediterranean.

125 In this study, we collected and successfully genotyped 50 individuals of *S. savaglia* across a depth
126 range of 8 – 60 m, and from five localities: one in the eastern Atlantic (Portugal), and four different
127 localities within the Mediterranean basin. Using a double digest restriction site-associated DNA
128 sequencing (ddRAD-Seq) approach, we retrieved 3,040 SNP markers, which were used to
129 reconstruct the population genomic structure at the Atlantic-Mediterranean scale. RAD-Seq data
130 were then coupled with the features of the sampling sites and reproductive behaviour of the species

131 to understand the observed patterns of connectivity. We further investigated potential correlations
132 between the geographic distance and the genetic structure, and we investigated if gene flow
133 occurred between the shallow (8-50 m) and deep (> 50 m) populations. Finally, this study aims to
134 highlight the importance of using genomic connectivity data to support and advance the
135 management and conservation plans, and to preserve vulnerable species such as the gold coral *S.*
136 *savaglia*.

137

138 **2. Materials and methods**

139 *2.1. Sampling*

140 A total of 50 specimens were collected by SCUBA diving from four localities of the Mediterranean
141 Sea (from west to east: Marseille - MAR, Northeast Sardinia - SAR, Puglia - PUG, Montenegro -
142 MON) and one in the eastern Atlantic (Southwest Portugal - POR) (Fig. 1; Fig. 2A). For traditional
143 population genetic studies based on microsatellites, a sample size of approximately 30 individuals
144 per population is generally useful to ascertain population structure and gene flow [47]. However,
145 with RAD-Seq methods even smaller sample size (i.e. ≤ 10) are likely to be enough to assess the
146 diversity and differentiation at population level [48-51]. For these reasons, and given the patchy
147 distribution of *S. savaglia* in western Mediterranean, at least 11 individuals were collected from
148 each of the Mediterranean localities, while five individuals were collected from the Atlantic
149 population. Detailed information on the sampling sites and depths is provided in Table 1 and
150 Supplementary Table 1; Appendix A.

151

152 *2.2. DNA extraction, library preparation and sequencing*

153 Genomic DNA was extracted using a modified CTAB protocol [52]. DNA yield was assessed with a
154 Qubit® fluorometer using a broad range assay kit (Invitrogen, Carlsbad, CA), whereas DNA quality
155 was visualized on a 1% agarose gel. Double digest restriction site-associated DNA sequencing
156 libraries (ddRAD-Seq) were built at the genomic unit of Admera Health (New Jersey, USA). For

157 each specimen, gDNA was digested with the restriction enzymes *EcoRI* and *MspI* and the resulting
158 fragments were ligated to barcodes and Illumina compatible adapters. The obtained libraries were
159 pooled and sequenced on an Illumina Hiseq platform using a 150 bp pair-end chemistry.

160

161 2.3. SNP calling

162 Sequenced raw reads were demultiplexed and further considered for bioinformatic analyses. The
163 quality of raw read sequences was first assessed using FastQC v.0.11.8 [53]. Raw sequences were
164 then filtered and trimmed to remove low quality base calls and PCR adapters using Trimmomatic
165 v.0.39 [54] with a ‘SLIDINGWINDOW’ parameter of 4:15, and both ‘LEADING’ and ‘TRAILING’
166 values set to 3. The obtained high-quality ddRAD tags were 32 bp in length, and were used for SNP
167 calling, which was performed in Stacks v.2.53 [55].

168 Stacks was run using the computer server of the Center for Strategic Research Project (CSRP) at the
169 University of the Ryukyus. The core module *ustacks* was used to align short sequence reads for each
170 individual into so-called ‘stacks’ with the aim of generating a set of loci by comparing the different
171 stacks. For this step, a combination of values for Stacks parameters *-M* and *-n* was tested (ranging 1-
172 9; fixing *-M* = *-n*; default = 2), while the *-m* parameter was set to 3. The loci obtained were then
173 matched across individuals to build a catalog of loci with the module *cstacks*. The number of
174 mismatches allowed between stacks (putative loci) during construction of the catalog (*-n* parameter)
175 was set to 3 (default = 1). Finally, the Stacks’ *populations* module was used to export the Single
176 Nucleotide Polymorphisms (SNPs) in genind format for downstream analysis in R v3.6.3 (R Core
177 Team 2018). Stacks’ *populations* module was run on the *M3n3m3* dataset (Supplementary File 1;
178 Appendix A), with *-min-mac* (minimum allele count) and *-min-maf* (minimum allele frequency)
179 parameters set to 2 and 0.1, respectively, which allowed us to exclude non-informative
180 monomorphic loci and spurious alleles. The selection of optimal *M*, *n* and *m* values that best fits our
181 data was performed following Paris et al. (2017) [56]. The value for the *-R* parameter (minimum
182 percentage of individuals across populations required to contain a locus for SNP calling) was set to

183 0.8 (80%), to minimize linkage loci, and *–write_single_snp* option was used to keep a maximum of
184 two SNPs per each variant site. Detailed information on the sampling sites and depths is provided in
185 Table 1.

186

187 *2.4. Statistical analyses to investigate populations structuring*

188 The retrieved SNPs exported from Stacks (genind format) were imported into R v.3.6.3 with the
189 package ‘adegenet’ [57] and were later filtered to remove loci out of Hardy-Weinberg equilibrium
190 (HWE) with ‘pegas’ [58], the outlier loci with the package ‘fsthet’ [59], and all individuals with
191 more than a third of missing loci. Any remaining missing loci, which were replaced with mean
192 allele frequency, were excluded using the package ‘poppr’ [60-61]. All downstream analyses were
193 performed on two datasets, using structure files containing 3,238 and 3,040 biallelic variant sites for
194 ‘all loci’ and ‘neutral loci’ datasets, respectively.

195 Overall population statistics parameters, such as observed (H_o) and expected heterozygosity (H_E)
196 values, and the inbreeding coefficient (F_{IS}) were computed at population level with the R package
197 ‘hierfstat’ [62]. Before running ‘LEA’ and STRUCTURE analyses, the R function
198 ‘genind2structure’ [63] was used to remove non-informative monomorphic loci and for converting
199 the R genind object into a structure format. Population genetic structure was assessed using the
200 Bayesian clustering method with STRUCTURE v.2.3.4 [64] and the R package ‘LEA’ [65]. Using
201 STRUCTURE we carried out a total of 10 runs using the Evanno method [66], for values of K
202 ranging from 1 to 10, with a model of correlated allele frequencies and a burn in of 50,000 followed
203 by 500,000 Markov Chain Monte Carlo steps. The web-based tools StructureSelector [67] and
204 Cluster Markov Packager Across K (CLUMPAK [68]) were then deployed to infer the most likely
205 number of K by averaging the results obtained from the 10 STRUCTURE runs. Regarding the
206 ‘LEA’ admixture analysis performed in R, the sparse non-negative matrix factorization (SNMF)
207 algorithm was used for estimating admixture coefficients at individual and population levels,
208 following Jenkins et al. (2019) [63].

209 In order to explore spatial patterns in genetic subdivision, additional analyses were conducted using
 210 a hierarchical design considering ‘Depth’ (two levels: ‘shallow’, corresponding to 8-50 m depth,
 211 ‘deep’, corresponding to > 50 m depth) and ‘Populations’ (five levels: ‘MAR’, ‘MON’, ‘POR’,
 212 ‘PUG’, ‘SAR’) nested within ‘Depth’. These analyses were conducted to test the effect of ‘Depth’
 213 and ‘Population’ on genetic structuring, and have included: (1) Goudet’s G statistics test (based on
 214 Nei’s F_{ST} values) with 999 permutations (Monte-Carlo test), performed with ‘hierfstat’; (2) principal
 215 coordinates analysis (PCoA) performed with the package ‘vegan’ [69,70]; (3) pairwise
 216 permutational multivariate analysis of variance (PERMANOVA) using the R wrapper function
 217 ‘pairwise.adonis’ [71]; and (4) a hierarchical analysis of molecular variance (AMOVA) executed in
 218 ‘poppr’, with 999 permutations computed in ‘ade4’ [72].

219 At the level of ‘Populations’ alone, pairwise Nei’s estimator of F_{ST} values [73] was computed for
 220 each population pair in ‘hierfstat’, using 999 permutations, and with the Benjamini and Yekutieli
 221 (2001) [74] False Discovery Rate (B-Y FDR) correction to calculate the respective p-values, similar
 222 to what was done by Carreras and colleagues (2019) [75]. An isolation by distance (IBD) analysis
 223 was performed with ‘adegenet’ using a Mantel test to investigate if there is a correlation between a
 224 matrix of genetic distances and a matrix of geographic distances.

225 Population structure was also explored through *a priori* assignment of each individual to its
 226 predetermined locality by conducting a discriminant analysis of principal components (DAPC) with
 227 the package ‘adegenet’. The package ‘poppr’ was used to assess whether certain individuals share
 228 the same Multi-Locus Genotype (MLG) with a 3% dissimilarity threshold. As no technical
 229 replicates were available for this dataset, we employed the same dissimilarity threshold utilised for
 230 2bRAD datasets for *Antipathella subpinnata* in Terzin et al. (2021) [12], and for *Parazoanthus*
 231 *axinellae* (Terzin et al. unpublished). Individuals sharing over 97% of genetic similarity were
 232 considered clonal. Aiming to assess whether asexual reproduction was the dominant strategy in the
 233 studied populations, we also verified if populations exhibit linkage across loci using Agapow and

234 Burt index of association (\bar{r}_d) [76]. Further details on the analyses performed are available in the
235 Supplementary File 1; Appendix A.

236

237 **3. Results**

238 *3.1. Locus identification*

239 We obtained $7,435,180 \pm 1,030,352$ (mean \pm SD) raw reads (146-151 bp) per individual, ranging
240 from 4,382,482 to 9,140,567 sequences per sample (Supplementary Table 2; Appendix A). After
241 removing the adapter sequences, *EcoRI* and *MspI* recognition sites, and low-quality base-calls
242 (Phred score < 20), $6,872,223 \pm 948,526$ high-quality ddRAD tags (32 bp) remained per individual
243 (Supplementary Table 2; Appendix A). None of the samples exhibited low sequence coverage, and
244 thereby all 50 individuals were considered for SNP calling in STACKS.

245 A total of 9,188 SNPs were exported from STACKS in genind format for downstream analysis in R.
246 None of the samples contained $> 30\%$ of missing loci and we thereby retained all 50 individuals.
247 Loci showing significant departures from HWE (p-value < 0.05 , based on 999 permutations and
248 after Benjamini Hochberg correction) were removed from the dataset and remaining missing loci
249 were replaced with mean allele frequency, which resulted in an R genind object containing 3,238
250 SNPs. Additionally, each of the four outlier loci detection methods found differing numbers of
251 outliers, equal to (1) 184 (Wright's F_{ST} [77]), (2) 184 (the β based on the variance in allele
252 frequencies [78]), (3) 197 (Cockerham and Weir's sample size corrected β (betahat) [78]), and (4)
253 189 outliers (Weir's θ (theta) F_{ST} estimator [79]) (Supplementary Fig. 1 and Supplementary Table
254 3; Appendix A). Following Carreras et al. (2019) [75], neutral SNPs were selected by removing the
255 198 loci that were found as outliers in two or more methods (Supplementary Figure 1 and
256 Supplementary Table 3; Appendix A). All downstream analyses were done using 'all loci' (3,238
257 SNPs) and 'neutral loci' only (3,040 SNPs) datasets, in both cases shared across 50 individuals. The
258 results obtained using neutral loci are shown in the main text, whereas those obtained using the 'all
259 loci' dataset can be found in the Supplementary material (Supplementary File 2; Appendix B).

260

261 3.2. Population structure and connectivity

262 The computed inbreeding coefficient (F_{IS}) values were negative in all localities. Additionally, an
263 excess of heterozygotes ($H_{OBSERVED} > H_{EXPECTED}$) was recorded in all shallow populations (MAR,
264 MON, SAR), whereas the calculated $H_{OBSERVED}$ for deeper populations (POR, PUG) had the same
265 values of $H_{EXPECTED}$ (Table 1). The hypothesis that alleles are linked across loci was significantly
266 supported by the linkage disequilibrium analysis ($p = 0.001$). Apart from Portugal, the observed
267 Agapow and Burt index of association (\bar{r}_d) resulted to be outside of the distribution expected under
268 no-linkage in each of the sampling localities (Supplementary Fig. 2; Appendix A). Thus, the null
269 hypothesis of no-linkage among loci was therefore rejected.

270 No monomorphic loci were detected prior to performing 'LEA' and STRUCTURE analyses. 'LEA'
271 admixture analysis detected three subpopulation clusters (K) (Fig. 2). Most individuals originated
272 from the MAR and SAR populations, which were associated to Cluster 2 (yellow) and Cluster 3
273 (black), respectively, with high scores for membership assignment. Samples from the remaining
274 populations (POR, PUG, MON) exhibited panmixia and formed the third 'LEA' cluster (mostly blue
275 in Fig. 2), although clonal individuals from MON were associated to Cluster 1 (blue) with high
276 admixture coefficient values (Fig. 2B). Bayesian clustering in STRUCTURE also inferred $K = 3$ as
277 the 'true' number of ancestral populations, with the same detected pattern as the one in 'LEA'
278 analysis (Supplementary Fig. 3; Appendix A).

279 Goudet's G statistics test revealed a significant effect of 'Depth' ($p = 0.01$, Fig. 3A) and
280 'Population' ($p = 0.01$, Fig. 3B) on overall genetic structuring. Pairwise F_{ST} values ranged between
281 0.01-0.39 for all population pairs. Corresponding p-values were significant for all shallow
282 population comparisons, and nearly all deep vs. shallow population comparisons, ranging from
283 0.005 to 0.044. Deep vs. deep population comparison was not statistically significant, and a very
284 low level of genetic differentiation was displayed between POR and PUG localities (pairwise F_{ST}

285 value = 0.01, p value = 0.744). The highest F_{ST} value was observed between the populations with
 286 the highest numbers of clones detected (MAR vs. SAR: $F_{ST} = 0.39$) (Fig. 3C).
 287 We retained 10 Principal Components (PCs) to perform the DAPC analysis, as suggested by the
 288 calculated alpha (α) and cross-validation scores (Supplementary Fig. 4; Appendix A). The DAPC
 289 plot also showed a clear genetic differentiation among three main clusters, with the MAR and SAR
 290 populations being particularly isolated from the remaining sampling localities, and from one
 291 another. Furthermore, the deep populations (POR and PUG) grouped together and seemed to exhibit
 292 high connectivity with the MON population (Fig. 4A). The composite bar plot based on population
 293 membership probabilities (computed in DAPC) showed that most of the individuals cluster within
 294 their *a priori* determined locality with high membership probability scores, although one individual
 295 from MAR (SS016), PUG (SS054) and SAR (SS146), and three individuals from MON (SS150,
 296 SS167, SS174) seemed more likely to originate from other populations (Fig. 4B).
 297 The MLG analysis showed that all shallow populations (MAR, MON, SAR) seemed to be partially
 298 clonal, while no clonality was observed among individuals sampled in deeper waters. In more
 299 detail, the number of clonal individuals within each of the shallow populations were: MAR = 10
 300 individuals within MLG-29, SAR = 8 individuals within MLG-12 + 2 individuals within MLG-3,
 301 and MON = 5 individuals within MLG-38 (Fig. 4C and Table 1).
 302 Concerning the PCoA analysis, after considering all deep and shallow populations together, we
 303 observed that the ‘Deep’ cluster was grouped within the ‘Shallow’ cluster, with all deep samples
 304 being located very closely to the ‘Deep’ group centroid (Supplementary Fig. 5A; Appendix A). In
 305 contrast, the majority of individuals from shallow localities exhibited greater distances to the
 306 ‘Shallow’ group centroid, with clonal shallow samples diverging the most (Supplementary Fig. 5A;
 307 Appendix A). The PCoA plot recorded a slightly different clustering pattern compared to the DAPC
 308 analysis, with the deep populations clustering very closely, and diverging from shallow localities
 309 (Supplementary Fig. 5B; Appendix A). PERMANOVA calculated with factor ‘Depth’ displayed
 310 significant structuring when all deep and shallow localities were compared (Holm p value = 0.001),

311 although with a low R squared value ($R^2 = 0.062$), which means that, while statistically significant,
312 ‘Depth’ did not account for a large portion of the dissimilarity between deep and shallow
313 populations (Table 2, Factor 1: Depth). Pairwise PERMANOVA computed for each population pair
314 also showed significant genetic differentiation for all population comparisons, except for POR-PUG
315 and POR-MON (Table 2, Factor 2: Populations).

316 Considering that the observed phi (ϕ) did not fall within the distribution expected from 999
317 permutations in ‘within Populations’ and ‘between Populations, nested within Depth’ cases
318 (Supplementary Fig. 6; Appendix A), the hierarchical AMOVA showed significant genetic
319 differentiation at two levels of variation: (1) within Populations ($p = 0.001$) and (2) between
320 Populations, nested within Depth ($p = 0.001$), but not (3) between Depths (Supplementary Table 4
321 in Appendix A; AMOVA: Testing significance with 999 Monte-Carlo tests). Lastly, no correlation
322 was observed between genetic and geographic distances based on the IBD analysis (Supplementary
323 Fig. 7; Appendix A).

324

325 4. Discussion

326 A total of 3,040 genome-wide SNPs retrieved from 50 individuals of *S. savaglia* from one
327 population in the eastern Atlantic (Portugal, POR) and four populations from the Mediterranean Sea
328 (Marseille MAR, Montenegro MON, Puglia PUG, Sardinia SAR) detected the presence of three
329 main clusters: (1) MAR, (2) SAR and (3) POR + PUG + MON. Interestingly, the deep populations
330 sampled at 60 m depth (one Mediterranean population (PUG) and the Atlantic POR population)
331 shared similar genetic profiles, and had a greater degree of population admixture compared to the
332 shallow populations. Additionally, no clonal individuals were detected from deep populations using
333 a 3% dissimilarity threshold in the MLG analysis. In contrast, each of the examined shallow
334 populations (MAR, MON, SAR) contained clones. Overall, a greater degree of genetic structuring
335 was observed for the shallow localities, which were all distinct from one another, with the highly
336 clonal MAR and SAR populations being particularly isolated. The high genetic structure found

among shallow populations could be caused by physical barriers to gene flow [80,81], and can possibly be explained by the patchy distribution of *S. savaglia* in the Mediterranean Sea [14,29]. The detection of clones in the MLG analysis coincides with our linkage disequilibrium analysis, as we found a strong linkage across loci in all Mediterranean populations (particularly the highly clonal MAR and SAR). Loci are expected to be combined into new genotypes during sexual reproduction due to recombination [76]. The strong linkage found in *S. savaglia* shows therefore that asexual reproduction prevails in all the Mediterranean populations considered and that sexual reproduction seems to be dominant only in the Atlantic POR population, as no linkage across loci was detected for this locality. Despite containing clonal colonies, the shallower populations considered (MAR, MON, SAR) reported an excess of heterozygotes ($H_O > H_E$) and negative inbreeding coefficient (F_{IS}) values. While a scenario of simultaneous clonal reproduction and excess of heterozygosity seems counter-intuitive at first, populations mostly maintained by clonal propagation have occasionally been documented to exhibit a significant heterozygote excess compared to expected values under the Hardy-Weinberg equilibrium, due to the long-term accumulation of mutations in clonal lineages [82-85] and the retention of more alleles [86]. This genetic feature has been hypothesized by some investigators as an evolutionary strategy to retain genetic diversity within individuals in asexual populations [87].

Similarly, Dahl et al. (2012) [88] found negative F_{IS} and high values of H_E in the eastern Atlantic scleractinian *Desmophyllum pertusum* (Linnaeus, 1758) (formerly known as *Lophelia pertusa*). High heterozygosity levels have also been reported in other anthozoans using allozymes and microsatellite markers [89,90], although heterozygote deficiencies also seem to be common among scleractinians [91,92]. Overall, heterozygosity deficiency can be attributed to inbreeding, restricted gamete dispersal, or caused by subpopulation structure [93,94], whereas biological features such as reproductive strategies, individuals' longevity, and/or selection against homozygotes might explain, in some instances, heterozygosity excess.

It is interesting to note that asexual reproduction in hexacorals has been documented to occur under

363 both favourable [95] and non-optimal [96] environmental conditions. For instance, Combosch and
364 Vollmer (2013) [95] observed that the common tropical scleractinian *Pocillopora damicornis*
365 (Linnaeus, 1758) deploys both sexual and asexual reproductive strategies and argued that sexual
366 reproduction increases the genetic diversity of individuals through recombination. Asexual
367 reproduction (which occurs among sexual recombinants in favourable conditions) would potentially
368 increase the abundance of those sexual recombinants which are best suited to local environmental
369 conditions, when local conditions are stable over time [95]. In contrast, Waller and Tyler (2005) [96]
370 observed that *D. pertusum* seemed to prefer asexual reproduction over sexual reproductive
371 strategies under stress conditions (e.g., high levels of trawling). While clonal fragmentation is
372 among the most common asexual reproductive strategies within anthozoans [97-99], it is still
373 unclear how asexual propagation affects the genetic architecture of populations, as well as their
374 genetic diversity, connectivity patterns, effective population sizes, and long-term persistence of
375 populations. One reason for this could be that many population genetic studies on asexual species
376 are designed to avoid the collection of clonal individuals. Otherwise, once detected, clones are
377 removed from the dataset [84,100-104]. While high levels of clonality may cause population
378 vulnerability under shifting environmental conditions due to low genetic diversity (as documented
379 by Carella et al. (2019) [105] in Antarctic demosponges), they may also play an important role in
380 the survival of corals in non-optimal environments, since asexual propagules may disperse into
381 adjacent habitats with more suitable conditions.

382 Concerning zoantharians, the majority of the species form colonies by budding [106]. Alternative
383 asexual propagation methods, such as transverse fission and budding from the base of the column
384 wall, have been only rarely reported in two zoantharian species: (1) *Sphenopus marsupialis*
385 (Gmelin, 1791) [107] and (2) *Acrozoanthus australiae* Saville-Kent, 1893 [108]. Overall,
386 fragmentation seems to be a common way of dispersal for coral reef zoantharians [109,110]. In
387 addition, an unusual form of growth fragmentation has been observed in eastern Atlantic (UK)
388 population of *P. axinellae* [106].

389 For *S. savaglia*, the high clonality rates detected in each of the shallow populations (MAR, MON,
390 SAR) are likely a consequence of different factors. For the Marseille population in particular, our
391 results indicate that the majority of the individuals collected within the Gulf of Marseilles are
392 clones. Interestingly, between 1967 and 1984, Zibrowius (1985) [111] observed a single colony of *S.*
393 *savaglia* at Grand Conglu, highlighting the rarity of the species within the gulf. The same author
394 explored the mechanisms of growth and the growing rates of the gold coral by transplanting small
395 fragments on different colonies of *Paramuricea clavata* at this site. The observations from
396 Zibrowius (1985) [111] showed two main growth stages. Before starting to deposit its own skeleton,
397 *S. savaglia* overgrows a host colony with an estimated speed ranging between 5 cm and 8 cm per
398 year [111]. The presence of clonal individuals collected at Gran Conglu (MAR) can be directly
399 linked to these transplantation experiments conducted more than 35 years ago within the Gulf of
400 Marseilles by Zibrowius (1985) [111]. Our results indicate that colonies from the Gulf of Marseilles
401 must be very poorly connected to other patches of *S. savaglia*, as during a period of over 35 years
402 (time elapsed from transplantation + the age of the transplanted colony) there has been no apparent
403 recruitment from other populations to the same site. In addition, the high level of clonality may
404 reflect the interesting capacity of this species to maintain itself in the absence of sexual recruitment.
405 Because of its extreme longevity, *S. savaglia* potentially has the opportunity to wait for the rare
406 event of a new arrival and then subsequently re-establish a sexual population, avoiding local
407 extinction in the face of environmental changes.

408 We also detected high clonality in northeastern Sardinia; yet unlike Marseilles, there are no
409 historical traces of human-induced transplantation. Recent surveys in the area have shown the
410 conspicuous presence of marine nets and lines, which represent a serious threat for the branched
411 anthozoans such as the gold coral, as well as to the associated species. Given the massive presence
412 of marine debris entanglement, as well as truncated living colonies and entirely extirpated dead
413 colonies [112, 113], see also Fig. 1 - SAR), we speculate that fragmentation (as a result of
414 mechanical damage) is the putative source of propagation of clones in northeastern Sardinia.

415 However, in order to confirm our hypothesis, further analyses involving a greater number of
416 individuals and more sites combined with field monitoring observations are needed.

417 While clonal reproduction in the Sardinia population may have been caused by human activities and
418 stressful conditions, clonal propagation in the Montenegro population could have resulted from
419 asexual reproduction of sexual recombinants that are best suited to local environmental conditions,
420 as hypothesized for the Indo-Pacific reef coral *Pocillopora damicornis* [95]. Overall, *S. savaglia* has
421 a scattered distribution in the Mediterranean Sea, yet, with more than 1000 colonies, Boka Kotorska
422 Bay in Montenegro has been documented to host the greatest abundance of this zoantharian species
423 within the entire Mediterranean basin [114,115], indicating that local environmental conditions in
424 the bay, especially in proximity to submarine springs, are favourable for the successful spread of
425 this hexacoral. The fragmentation and transportation of the gold coral's fragments in the Boka
426 Kotorska Bay have been associated to the activity of local freshwater streams that, over the
427 winter/spring months, may carry coarse sand and gravel which sometimes damage part of the
428 benthic fauna, including the small *S. savaglia* colonies [114]. It has also been observed that when
429 colonies of *S. savaglia* grow in areas with a high density of gorgonians, this species can rapidly
430 spread from one colony to another if physical continuity is present. Although being distributed on a
431 wider area and with a large spatial distribution, the only other geographic area where monospecific
432 facies of hundreds of *S. savaglia* colonies have been recorded so far is in the Canary Islands [116].
433 It is additionally interesting to note that some of the Montenegro individuals share a similar
434 genomic profile with the Ionian deep (PUG) population (Fig. 2B). While some previous genetic
435 studies conducted on Mediterranean corals have not recorded gene flow between shallow and deep
436 coral populations [12,117,118], our molecular data on *S. savaglia* indicate that there is gene flow
437 across approximately 200 km between Puglia (deep) and Montenegro (shallow) populations.
438 However, it is uncertain which population acts as the 'source' of genetic diversity through provision
439 of coral propagules. Coupling our data with physical oceanographic parameters (i.e., ocean currents)
440 and conducting experiments in the field (i.e., larvae tracking) will be crucial to better understand

441 which population acts as the source of genetic diversity. Therefore, we argue that directional gene
442 flow analyses should be pursued in future studies on the population dynamics of *S. savaglia*.
443 Similarly, investigating the characteristics of zoantharian larvae is another research direction to
444 follow, particularly when considering the overall lack of knowledge on zoantharian reproductive
445 biology. In this regard, the study of Babcock and Ryland (1990) [119] represents the only
446 description available so far on macrocnemic zoantharian development from spawning to settlement.
447 In addition, it is thought that zoantharians of the suborder Macrocnemina do not reproduce through
448 pelagic larvae as seen in species of suborder Brachycnemina, but instead through planulae that
449 likely settle in proximity of parent colonies. As *S. savaglia* is found within the suborder
450 Macrocnemina, this hypothesis would explain the great densities of *S. savaglia* observed in some
451 areas (i.e., Canary Island and Montenegro), leaving however some doubts on the patchy distribution
452 documented in the majority of Mediterranean sites [18]. Similar to other widespread Macrocnemina
453 genera (e.g., *Epizoanthus*, *Parazoanthus*), *S. savaglia* can be found across sites that are thousands of
454 kilometres apart. Connectivity and colonization would potentially be facilitated by the presence of
455 long-lived pelagic larval stages, which can instead be conversely hampered in cases of species with
456 short pelagic stages. Given the recent observations and the genetic data obtained, we hypothesize
457 that the pelagic larval duration (PLD) of the gold coral *S. savaglia* might be of medium-long
458 duration, as has been supposed already for other zoantharians (i.e., Macrocnemina [120]; *Palythoa*
459 [121]). This hypothesis is supported by the lack of correlation between genetic and geographic
460 distances observed in this study, although –as already emphasized– experimental studies are needed
461 to fully describe the larval features of *S. savaglia*.
462 Southwestern Portugal individuals were collected from an average depth of 60 m, nonetheless it is
463 likely that unseen larger populations are present even deeper and are more widely distributed within
464 the mesophotic zone [122], which is to date still poorly explored [24-26]. Although the Almeria-
465 Oran front constitutes a strong oceanographic barrier against the migration of Atlantic individuals
466 into the Mediterranean Sea for some species [123,124], connectivity between *S. savaglia*

populations at this wider spatial scale is coherent with the dominant surface circulation pattern of Atlantic inflow water into the Mediterranean Sea. Moderate larval dispersal ability via the surface (hypothesized), even in a stepping-stone manner, may favour such a large-scale connectivity via for instance the Algeria current [125,126], as observed for other coral species (e.g., [25]). The Atlantic-Mediterranean connectivity in turn explains the widely shared genetic ancestry, such as that between *S. savaglia* in Portugal and the Ionian and Adriatic Sea populations (ancestry with clusters 1, 2 and 3 simultaneously; Fig. 2). One can assume that *S. savaglia* was historically distributed in eastern Atlantic deep-sea waters (> 50 m), as it is still nowadays, and that its presence within the Mediterranean Sea is the consequence of oceanographic dynamics events. Nonetheless, post-glacial colonization strategies have been shown to vary greatly even among ecologically close coral species. For instance, the cold-water corals *D. pertusum* and *Madrepora oculata* Linnaeus, 1758 are presumed to have undergone a fast and long-range post-glacial recolonisation of the northeastern Atlantic from Mediterranean glacial refugia populations for one species and more complex scenarios of multiple refugia and secondary contacts for the other species [127]. We observed gene flow between Atlantic and Mediterranean populations, yet the data obtained do not allow us to examine whether the Mediterranean Sea acted as a recolonisation source for the Atlantic Ocean, or if alternately the Atlantic individuals have colonized the Mediterranean Sea. In particular, we found that some of the Mediterranean populations (PUG and MON) are genetically closer to the Atlantic (POR) population than to other Mediterranean ones (Figs. 2A-B). Additionally, the genetic distances between populations do not increase with geographic distance. Most likely the major structuring differences are driven by the extreme clonality observed in the western Mediterranean Marseilles and Sardinia populations. Such extreme clonality explains why the western Mediterranean populations are so genetically isolated from the eastern ones (PUG and MON). Recent ecological surveys have detected an overall expansion of the range of *S. savaglia* in the Mediterranean Sea [128]. This expansion could be explained by the recent general warming of the Mediterranean Sea waters –*S. savaglia* is a thermophilic species [129]– and by an overall decline of

493 sea-fan populations [130,131], which could then potentially increase the spreading of parasitic
494 species such as the gold coral. On the other hand, the recent finding of a great number of *S. savaglia*
495 colonies in the Mediterranean Sea can be attributed to the increase in mesophotic surveys and
496 biodiversity expeditions undertaken as already reported for other Mediterranean marine
497 invertebrates [132-134].

498 Preserving habitat forming corals, such as *S. savaglia*, is of crucial importance as the loss of
499 ecosystem engineers is known to cause population declines across multiple taxa of coral-associated
500 flora and fauna [135-137]. Such wide population declines affect the stability of the entire
501 ecosystem. In the case of *S. savaglia*, it would be valuable to assess how this species reacts to
502 shifting climate conditions such as ocean warming and acidification. Particularly, this will be useful
503 to determine whether *S. savaglia* will be threatened under future climate scenarios in the
504 Mediterranean Sea, and to what extent this ecologically important species needs to be protected
505 from anthropogenic impacts.

506

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522

523 **Data**

524 Raw DNA sequence reads for each specimen have been deposited in the National Center for
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534

535 **References**

- 536 [1] M. Ungerer, L. Johnson, M. Herman, Ecological genomics: understanding gene and genome
537 function in the natural environment, *Heredity* 100 (2008) 178–183.
- 538 [2] D. Tagu, J.K. Colbourne, N. Nègre, Genomic data integration for ecological and evolutionary
539 traits in non-model organisms, *BMC Genomics* 15 (2014) 490.
- 540 [3] S.M. Rudman, M.A. Barbour, K. Csilléry, P. Gienapp, F. Guillaume, N.G. Hairston Jr, A.P.
541 Hendry, J.R. Lasky, M. Rafajlović, K. Räsänen, P.S. Schmidt, O. Seehausen, N.O. Therkildsen,
542 M.M. Turcotte, J.M. Levine, What genomic data can reveal about eco-evolutionary dynamics,
543 *Nat. Ecol. Evol.* 2 (2018) 9–15.
- 544 [4] W.C. Funk, J.K. McKay, P.A. Hohenlohe, F.W. Allendorf, Harnessing genomics for delineating
545 conservation units. *Trends in Ecol. Evol.* 27 (2012) 489–496.
- 546 [5] E.S. Nielsen, M. Beger, R. Henriques, K.A. Selkoe, S. von der Heyden, Multispecies genetic
547 objectives in spatial conservation planning, *Conserv. Biol.* 31 (2017) 872–882.
- 548 [6] S. Barbosa, F. Mestre, T.A. White, J. Paupério, P.C. Alves, J.B. Searle, Integrative approaches to
549 guide conservation decisions: using genomics to define conservation units and functional
550 corridors, *Mol. Ecol.* 27 (2018) 3452–3465.
- 551 [7] S.J. Bourlat, A. Borja, J. Gilbert, M.I. Taylor, N. Davies, S.B. Weisberg, J.F. Griffith, T. Lettieri,
552 D. Field, J. Benzie, F.O. Glöckner, N. Rodríguez-Ezpeleta, D.P. Faith, T.P. Bean, M. Obst,
553 Genomics in marine monitoring: New opportunities for assessing marine health status, *Mar.*
554 *Pollut. Bull.* 74 (2013) 19–31.

- 555 [8] A.M. Waldvogel, D. Schreiber, M. Pfenninger, B. Feldmeyer, Climate change genomics calls for
556 standardized data reporting, *Front. Ecol. Evol.* 8 (2020) 242.
- 557 [9] A.M. Waldvogel, B. Feldmeyer, G. Rolshausen, M. Exposito-Alonso, C. Rellstab, R. Kofler, T.
558 Mock, K. Schmid, I. Schmitt, T. Bataillon, O. Savolainen, A. Bergland, T. Flatt, F. Guillaume,
559 M. Pfenninger, Evolutionary genomics can improve prediction of species' responses to climate
560 change, *Evol. Lett.* 4 (2020) 4–18.
- 561 [10] G.R. Almany, M.L. Berumen, S.R. Thorrold, S. Planes, G.P. Jones, (2007) Local replenishment
562 of coral reef fish populations in a marine reserve, *Science* 316 (2007) 742–744.
- 563 [11] M. Jahnke, P.R. Jonsson, P.O. Moksnes, L.O. Loo, M.N. Jacobi, J.L. Olsen, Seascape genetics
564 and biophysical connectivity modelling support conservation of the seagrass *Zostera marina* in
565 the Skagerrak-Kattegat region of the eastern North Sea, *Evol. Appl.* 11 (2018) 645–661.
- 566 [12] M. Terzin, M.G. Paletta, K. Matterson, M. Coppari, G. Bavestrello, M. Abbiati, M. Bo, F.
567 Costantini, Population genomic structure of the black coral *Antipathella subpinnata* in
568 Mediterranean vulnerable marine ecosystems, *Coral Reefs* 40 (2021) 751–766.
- 569 [13] F. Prada, L. Musco, A. Alagna, D. Agnetta, E. Beccari, G. D'Anna, V.M. Giacalone, C.
570 Pipitone, T. Vega Fernández, S. Goffredo, F. Badalamenti, Anthropogenic impact is negatively
571 related to coral health in Sicily (Mediterranean Sea), *Sci. Rep.* 9 (2019) 13469.
- 572 [14] M. Previati, M. Palma, G. Bavestrello, C. Falugi, C. Cerrano, Reproductive biology of
573 *Parazoanthus axinellae* (Schmidt, 1862) and *Savalia savaglia* (Bertoloni, 1819) (Cnidaria,
574 Zoantharia) from the NW Mediterranean coast, *Mar. Ecol.* 31 (2010) 555–565.
- 575 [15] E.B. Roark, T.P. Guilderson, R.B. Dunbar, S.J. Fallon, D.A. Mucciarone, (2009) Extreme
576 longevity in proteinaceous deep-sea corals. *Proc. Natl. Acad. Sciences. USA* 106 (2009) 5204–
577 5208.
- 578 [16] F. Sinniger, O.V. Ocaña, A.R. Baco, (2013) Diversity of zoanthids (Anthozoa: Hexacorallia) on
579 Hawaiian seamounts: Description of the Hawaiian gold coral and additional zoanthids, *PLoS*
580 *ONE* 8 (2013) e52607.
- 581 [17] V. Häussermann, Ordnung Zoantharia (=Zoanthiniaria, Zoanthidae) (Krustenanemonen), in R.
582 Hofrichter (Ed.) *Das Mittelmeer. Fauna, Flora, Ökologie*, Volume II/1. Heidelberg: Spektrum
583 Akademischer Verlag, 2003 pp.501–505.
- 584 [18] M. Giusti, C. Cerrano, M. Angiolillo, L. Tunesi, S. Canese, An updated overview of the
585 geographic and bathymetric distribution of *Savalia savaglia*, *Mediterr. Mar. Sci.* 16 (2015) 128–
586 135.
- 587 [19] A. Altuna, A. Polisenio, Taxonomy, genetics and biodiversity of Mediterranean deep-sea corals
588 and cold-water corals, in: O. Covadonga, C. Jiménez (Eds.), *Mediterranean Cold-Water Corals:*
589 *Past, Present and Future*. Springer, 2019 121–156.
- 590 [20] O. Ocaña, A. Brito, J. Núñez, J.J. Bacallado, Redescrición de *Gerardia savaglia* (Bertoloni,
591 1819) (Anthozoa: Zoantharia: Gerardiidae), *Vieraea* 24 (1995) 153–164.
- 592 [21] M.İ. Artüz, M. Artüz, O.B. Artüz, Mercan Türlerine Getirilen Yasaklar İle İlgili Görüşler. T. C.
593 Çevre Bakanlığı Raporu K. K. G. M. Su Ürünleri Sirküleri Düzenlemeleri 1990.
- 594 [22] B. Öztürk, J.P. Bourguet, Données préliminaires sur le corail noir de la Mer de Marmara
595 (Turquie) *Gerardia savaglia* (Bertolini, 1819). *Istanbul University Journal of Aquatic Products* 4
596 (1990) 45–48.
- 597 [23] A. Altuna, F. Sinniger, J.M. Aldrey, Occurrence of *Savalia savaglia* (Anthozoa: Zoantharia) in
598 the Ría de Arousa (Galicia, north-western Spain, north- eastern Atlantic), *Mar. Biodivers. Rec.* 3
599 (2010) e110.
- 600 [24] J. Boavida, J. Assis, J. Reed, E.A. Serrão, J.M.S. Gonçalves, Comparison of small remotely
601 operated vehicles and diver-operated video of circalittoral benthos. *Hydrobiologia* 766 (2016)
602 247–260.
- 603 [25] J. Boavida, D. Paulo, D. Aurelle, S. Arnaud-Haond, C. Marschal, J. Reed, J.M.S. Gonçalves,
604 E.A. Serrão, A well-kept treasure at depth: Precious red coral rediscovered in Atlantic deep coral
605 gardens (SW Portugal) after 300 years. *PLoS One* 11 (2016) e0147228.

- [26] V. Dias, F. Oliveira, J. Boavida, E.A. Serrão, J.M.S. Gonçalves, M.A.G. Coelho, High coral bycatch in bottom-set gillnet coastal fisheries reveals rich coral habitats in Southern Portugal. *Front. Mar. Sci.* 7 (2020) 1–16.
- [27] A. Brito, Hábitat y distribución de *Gerardia savaglia* (Bertoloni, 1819) (Anthozoa: Zoantharia) en las Islas Canarias (Océano Atlántico), *Téthys* 11 (1983) 89–91.
- [28] O. Ocaña, A. Brito, A review of Gerardiidae (Anthozoa: Zoantharia) from Macaronesian Islands and the Mediterranean sea with the description of a new species, *Revista de la Academia Canaria de Ciencias* 15 (2004) 159–189.
- [29] C. Cerrano, R. Danovaro, C. Gambi, A. Pusceddu, A. Riva, S. Schiaparelli, Gold coral (*Savalia savaglia*) and gorgonian forests enhance benthic biodiversity and ecosystem functioning in the mesophotic zone, *Biodivers. Conserv.* 19 (2010) 153–167.
- [30] P. Arena, F.L. Li Greci, (1973) Indagine sulle condizioni faunistiche e sui rendimenti di pesca dei fondali batiali della Sicilia occidentale e della bordura settentrionale dei banchi della soglia Siculo-Tunisina. *Quaderni del Laboratorio di Tecnologia della Pesca* 1 (1973) 157–201.
- [31] Oceana (2010) Seamounts of The Balearic Islands. Proposal for a Marine Protected Area in the Mallorca Channel (Western Mediterranean). pp. 62.
- [32] M.M. Otero, C. Numa, M. Bo et al., Overview of the conservation status of Mediterranean anthozoans, IUCN, Malaga, Spain, 2016 pp. 73.
- [33] L. Thomas, J. Underwood, A. Adam, Z. Richards, L. Dugal, K. Miller, J. Gilmour, Contrasting patterns of genetic connectivity in brooding and spawning corals across a remote atoll system in northwest Australia, *Coral Reefs* 39 (2019) 55–60.
- [34] D. Huang, R. Meier, P.A. Todd, L.M. Chou, Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding, *J. Mol. Evol.* 66 (2008) 167–174.
- [35] F. Costantini, A.M. Addamo, A. Machordom, M. Abbiati, Genetic connectivity and conservation of temperate and cold-water habitat-forming corals, in: S. Rossi, L. Bramanti, A. Gori, C. Orejas (Eds.), *Marine animal forests*. Springer, Cham 2017, pp. 1–22.
- [36] D. Albinsky, D. Wham, N. Shinzato, J.D. Reimer, Population connectivity in the common reef zoantharian *Zoanthus sansibaricus* (Anthozoa: Hexacorallia) in southern Japan, *Zool. Sci.* 35 (2018) 321–329.
- [37] A. Villamor, L.F. Signorini, F. Costantini, M. Terzin, M. Abbiati, Evidence of genetic isolation between two Mediterranean morphotypes of *Parazoanthus axinellae*, *Sci. Rep.* 10 (2020) 13938.
- [38] F. Sinniger, J.D. Reimer, J. Pawlowski, Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia), *Zool. Sci.* 25 (2008) 1253–1260.
- [39] T.D. Swain, Revisiting the phylogeny of Zoanthidea (Cnidaria: Anthozoa): staggered alignment of hypervariable sequences improves species tree inference, *Mol. Phylogenetics Evol.* 118 (2018) 1–12.
- [40] F. Sinniger, P. Chevaldonné, J. Pawlowski, Mitochondrial genome of *Savalia savaglia* (Cnidaria, Hexacorallia) and early metazoan phylogeny, *J. Mol. Evol.* 64 (2007) 196–203.
- [41] A. Poliseno, M.E.A. Santos, H. Kise, B. Macdonald, A.M. Quattrini, C.S. McFadden, J.D. Reimer, Evolutionary implications of analyses of complete mitochondrial genomes across order Zoantharia (Cnidaria: Hexacorallia), *J. Zool. Syst. Evol. Res.* 58 (2020) 858–868.
- [42] N.A. Baird, P.D. Etter, T.S. Atwood, M.C. Currey, A.L. Shiver, Z.A. Lewis, E.U. Selker, W.A. Cresko, E.A. Johnson, Rapid SNP discovery and genetic mapping using sequenced RAD markers, *PLoS ONE* 3 (2008) e3376.
- [43] G.C.B. Schopen, H. Bovenhuis, M.H.P.W. Visker, J.A.M. Van Arendonk, Comparison of information content for microsatellites and SNPs in poultry and cattle, *Anim. Genet.* 39 (2008) 451–453.
- [44] A.M. Reitzel, S. Herrera, M.J. Layden, M.Q. Martindale, T.M. Shank, Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics, *Mol. Ecol.* 22 (2013) 2953–2970.

658 [45] R.G.J. Hodel, S. Chen, A.C. Payton, S.F. McDaniel, P. Soltis, D.E. Soltis, Adding loci improves
659 phylogeographic resolution in red mangroves despite increased missing data: comparing
660 microsatellites and RAD-Seq and investigating loci filtering, *Sci. Rep.* 7 (2017) 17598.

661 [46] Z.B.R. Quek, D. Huang, Application of phylogenomic tools to unravel anthozoan evolution,
662 *Coral Reefs* (2021) <https://doi.org/10.1007/s00338-021-02072-3>.

663 [47] M.L. Hale, T.M. Burg, T.E. Steeves, Sampling for microsatellite-based population genetic
664 studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies,
665 *PLoS ONE* 7 (2012) e45170.

666 [48] D.L. Jeffries, G.H. Copp, L.L. Handley, H.K. Olsén, C.D. Sayer, B. Hänfling, Comparing
667 RADseq and microsatellites to infer complex phylogeographic patterns, an empirical
668 perspective in the Crucian carp, *Carassius Carassius*, *Mol. Ecol.* 25 (2016) 2997–3018.

669 [49] A.G. Nazareno, J.B. Bemmels, C.W. Dick, L.G. Lohmann, Minimum sample sizes for
670 population genomics: An empirical study from an Amazonian plant species, *Mol. Ecol. Resour.*
671 17 (2017) 1136–1147.

672 [50] E.P. Flesch, J.J. Rotella, J.M. Thomson, T.A. Graves, R.A. Garrott, Evaluating sample size to
673 estimate genetic management metrics in the genomics era, *Mol. Ecol. Resour.* 18 (2018) 1077–
674 1091.

675 [51] W.M. Qu, N. Liang, Z.K. Wu, Y.G. Zhao, D. Chu, Minimum sample sizes for invasion
676 genomics: Empirical investigation in an invasive whitefly, *Ecol. Evol.* 10 (2020) 38–49.

677 [52] S. Porebski, L.G. Bailey, B. Baum, Modification of a CTAB DNA extraction protocol for
678 plants containing high polysaccharide and polyphenol components, *Plant. Mol. Biol. Rep.* 15
679 (1997) 8–15.

680 [53] S. Andrews, FastQC: a quality control tool for high throughput sequence data, 2010, Available
681 online at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.

682 [54] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: A flexible trimmer for Illumina Sequence
683 Data, *Bioinformatics* 30 (2014) 2114–2120.

684 [55] N. Rochette, A. Rivera-Colón, J. Catchen, Stacks 2: Analytical methods for paired-end
685 sequencing improve RADseq-based population genomics, *Mol. Ecol.* 28 (2019) 4737–4754.

686 [56] J. Paris, J. Stevens, J. Catchen, Lost in parameter space: a road map for Stacks, *Methods in*
687 *Ecol. Evol.* 188 (2017) 799–814.

688 [57] T. Jombart, Adegenet: a R package for the multivariate analysis of genetic markers,
689 *Bioinformatics* 24 (2008) 1403–1405.

690 [58] E. Paradis, Pegas: an R package for population genetics with an integrated-modular approach,
691 *Bioinformatics* 26 (2010) 419–420.

692 [59] S. Flanagan, A. Jones, Constraints on the F_{ST} -heterozygosity outlier approach, *J. Hered.* 108
693 (2017) 561–573.

694 [60] Z. Kamvar, J. Tabima, N. Grünwald, Poppr: an R package for genetic analysis of populations
695 with clonal, partially clonal, and/or sexual reproduction, *PeerJ* 2 (2014) p.e281.

696 [61] Z. Kamvar, J. Brooks, N. Grünwald, Novel R tools for analysis of genome-wide population
697 genetic data with emphasis on clonality, *Front. Genet.* 6 (2015) 208.

698 [62] J. Goudet, hierfstat, a package for r to compute and test hierarchical F-statistics, *Mol. Ecol.*
699 *Notes* 5 (2005) 184–186.

700 [63] T. Jenkins, C. Ellis, A. Triantafyllidis, J. Stevens, Single nucleotide polymorphisms reveal a
701 genetic cline across the north-east Atlantic and enable powerful population assignment in the
702 European lobster, *Evol. Appl.* 12 (2019) 1881–1899.

703 [64] J.K. Pritchard, M. Stephens, P. Donnelly, Inference of population structure using multilocus
704 genotype data, *Genetics* 155 (2000) 945–959.

705 [65] E. Frichot, O. François, LEA: an R package for landscape and ecological association studies,
706 *Methods Ecol. Evol.* 6 (2015) 925–929.

707 [66] G. Evanno, S. Regnaut, J. Goudet, Detecting the number of clusters of individuals using the
708 software structure: a simulation study, *Mol. Ecol.* 14 (2005) 2611–2620.

- [67] Y. Li, J. Liu, StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods, *Mol. Ecol. Resour.* 18 (2017) 176–177.
- [68] N.M. Kopelman, J. Mayzel, M. Jakobsson, N.A. Rosenberg, I. Mayrose, Clumpak: a program for identifying clustering modes and packaging population structure inferences across K, *Mol. Ecol. Resour.* 15 (2015) 1179–1191.
- [69] P. Dixon, VEGAN, a package of R functions for community ecology, *J. Veg. Sci.* 14 (2003) 927–930.
- [70] J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, H. Wagner, Vegan: Community Ecology Package (Version 2.5-6), 2020, [Software]. Retrieved from <https://CRAN.R-project.org/package=vegan>.
- [71] P. Martinez Arbizu, pairwiseAdonis: Pairwise multilevel comparison using adonis, R package version 0.3, 2019.
- [72] S. Bougeard, S. Dray, Supervised multiblock analysis in R with the ade4 Package, *J. Stat. Softw.* 86 (2018) doi: 10.18637/jss.v086.i01.
- [73] M. Nei, Genetic distance and molecular phylogeny, in: N. Ryman, F. Utter (Eds.), *Population Genetics and Fishery Management* University of Washington Press, Seattle, WA, (1987) 193–223.
- [74] Y. Benjamini, D. Yekutieli, The control of the false discovery rate in multiple testing under dependency, *Ann. Stat.* 29 (2001) 1165–1188.
- [75] C. Carreras, A. García-Cisneros, O. Wangenstein, V. Ordóñez, C. Palacín, M. Pascual, X. Turon, East is east and west is west: population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species *Paracentrotus lividus* (Echinoidea), *Divers. Distrib.* 26 (2019) 382–398.
- [76] P. Agapow, A. Burt, Indices of multilocus linkage disequilibrium, *Mol. Ecol. Notes* 1 (2001) 101–102.
- [77] S. Wright, Isolation by distance, *Genetics* 28 (1943) 114–138.
- [78] C.C. Cockerham, B.S. Weir, Estimation of gene flow from F-statistics, *Evolution* 7 (1993) 855–863.
- [79] B. Weir, *Genetic data analysis II*, Sunderland, Massachusetts, 1990, Sinauer Associates.
- [80] F.E. Werner, R.K. Cowen, C.B. Paris, Coupled biological and physical models, *Oceanography* 20 (2007) 54–69.
- [81] M. Pascual, B. Rives, C. Schunter, E. Macpherson, Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLoS ONE* 12 (2017) e0176419.
- [82] T. De Meeûs, F. Balloux, F-statistics of clonal diploids structured in numerous demes, *Mol. Ecol.* 14 (2005) 2695–702.
- [83] M. Hellberg, Footprints on water: the genetic wake of dispersal among reefs, *Coral Reefs* 26 (2007) 463–473.
- [84] M. Adjerdoud, A. Guérécheau, J. Vidal-Dupiol, J.F. Flot, S. Arnaud-Haond, F. Bonhomme, Genetic diversity, clonality and connectivity in the scleractinian coral *Pocillopora damicornis*: a multi-scale analysis in an insular, fragmented reef system, *Mar. Biol.* 161 (2014) 531–541.
- [85] A. García-Cisneros, C. Palacín, R. Ventura, B. Feital, P.C. Paiva, R. Pérez-Portela, Intraspecific genetic structure, divergence and high rates of clonality in an amphi-Atlantic starfish, *Mol. Ecol.* 27 (2017) 752–772.
- [86] F. Balloux, W. Amos, T. Coulson, Does heterozygosity estimate inbreeding in real populations? *Mol. Ecol.* 13 (2004) 3021–3031.
- [87] T.N. Marriage, M.E. Orive, Mutation-selection balance and mixed mating with asexual reproduction, *J. Theor. Biol.* 308 (2012) 25–35.
- [88] M.P. Dahl, R.T. Pereyra, T. Lundälv, C. André, Fine-scale spatial genetic structure and clonal distribution of the cold-water coral *Lophelia pertusa*, *Coral Reefs* 31 (2012) 1135–1148.

- 760 [89] M.J.H. van Oppen, G. Worheide, M. Takabayashi, Nuclear markers in evolutionary and
761 population genetic studies of scleractinian corals and sponges. *Proceedings 9th International*
762 *Coral Reef Symposium 1* (2000) 131–138.
- 763 [90] I.B. Baums, A restoration genetics guide for coral reef conservation, *Mol. Ecol.* 17 (2008)
764 2796–2811.
- 765 [91] J.N. Underwood, P.B. Souter, E.R. Ballment, A.H. Lutz, M.J.H. van Oppen, Development of 10
766 polymorphic microsatellite markers from herbicide bleached tissues of the brooding pocilloporid
767 coral *Seriatopora hystrix*, *Mol. Ecol. Notes* 6 (2006) 176–178.
- 768 [92] M.J.H. Van Oppen, J.N. Underwood, A.N. Muirhead, L. Peplow, Ten microsatellite loci for the
769 reef-building coral *Acropora millepora* (Cnidaria, Scleractinia) from the Great Barrier Reef,
770 Australia, *Mol. Ecol. Notes* 7 (2007) 436–438.
- 771 [93] E. Maier, R. Tollrian, B. Rinkevich, B. Nurnberger, Isolation by distance in the scleractinian
772 coral *Seriatopora hystrix* from the Red Sea, *Mar. Biol.* 147 (2005) 1109–1120.
- 773 [94] J.N. Underwood, L.D. Smith, M.J. Van Oppen, J.P. Gilmour, Multiple scales of genetic
774 connectivity in a brooding coral on isolated reefs following catastrophic bleaching, *Mol. Ecol.*
775 16 (2007) 771–784.
- 776 [95] D.J. Combosch, S.V. Vollmer, Mixed asexual and sexual reproduction in the Indo-Pacific reef
777 coral *Pocillopora damicornis*, *Ecol. Evol.* 3 (2013) 3379–3387.
- 778 [96] R.G. Waller, P.A. Tyler, The reproductive biology of two deep-water, reef-building
779 scleractinians from the NE Atlantic Ocean, *Coral Reefs* 3 (2005) 514–522.
- 780 [97] R.N. Hughes, J.M. Cancino, An ecological overview of cloning in Metazoa, in: J.B.C. Jackson,
781 L.W. Buss, R.E. Cook (Eds.), *Population biology and evolution of clonal organisms*, Yale
782 University Press, New Haven, 1985, pp. 153–186.
- 783 [98] R.N. Hughes, *A functional biology of clonal animals*, Chapman and Hall, London, 1989.
- 784 [99] A. Acosta, P.W. Sammarco, L.F. Duarte, Asexual reproduction in a zoanthid by fragmentation:
785 The role of exogenous factors, *Bull. Mar. Sci.* 68 (2001) 363–381.
- 786 [100] C. Drury, K.E. Dale, J.M. Panlilio, S.V. Miller, D. Lirman, E.A. Larson, E. Bartels, D.L.
787 Crawford, M.F. Oleksiak, Genomic variation among populations of threatened coral: *Acropora*
788 *cervicornis*, *BMC Genomics* 17 (2016) 286.
- 789 [101] V. Lukoschek, C. Riginos, M.J.H. van Oppen, Congruent patterns of connectivity can inform
790 management for broadcast spawning corals on the Great Barrier Reef, *Mol. Ecol.* 25 (2016)
791 3065–3080.
- 792 [102] X.M. Serrano, I.B. Baums, K. O'Reilly, T.B. Smith, R.J. Jones, T.L. Sheare, F.L.D. Nunes,
793 A.C. Baker, Geographic differences in vertical connectivity in the Caribbean coral *Montastraea*
794 *cavernosa* despite high levels of horizontal connectivity at shallow depths, *Mol. Ecol.* 23 (2014)
795 4226–4040.
- 796 [103] X.M. Serrano, I.B. Baums, T.B. Smith, R.J. Jones, T.L. Shearer, A.C. Baker, Long distance
797 dispersal and vertical gene flow in the Caribbean brooding coral *Porites astreoides*, *Sci. Rep.* 6
798 (2016) 21619.
- 799 [104] G. Suzuki, S. Keshavmurthy, T. Hayashibara, C.C. Wallace, Y. Shirayama, C.A. Chen, H.
800 Fukami, Genetic evidence of peripheral isolation and low diversity in marginal populations of
801 the *Acropora hyacinthus* complex, *Coral Reefs* 35 (2016) 1419–1432.
- 802 [105] M. Carella, G. Agell, M.J. Uriz, Asexual reproduction and heterozygote selection in an
803 Antarctic demosponge (*Stylocordyla chupachus*, Suberitida), *Polar. Biol.* 42 (2019) 475–483.
- 804 [106] J.S. Ryland, Reproduction in Zoanthidea (Anthozoa: Hexacorallia), *Invertebr. Reprod. Dev.*
805 31 (1997) 177–188.
- 806 [107] K. Soong, Y.S. Shiau, C.P. Chen, Morphological and reproductive variations of the zoanthid,
807 *Sphenopus marsupialis*, from two locations in Taiwan, in: 6th International Conference on
808 Coelenterate Biology, 1995, Abstracts, p. 91.
- 809 [108] J.S. Ryland, Budding in *Acrozoanthus* Saville-Kent, 1893 [Anthoma: Zoanthidea], in: J.C. den
810 Hartog (Ed.) *Proceedings of the 6th International Conference on Coelenterate Biology*, 1996.

- 811 [109] W.J. Cooke, Reproduction, growth, and some tolerances of *Zoanthus pacificus* and *Palythoa*
812 *vestitus* in Kaneohe Bay, Hawaii, in: G.O. Mackie (Ed.), Coelenterate Ecology and Behavior,
813 Third International Symposium on Coelenterate Biology, Plenum Press, New York and London,
814 1976 pp. 281–288
- 815 [110] R.H. Karlson, Disturbance, colonial fragmentation, and size-dependent life history variation
816 in two coral reef cnidarians, *Mar. Ecol. Prog. Ser.* 28 (1986) 245–249.
- 817 [111] H. Zibrowius, Comportement agressif du Zoanthaire *Gerardia savaglia* contre le Gorgonaire
818 *Paramuricea clavata* (Cnidaria: Anthozoa), *Commission Internationale pour l'exploration*
819 *Scientifique de la Mer Méditerranée* 29 (1995) 351–353.
- 820 [112] M. Canessa, Valutazione sperimentale dello stato di conservazione del Coralligeno dell'AMP
821 Tavolara Punta Coda Cavallo nell'ambito della Strategia Marina, Final Report, Conisma, Distav,
822 MPA Tavolara 2018 (In Italian).
- 823 [113] E. Trainito, Valutazione sperimentale dello stato di conservazione del coralligeno dell'AMP
824 Tavolara - Punta Coda Cavallo nell'ambito della strategia marina, CoNISMA, MPA Tavolara
825 Punta Coda Cavallo, Sardinia, Italy, 2018, pp. 45 (In Italian).
- 826 [114] A. Eusebio, R. Bordin, R. Jarre, G. Minciotti, Recenti esplorazioni speleosubacquee nel golfo
827 di Kotor (Montenegro), *Thalassia Salentina* 30 (2007) 25–37.
- 828 [115] E. Trainito, Hard bottoms habitats in Boka Kotorska, Montenegro. PAP/RAC Kraj Sv. Ivana
829 11 21000 Split, Croatia 2019, pp. 74.
- 830 [116] O. Ocaña, A. Brito, G. González, R. Herrera, Additions in relation to Gerardiidae from the
831 Macaronesian waters and the Mediterranean Sea (Anthozoa: Zoantharia), *Vieraea* 35 (2007)
832 163–168.
- 833 [117] F. Costantini, D. Aurelle, J.B. Ledoux, M. Abbiati, Population genetic structure of *Corallium*
834 *rubrum* in the Mediterranean Sea: Diversity, phylogeography, and bathymetric patterns. in: S.
835 Goffredo, Z. Dubinsky Z (Eds.), *The cnidaria, past, present and future*, Springer, Cham, 2016.
- 836 [118] F. Costantini, A. Gori, P. Lopez-González, L. Bramanti, S. Rossi, J.M. Gili, M. Abbiati,
837 Limited genetic connectivity between gorgonian morphotypes along a depth gradient, *PLoS*
838 *ONE* 11 (2016) e0160678.
- 839 [119] R.C. Babcock, J.S. Ryland, Larval development of a tropical zoanthid (*Protopalythoa* sp.),
840 *Invertebr. Reprod. Dev.* 17 (1990) 229–236.
- 841 [120] J.S. Ryland, S.D. Putron, R.S. Scheltema, P.J. Chimonides, D.G. Zhadan, Semper's (zoanthid)
842 larvae: pelagic life, parentage and other problems, *Hydrobiologia* 440 (2000) 191–198.
- 843
- 844 [121] O. Polak, Y. Loya, I. Brickner, E. Kramarski-Winter, Y. Benayahu, The widely-distributed
845 Indo-Pacific zoanthid *Palythoa tuberculosa*: A sexually conservative strategist, *Bull. Mar. Sci.*
846 87 (2011) 605–621.
- 847 [122] M. Soares, J.T. de Araújo, S.M.C. Ferreira, B.A. Santos, J.R.H. Boavida, F. Costantini, S.
848 Rossi, Why do mesophotic coral ecosystems have to be protected? *Sci. Total. Environ.* 726
849 (2020) 138456.
- 850 [123] R. Fernández, S. Lemer, E. McIntyre, G. Giribet, Comparative phylogeography and
851 population genetic structure of three widespread mollusc species in the Mediterranean and near
852 Atlantic, *Mar. Ecol.* 36 (2015) 701–715.
- 853 [124] A. Garcia-Cisneros, C. Palacín, Y.B. Khadra, R. Pérez-Portela, Low genetic diversity and
854 recent demographic expansion in the red starfish *Echinaster sepositus* (Retzius 1816), *Sci. Rep.*
855 6 (2016) 33269.
- 856 [125] N. Pinardi, E. Mosetti, Variability of the large-scale general circulation of the Mediterranean
857 Sea from observations and modelling: a review, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 158
858 (2000) 153–174.
- 859 [126] J.T. Allen, D.A. Smeed, J. Tintore, Mesoscale subduction at the Almeria—Oran front Part 1:
860 Ageostrophic flow, *J. Mar. Sys.* 30 (2001) 263–285.

- [127] J.R. Boavida, R. Becheler, M. Choquet, N. Frank, M. Taviani, J.F. Bourillet, A.L. Meistertzheim, A. Grehan, A. Savini, S. Arnaud-Haond, Out of the Mediterranean? Post-glacial colonisation pathways varied among cold-water coral species, *J. Biogeogr.* 46 (2019) 915–931.
- [128] M. Canessa, G. Bavestrello, E. Trainito, C.N. Bianchi, C. Morri, A. Navone, R. Cattaneo-Vietti, A large and erected sponge assemblage on granite outcrops in Marine Mediterranean Protected Area (NE Sardinia), *Reg. Stud. Mar. Sci.* 44 (2021) 101734.
- [129] L. Rossi, Primo rinvenimento di *Gerardia savaglia* (Bert) (Zoantharia) nei mari italiani (Golfo di Genova). *Doriana* 2, 1958, 8 pp.
- [130] M. Ponti, R.A. Perlini, V. Ventra, D. Grech, M. Abbiati, C. Cerrano, Ecological shifts in Mediterranean coralligenous assemblages related to gorgonian forest loss, *PLoS ONE* 9 (2014) e102782.
- [131] J. Verdura, C. Linares, E. Ballesteros, R. Coma, M.J. Uriz, N. Bensoussan, E. Cebrian, Biodiversity loss in a Mediterranean ecosystem due to an extreme warming event unveils the role of an engineering gorgonian species, *Sci. Rep.* 9 (2019) 5911.
- [132] P.G. Albano, M. Azzarone, B. Amati, C. Bogi, B. Sabelli, G. Rilov, Low diversity or poorly explored? Mesophotic molluscs highlight undersampling in the Eastern Mediterranean, *Biodivers. Conserv.* 29 (2020) 4059–4072.
- [133] F. Cardone, G. Corriero, C. Longo, M. Mercurio, S.O. Tarantini, M.F. Gravina, S. Lisco, M. Moretti, F. De Giosa, A. Giangrande, C. Nonnis Marzano, C. Pierri, Massive bioconstructions built by *Neopycnodonte cochlear* (Mollusca, Bivalvia) in a mesophotic environment in the central Mediterranean Sea, *Sci. Rep.* 10 (2020) 6337.
- [134] G. Chimienti, D. De Padova, M. Mossa, F. Mastrototaro, A mesophotic black coral forest in the Adriatic Sea, *Sci. Rep.* 10 (2020) 8504.
- [135] C. Cerrano, G. Bavestrello, C.N. Bianchi, R. Cattaneo-vietti, S. Bava, C. Morganti, C. Morri, P. Picco, G. Sara, S. Schiaparelli, A. Siccardi, F. Sponga, A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-Western Mediterranean), summer 1999, *Ecol. Lett.* 3 (2000) 284–293.
- [136] C. Wild, O. Hoegh-Guldberg, M.S. Naumann, M.F. Colombo-Pallotta, M. Ateweberhan, W.K. Fitt, R. Iglesias-Prieto, C. Palmer, J.C. Bythell, J.C. Ortiz, Y. Loya, R. Van Woesik, Climate change impedes scleractinian corals as primary reef ecosystem engineers, *Mar. Freshw. Res.* 62 (2011) 205–215.
- [137] M.S. Pratchett, A.S. Hoey, S.K. Wilson, Reef degradation and the loss of critical ecosystem goods and services provided by coral reef fishes, *Curr. Opin. Environ. Sustain.* 7 (2014) 37–43.

FIGURES

Fig. 1. Overview of *Savalia savaglia* colonies from eastern Atlantic and Mediterranean Sea. POR: Portugal; MAR: Marseille; SAR: Sardinia; PUG: Puglia; MON: Montenegro. Damaged and truncated colonies from Sardinia (SAR) are pointed by white arrows. Photo credits: PUG Cesare Petrelli.

Fig. 2. Admixture analysis. Three clusters were detected as the true number of Ks (ancestral populations) and are shown in blue (Cluster 1), yellow (Cluster 2) and black (Cluster 3). A) Pie charts represent the mean population admixture proportions at each of the five sampling localities.

905 The black arrows represent the main surface ocean currents of the Mediterranean Sea. B) Bar plots
 906 indicate individual admixture proportions, with individuals being clustered within their sampling
 907 locality. Sampling localities were ordered geographically (from West to East) within ‘Shallow’ and
 908 ‘Deep’ categories, respectively.

909
 910 **Fig. 3.** F_{ST} statistics. Goudet’s G statistics with 999 permutations was deployed to test the effect of
 911 A) ‘Depth’ and B) ‘Populations’ on overall genetic structuring. C) Heatmap of the Nei’s F_{ST}
 912 pairwise distances for each population pair. Respective p-values are shown in the upper triangle.
 913 Significant B-Y adjusted p-values (< 0.05) are marked in red. Population pairs that significantly
 914 differ have their Nei’s F_{ST} values in bold

915
 916 **Fig. 4.** DAPC and MLG analyses. A) Discriminant Analysis of Principal Components (DAPC) was
 917 performed by assigning individual samples to their sampling localities *a priori*. B) The posterior
 918 assignment probabilities of individuals to their predetermined localities are represented by stacked
 919 bar plots. C) Multi-locus genotype (MLG) analysis was performed using a 3% dissimilarity
 920 threshold. Individual samples that exhibited a genetic similarity $> 97\%$ based on Nei distances were
 921 considered to be clonal and were clustered into the same MLG. Specimens and MLGs are shown on
 922 the x and y axes, respectively.

923 TABLES

924
 925 **Table 1** Information on the *S. savaglia* specimens investigated. This includes details on the locality,
 926 date of sampling, geographic coordinates, depth, average number of reads per population, number
 927 of colonies sampled (N. col), number of genotyped individuals (Ind) and unique multi-locus
 928 genotypes (MLG). The observed and expected Heterozygosity values (H_O and H_E), and inbreeding
 929 coefficient values (F_{IS}) are also reported. The total number of individuals and multi-locus genotypes
 930 is given on the bottom of ‘Ind’ and ‘MLG’ columns.

931
 932 **Table 2** Permutational multivariate analysis of variance (PERMANOVA). The result of a F-test to
 933 compare within-group to between-group variance can be found in column ‘F model’. The ‘ R^2 ’ col-
 934 umn shows the variation from the distance matrix that is explained by the grouping being tested, al-
 935 so expressed in percentages (column: $R^2[\%]$). Non-adjusted P values were inferred from 999 permu-
 936 tations (column: P value) and have later undergone Holm FDR correction for multiple comparisons
 937 (column: P adjusted). Adjusted p values showing significance (< 0.05) after Holm FDR correction
 938 were marked in bold to show population pairs that significantly differ. Sampling codes are as fol-

low: shallow – 1) MAR: Marseille; 2) MON: Montenegro; 3) SAR: Sardinia, and deep – 4) POR: Portugal; 6) PUG: Puglia.

Appendix A. Supplementary data

Supplementary File 1

Additional information on the data analysis.

Supplementary Fig. 1. Outlier loci detection based on the F_{ST} - H_t distribution. Smoothed quantiles were computed from the existing dataset to identify loci with extreme F_{ST} values relative to their heterozygosity and are shown as red lines on the plot. Loci found outside the red line were considered to be under selection. F_{ST} values were computed using four commonly used F_{ST} calculation methods: A) Wright's F_{ST} , B) Cockerham and Weir's β based on the variance in allele frequencies, C) Cockerham and Weir's sample size corrected β (betahat), and D) Weir's θ (theta) F_{ST} estimator.

Supplementary Fig. 2. Assessment of linkage across loci. Agapow and Burt index of association (\bar{r}_d) was computed with 999 permutations for each of the sampling localities: A) PUG; B) POR; C) MAR; D) MON; E) SAR.

Supplementary Fig. 3. STRUCTURE analysis: Number of inferred genetic groups. A) Posterior probabilities of individual assignments to the most probable number of clusters ($K = 3$), computed using STRUCTURE. B) Detection of the most likely number of genetic groups (K) were determined following ad hoc statistic $\Delta(K)$ (Evanno method) and visualized using StructureSelector. The numbers on the x axis correspond to the following sampling localities: (1) – MAR; (2) – PUG; (3) – POR; (4) – SAR; (5) – MON.

Supplementary Fig. 4. Determination of the optimal number of PCs to retain for DAPC analysis. A) Alpha (α) optimization. B) Cross validation scores computed using 999 permutations.

Supplementary Fig. 5. PCoA plot. Depth A) and sampling locality B) were used as PCA grouping factors. Sampling localities are as follows: Shallow, MAR; MON; SAR and Deep, POR and PUG.

Supplementary Fig. 6. Hierarchical AMOVA with two factors: 1) 'Depth' and 2) 'Population'. AMOVA based on Euclidean distances was computed to test genetic structuring at all levels: A) within Populations and B) between Populations (nested within Depth) and C) between Depths.

Supplementary Fig. 7. Isolation by distance (IBD). Correlation between genetic and geographic distances.

Supplementary Table 1 Information on the *S. savaglia* specimens investigated including sampling locality and site, sampling code, specimen numbers and depth. N/A not available.

Supplementary Table 2 Read summary statistics. Number of sequences before and after quality filtering for each specimen are shown in columns. The number of raw reads (146-151 bp) and the number of filtered ddRAD tags (32 bp) are shown in the third and fifth column, respectively. Summarized information across all specimens can be found at the bottom of the table.

Supplementary Table 3 Outlier loci selection. The number of outlier loci detected in the *fsthet* R package is shown for each of the F_{ST} calculation methods: 1) Wright's F_{ST} , 2) Cockerham and

989 Weir's β (beta), 3) Cockerham and Weir's sample size corrected β (betahat), 4) Weir's θ (theta), as
990 well as for those outliers detected simultaneously in multiple calculation methods.

991

992 **Supplementary Table 4** AMOVA summary statistics. The 'AMOVA: Overall results' table shows
993 degrees of freedom (Df), sum of squares (Sum Sq) and mean of squares (Mean Sq) for each hierar-
994 chical level. The 'AMOVA: Components of covariance' table shows how much variance is detected
995 at each hierarchical level, with sigma (σ) representing the variance [Sigma (σ) column], also ex-
996 pressed in percentages [% column]. Most variance is expected to derive from the 'Within Popula-
997 tions' level for groups that are not genetically structured. The 'AMOVA: Phi statistics' table pro-
998 vides the ϕ population differentiation statistics, and a higher ϕ statistic is expected to represent a
999 higher amount of differentiation. The 'AMOVA: Testing significance with 999 Monte-Carlo tests'
1000 table shows p values inferred based on 999 permutations at each hierarchical level.

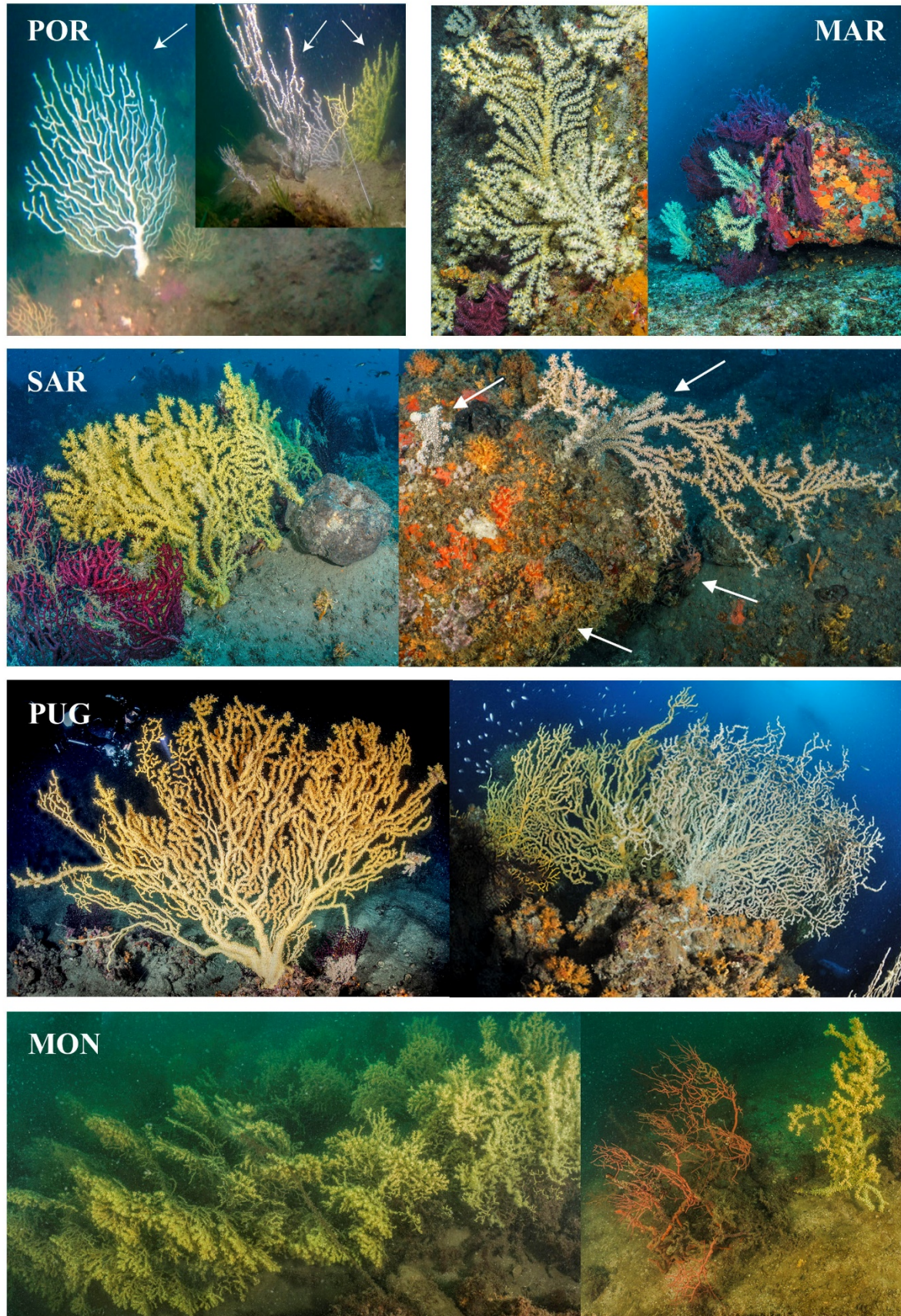
1001

1002 **Appendix B. Supplementary data**

1003

1004 **Supplementary File 2**

1005 Results obtained using the "all loci" dataset.



1006
 1007 **Fig. 1.** Overview of *Savalia savaglia* colonies from eastern Atlantic and Mediterranean Sea. POR: Portugal; MAR:
 1008 Marseille; SAR: Sardinia; PUG: Puglia; MON: Montenegro. Damaged and truncated colonies from Sardinia (SAR) are
 1009 pointed by white arrows. Photo credits: PUG Cesare Petrelli.

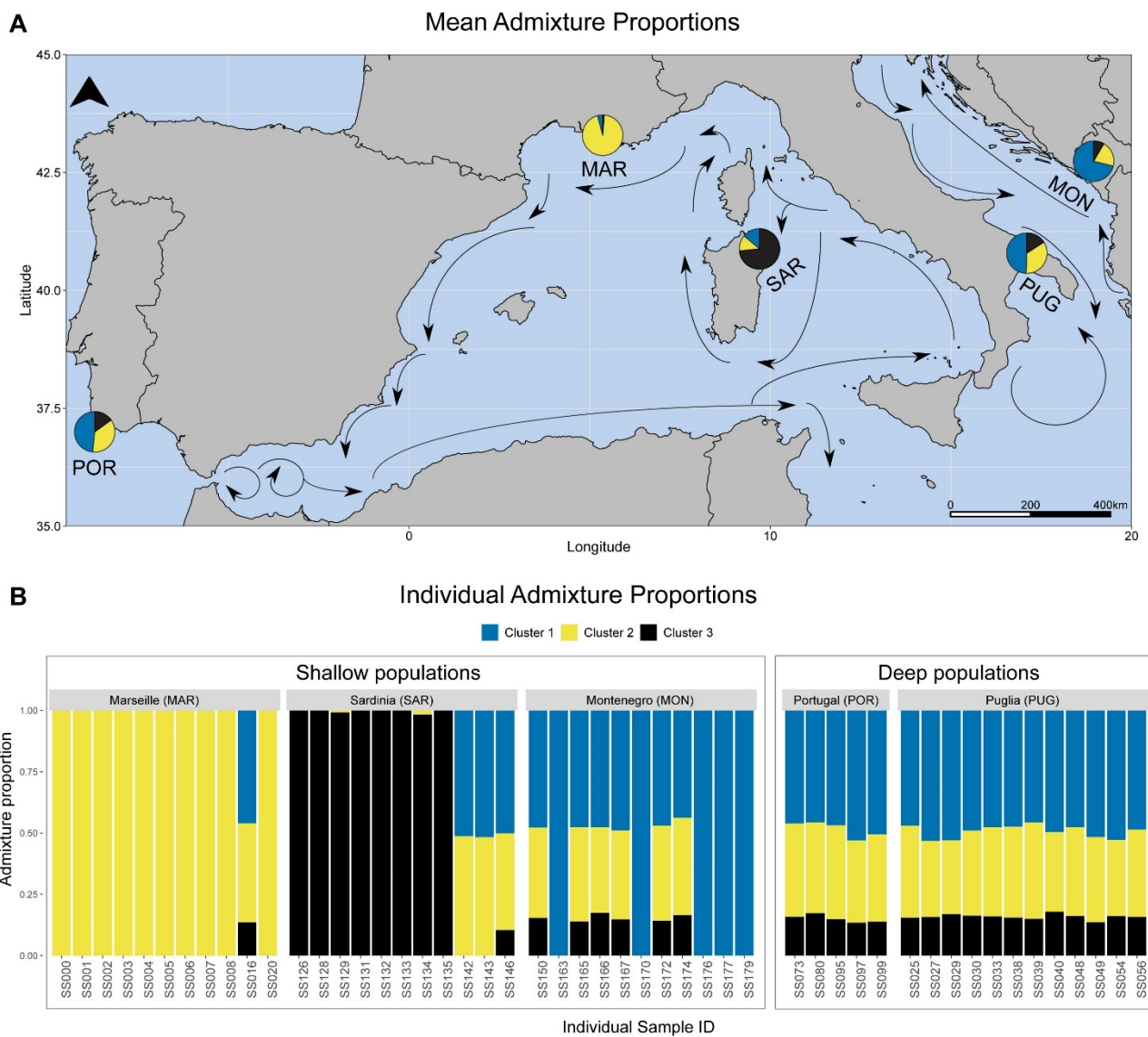
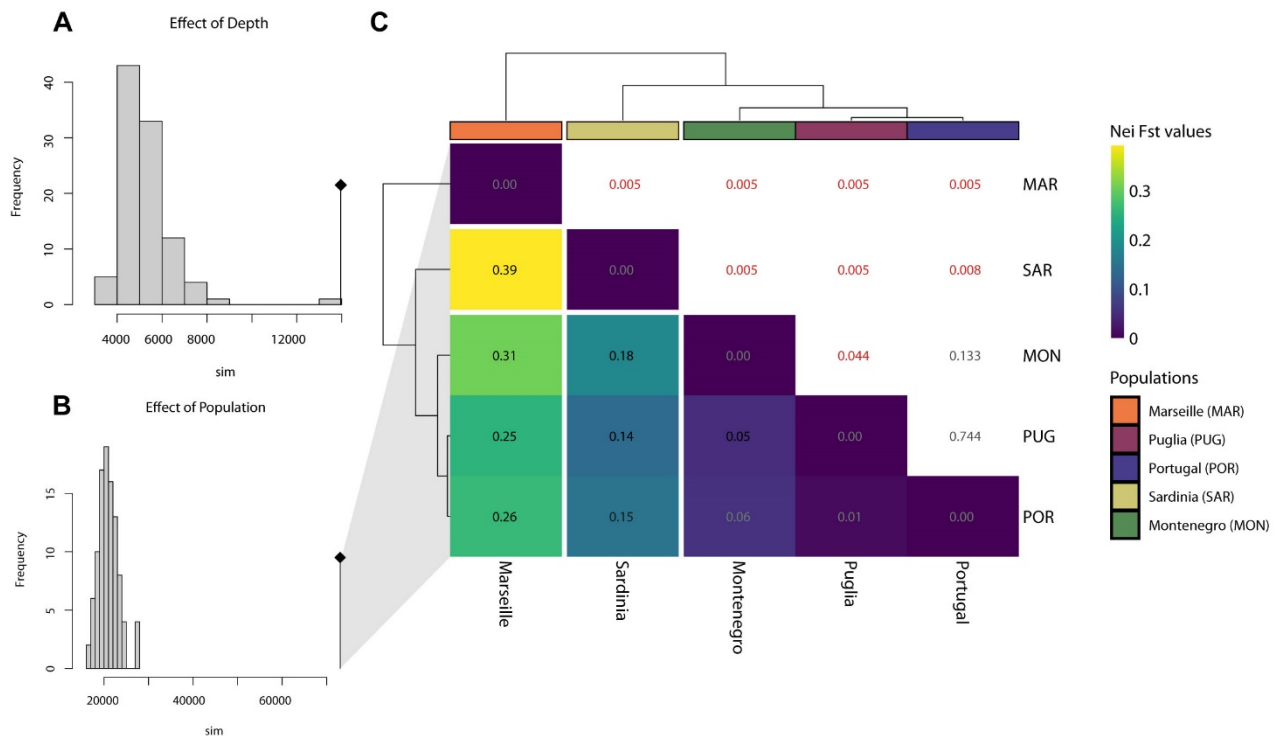


Fig. 2. Admixture analysis. Three clusters were detected as the true number of Ks (ancestral populations) and are shown in blue (Cluster 1), yellow (Cluster 2) and black (Cluster 3). A) Pie charts represent the mean population admixture proportions at each of the five sampling localities. The black arrows represent the main surface ocean currents of the Mediterranean Sea. B) Bar plots indicate individual admixture proportions, with individuals being clustered within their sampling locality. Sampling localities were ordered geographically (from West to East) within ‘Shallow’ and ‘Deep’ categories, respectively.



1031
 1032 **Fig. 3.** F_{ST} statistics. Goudet's G statistics with 999 permutations was deployed to test the effect of A) 'Depth' and B)
 1033 'Populations' on overall genetic structuring. C) Heatmap of the Nei's F_{ST} pairwise distances for each population pair.
 1034 Respective p-values are shown in the upper triangle. Significant B-Y adjusted p-values (< 0.05) are marked in red.
 1035 Population pairs that significantly differ have their Nei's F_{ST} values in bold.
 1036

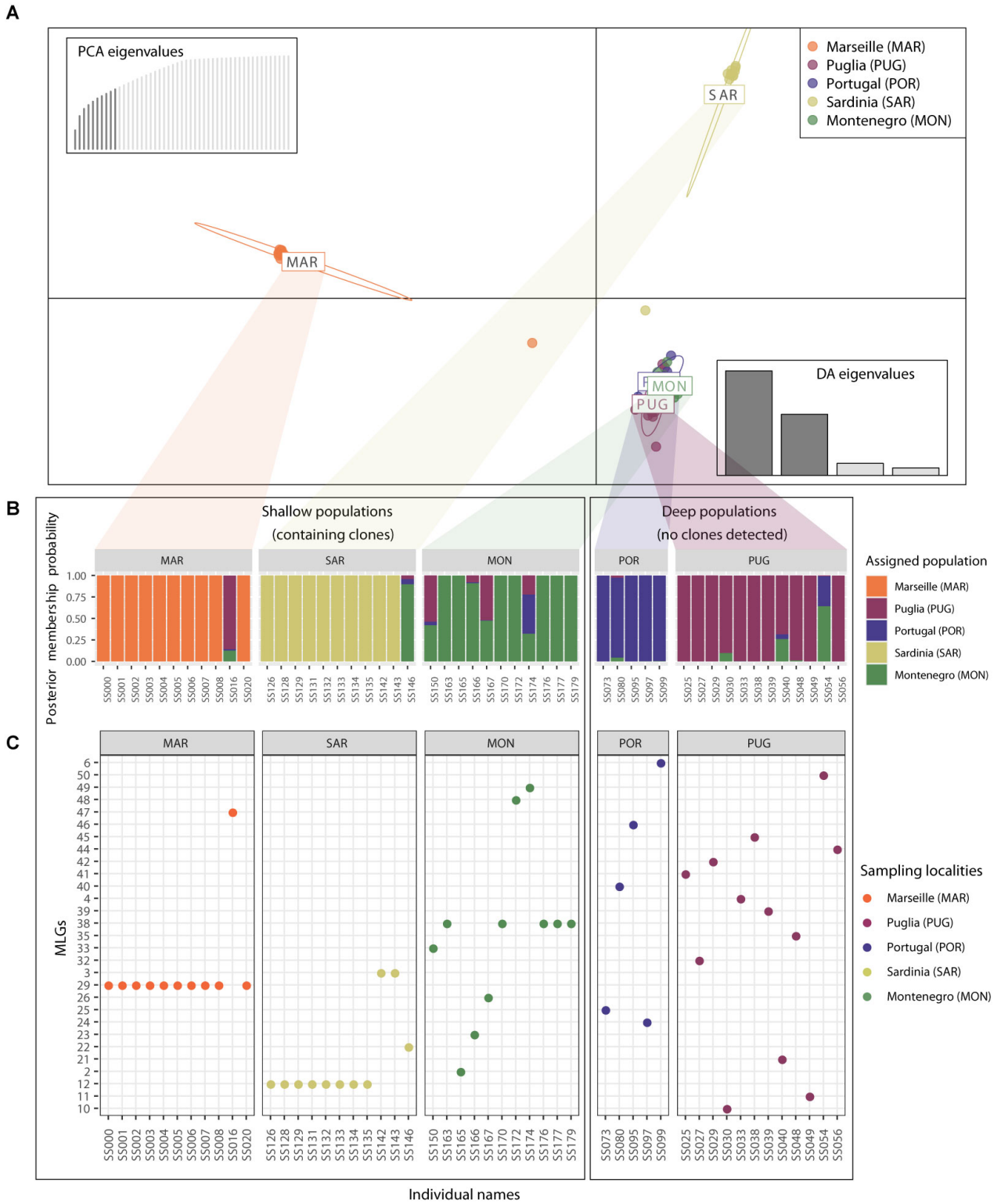


Fig. 4. DAPC and MLG analyses. A) Discriminant Analysis of Principal Components (DAPC) was performed by assigning individual samples to their sampling localities *a priori*. B) The posterior assignment probabilities of individuals to their predetermined localities are represented by stacked bar plots. C) Multi-locus genotype (MLG) analysis was performed using a 3% dissimilarity threshold. Individual samples that exhibited a genetic similarity > 97% based on Nei distances were considered to be clonal and were clustered into the same MLG. Specimens and MLGs are shown on the x and y axes, respectively.

1047 **Table 1** Information on the *S. savaglia* specimens investigated. This includes details on the locality, date of sampling,
1048 geographic coordinates, depth, average number of reads per population, number of colonies sampled (N. col), number of
1049 genotyped individuals (Ind) and unique multi-locus genotypes (MLG). The observed and expected Heterozygosity val-
1050 ues (H_o and H_e), and inbreeding coefficient values (F_{IS}) are also reported. The total number of individuals and multi-
1051 locus genotypes is given on the bottom of 'Ind' and 'MLG' columns.
1052

Sampling locality	Code	Date	Lat (N)	Long (E)	Depth (category)	Depth (m)	Reads (mean \pm SD)	N. col	Ind	MLG	H_o	H_e	F_{IS}
Marseille	MAR	2018	43.29	5.36	Shallow	8-40	7,116,491 \pm 494,201	11	11	2	0.12	0.09	-0.07
Sardinia	SAR	2018	40.90	9.71	Shallow	44-50	6,763,228 \pm 632,202	11	11	3	0.39	0.30	-0.22
Montenegro	MON	2018	42.74	18.95	Shallow	14-20	7,193,841 \pm 1,134,391	11	11	7	0.41	0.36	-0.10
Portugal	POR	2011-12	37.00	-8.70	Deep	60	7,045,775 \pm 475,165	5	5	5	0.39	0.39	-0.02
Puglia	PUG	2014-15	40.00	17.55	Deep	60	6,381,094 \pm 1,260,654	12	12	12	0.39	0.39	-0.01
									50	29			

1053
1054
1055 **Table 2** Permutational multivariate analysis of variance (PERMANOVA). The result of a F-test to compare within-
1056 group to between-group variance can be found in column 'F model'. The ' R^2 ' column shows the variation from the dis-
1057 tance matrix that is explained by the grouping being tested, also expressed in percentages (column: R^2 [%]). Non-
1058 adjusted P values were inferred from 999 permutations (column: P value) and have later undergone Holm FDR correc-
1059 tion for multiple comparisons (column: P adjusted). Adjusted p values showing significance (< 0.05) after Holm FDR
1060 correction were marked in bold to show population pairs that significantly differ. Sampling codes are as follow: shallow
1061 – 1) MAR: Marseille; 2) MON: Montenegro; 3) SAR: Sardinia, and deep – 4) POR: Portugal; 6) PUG: Puglia.
1062

Factor 1: Depth	pairs	F Model	R^2	P value	P adjusted
1	Shallow vs Deep	3.175	0.062	0.001	0.001
Factor 2: Populations	pairs	F Model	R^2	P value	P adjusted
1	MAR vs PUG	8.332	0.284	0.001	0.01
2	MAR vs POR	8.389	0.375	0.001	0.01
3	MAR vs SAR	20.202	0.503	0.001	0.01
4	MAR vs MON	11.797	0.371	0.001	0.01
5	PUG vs POR	1.192	0.074	0.076	0.08
6	PUG vs SAR	5.371	0.204	0.001	0.01
7	PUG vs MON	2.309	0.099	0.002	0.01
8	POR vs SAR	4.571	0.246	0.001	0.01
9	POR vs MON	1.918	0.120	0.040	0.08
10	SAR vs MON	7.321	0.268	0.001	0.01

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