

1 **Title**

2 **Species and population genomic differentiation in *Pocillopora* corals (Cnidaria,**  
3 **Hexacorallia)**

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53

54 **Abstract:**

55 Correctly delimiting species and populations is a prerequisite for studies of connectivity, adaptation  
56 and conservation. Genomic data are particularly useful to test for species differentiation for  
57 organisms with few informative morphological characters or low discrimination of cytoplasmic  
58 markers, as in Scleractinians. Here we applied Restriction site Associated DNA sequencing (RAD-  
59 sequencing) to the study of species differentiation and genetic structure in populations of  
60 *Pocillopora* spp. from Oman and French Polynesia, with the objectives to test species hypotheses,  
61 and to study the genetic structure among sampling sites within species. We focused here on coral  
62 colonies morphologically similar to *P. acuta* (*damicornis* type  $\beta$ ). We tested the impact of different  
63 filtering strategies on the stability of the results. The main genetic differentiation was observed  
64 between samples from Oman and French Polynesia. These samples corresponded to different  
65 previously defined primary species hypotheses (PSH), i.e. PSHs 12 and 13 in Oman, and PSH 5 in  
66 French Polynesia. In Oman, we did not observe any clear differentiation between the two putative  
67 species PSH 12 and 13, nor between sampling sites. In French Polynesia, where a single species  
68 hypothesis was studied, there was no differentiation between sites. Our analyses allowed the  
69 identification of clonal lineages in Oman and French Polynesia. The impact of clonality on genetic  
70 diversity is discussed in light of individual-based simulations.

71

72 **Keywords**

73 coral, species delineation, RAD sequencing, *Pocillopora*, genetic structure, clonal reproduction

74

75 **Compliance with Ethical Standards**

76 The authors declare that they have no conflict of interest.

## 77 **Introduction**

78 Anthozoans, i.e. hexacorals and octocorals, are ecologically key species in various marine  
79 ecosystems, from tropical shallow reefs to deep ecosystems. They are the subject of numerous  
80 studies on the impact of climate change, as heat waves can lead to bleaching or necrosis events in  
81 tropical and temperate species (Garrahou et al. 2009; Hughes et al. 2018). Anthozoans are also  
82 important models in evolutionary biology, from phylogenetic studies to better understand their long-  
83 term evolution (Kayal et al. 2018; Pratlong et al. 2017b), to population genetic studies dealing with  
84 dispersal, parentage analysis or sex determinism (Underwood et al. 2007; Ledoux et al. 2010;  
85 Mokhtar-Jamaï et al. 2013; Pratlong et al. 2017a; Sheets et al. 2018). Population genetic studies  
86 should rely on adequate species delineation, as evidenced in studies assessing the diversity of  
87 thermotolerance and making inferences on population connectivity (Pante et al. 2015b; Brenner-  
88 Raffali et al. 2019). Nevertheless, in Anthozoans species limits can be difficult to infer because of  
89 morphological plasticity, slow evolution of mitochondrial DNA, and hybridization (Calderón et al.  
90 2006; Marti-Puig et al. 2014; Aurelle et al. 2017; Gélín et al. 2017b).

91 Hexacorals of the *Pocillopora* genus (Lamarck, 1816), such as the morpho-species *P. damicornis*,  
92 *P. grandis*, and *P. acuta*, are common corals found in shallow waters of the Red Sea, Indian and  
93 Pacific Oceans. The taxonomy of the *Pocillopora* genus is complicated by the effects of an  
94 important morphological variability, plasticity, and hybridization (Veron 2013; Schmidt-Roach et al.  
95 2014). Considering these difficulties, several studies have used genetic data to refine species  
96 delineation in *Pocillopora*. Pinzón et al. (2013) used internal transcribed spacer 2 (ITS2), and the  
97 so-called mitochondrial ORF (which corresponds to the *tmp362* gene; Banguera-Hinestroza et al.  
98 2019). The different lineages identified with these markers were not correlated with the morphology  
99 of *Pocillopora* corals, and there was no or reduced gene flow among lineages found in sympatry.

100 Schmidt-Roach et al. (2014) made a taxonomic revision of the *P. damicornis* species complex on  
101 the basis of morphological characters, including micromorphology, and on mitochondrial ORF and  
102 nuclear HSP70B loci. These authors have shown a concordance between mitochondrial lineages  
103 and morphology, though the separation of some genetic lineages was in some cases blurred by  
104 morphological variability. Gélín et al. (2017b) put the study of species delineation within  
105 *Pocillopora* in a framework of testing species hypotheses. They proposed primary species  
106 hypotheses (PSH) based on mitochondrial DNA, and then tested secondary species hypotheses  
107 (SSH) with microsatellite data (see Figure 1 in Pante et al. 2015b, for the PSH and SSH process).  
108 Gélín et al. (2017b) have shown that PSH and SSH defined with molecular markers were not  
109 always congruent with species hypotheses based on morphology (i.e. morpho-species).

110 Genomic data can now be used to test species delineations on the basis of previous species  
111 hypotheses based on morphology or on a reduced number of markers. Restriction Sites Associated  
112 DNA sequencing (RAD-sequencing), allows the simultaneous discovery and genotyping of Single

113 Nucleotide Polymorphism (SNPs) in non-model organisms (Baird et al. 2008). RAD-sequencing  
114 has been used to test species delineations in octocorals (Pante et al. 2015a) and hexacorals (Forsman  
115 et al. 2017), including *Pocillopora*. The first RAD-sequencing study dealing with *Pocillopora*  
116 corals suggested the possibility of hybridization of *P. damicornis* (Linnaeus, 1758) with *P. grandis*  
117 Dana, 1846, and *P. elegans* Dana, 1846 (Combosch and Vollmer 2015). Based on RAD-Sequencing  
118 analyses of seven *Pocillopora* species (*P. acuta*, *P. damicornis*, *P. grandis*, *P. ligulata*, *P. meandrina*,  
119 *P. verrucosa*, *P. sp. B*), Johnston et al. (2017) found a good concordance with the phylogenetic  
120 relationships inferred from mitochondrial DNA. Their results suggested a possibility of  
121 hybridization between the closest sister species corresponding to mitochondrial haplotypes 4 (*P.*  
122 *damicornis*) and 5 (*P. acuta*). RAD-sequencing has also been used to assess levels of intraspecific  
123 variation in *P. damicornis*, demonstrating genetic by environment interactions, and therefore  
124 potential local adaptation, when comparing populations in the Great Barrier Reef (van Oppen et al.  
125 2018).

126 Additional genomic studies of *Pocillopora* spp. species and populations may provide insights into  
127 important evolutionary questions regarding the population dynamics and species delineation of  
128 corals. First, genomic data would allow testing more precisely the possibility of hybridization (i.e.  
129 the existence of individuals with mixed ancestry) or introgression (directional gene flow) among  
130 putative species, particularly in situation of sympatry. Second, on the basis of sound species  
131 delineations, it would be useful to study levels of genetic differentiation and connectivity among  
132 populations within species (Pante et al. 2015b). Third, RAD-sequencing could be used to study the  
133 potential impact of clonality on the genomic diversity of these corals. Indeed, as in many other  
134 scleractinian species, clonality has been demonstrated in *P. acuta* (Gélin et al., 2017a). It is expected  
135 that clonality will have a significant impact on the populations genetic structure (Balloux et al.  
136 2003; Adjeroud et al. 2014).

137 Here we used a hierarchical sampling design to study the genomic diversity of *Pocillopora* lineages  
138 at different scales. Specifically, we sampled *Pocillopora* spp. in two distant regions located at the  
139 margins of the distribution range of the genus: French Polynesia, Central Pacific Ocean, and Oman,  
140 Northwestern Indian Ocean, with multiple sampling sites within each region. This sampling scheme  
141 corresponded to a previous study on the diversity of thermotolerance in *Pocillopora* associated with  
142 different thermal regimes (see Brener-Raffali et al. 2022). Despite sampling morphologically  
143 similar colonies in French Polynesia and Oman, our samples included different mitochondrial  
144 haplotypes associated with distinct putative species. In order to minimize taxonomic assumptions,  
145 we will use throughout the manuscript the nomenclature of mitochondrial lineages from Pinzón et  
146 al. (2013). We indicate in Table 1 the correspondence between the nomenclature of mitochondrial  
147 lineages used in main text, the ORF haplotype number, the PSH and SSH defined based on Gélin et  
148 al. (2017b), and potential nominal species. Specifically, in this study we mainly compared

149 individuals of mitochondrial lineages 5 (corresponding to *P. acuta*; PSH 5) in French Polynesia, and  
150 3 and 7 (corresponding to *P. verrucosa*; PSHs 13 and 12 respectively) in Oman (see Results).  
151 Previous studies with microsatellite loci showed a distinction between mitochondrial lineages 5 and  
152 3/7; however, mitochondrial lineages 3 and 7 were partly separated as distinct SSHs (Gélin et al.  
153 2017b). Conversely, microsatellite data did not separate individuals with mitochondrial haplotypes  
154 3 and 7 in the Red Sea and Arabian Gulf (Pinzón et al. 2013). We therefore applied RAD-  
155 sequencing to these samples to test genetic differentiation among lineages identified with  
156 mitochondrial sequences, and among sampling sites. We further simulated data with different levels  
157 of clonal reproduction to help in the interpretation of results obtained with RAD-sequencing.

158

## 159 **Materials and methods**

### 160 ***Sampling and DNA extraction***

161 Five sites were sampled in Oman (hereafter identified as O1 to O5 sample sites, export CITES n°  
162 37/2014 / import CITES n° FR1406600081-I), and six sites at two islands in French Polynesia (with  
163 sites MH, MV and MT, at Moorea, and TF, TV and TT at Tahiti; export CITES n° FR1398700171-E  
164 / import CITES n° FR1306600053-I). The list and location of sampling sites are presented in Table  
165 2 and in Supplementary Figure S1. Thirty colonies were sampled in each site in Oman (except at  
166 O3, which included 13 sampled colonies), and ten colonies per site in French Polynesia. The  
167 sampling was focused on coral colonies morphologically similar to *P. acuta* (*damicornis* type  $\beta$ )-  
168 like *corallum* morphology. Both in Oman and French Polynesia, we also sampled additional  
169 colonies potentially belonging to other species, to be used as outgroups. The corresponding species  
170 hypotheses were checked through sequencing of part of the mitochondrial DNA (see below).

171 After sampling, all colony fragments were bleached with menthol according to previously described  
172 protocols (Wang et al. 2012; Vidal-Dupiol et al. 2020). Samples were then preserved in 95 %  
173 ethanol and stored at -20 °C until DNA extraction. Total genomic DNA was extracted according to  
174 the protocol described by Sambrook et al. (1989). After precipitation of DNA by isopropanol and  
175 ethanol, DNA was resuspended in 50  $\mu$ L water. This DNA solution was then purified with DNeasy  
176 blood and tissue spin columns (Qiagen, Hilden, Germany) according to the manufacturer's protocol,  
177 with elution in 100  $\mu$ L water, which were put on the column for a second centrifugation. Genomic  
178 DNA concentration was quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad,  
179 CA).

180

### 181 ***Mitochondrial ORF sequencing and microsatellite genotyping:***

182 Based on Gélin et al. (2017b), we used mitochondrial sequencing and microsatellite genotyping to  
183 assign the colonies to Primary Species Hypothesis (PSH) and to Secondary Species Hypothesis  
184 (SSH), respectively. The mitochondrial locus ORF was amplified with the FATP6.1 (5'-

185 TTTGGGSATTCGTTTAGCAG-3') and RORF (5'-SCCAATATGTTAAACASCATGTCA-3')  
186 primers (Flot and Tillier 2007) and submitted for Sanger sequencing in both directions. GenBank  
187 accession numbers for all mitochondrial haplotypes used for PSH definition (obtained here and  
188 from previous works) are indicated in G lin et al. (2017b). Nucleotide sequences were analyzed  
189 using MEGA version 6 (Tamura et al. 2013). We built a network of ORF sequences with the  
190 minimum spanning method using PopART v.1.7 (Bandelt et al. 1999; Leigh and Bryant 2015).  
191 Haplotypes were colored and sized according to localities, and numbers of occurrences,  
192 respectively. Characterization of haplotypes were based on comparisons with the sequences used in  
193 G lin et al. (2017b). Additionally, a subset of colonies (N = 165) were genotyped with 13  
194 microsatellite loci, and assigned to SSHs with Bayesian clustering as described in G lin et al.  
195 (2017b). Furthermore, as PSH05 (*P. acuta* or *P. damicornis* type  $\beta$ ) is known to propagate asexually  
196 (G lin et al. 2017a, 2018), microsatellite genotyping was used to search for repeated multilocus  
197 genotypes (MLGs) as a benchmark for the delineation of clonal lineages with RAD-sequencing (see  
198 G lin et al. 2017a for description of methods).

199

#### 200 ***RAD sequencing and analyses***

201 The preparation and sequencing of RAD-sequencing libraries was performed with the PstI  
202 restriction enzyme as described in Pratlong et al. (2021). We started with an initial number of 211  
203 individuals, distributed among seven libraries. Libraries were sequenced on an Illumina HiSeq2000  
204 using 100 bp single-end reads, at the Biology Institute of Lille (France, IBL, UMR 8199 CNRS)  
205 and at the MGX sequencing platform in Montpellier (France). The sequences were first  
206 demultiplexed, filtered by quality, and searched for adapters contamination using iPyrad v0.7.28  
207 with default parameters (Eaton and Overcast 2020; <https://ipyrad.readthedocs.io/>). We then checked  
208 the quality of the sequences and the absence of adapters with FastQC (Andrews 2010). The  
209 assembly of RAD loci was performed with Stacks 2.3 (Catchen et al. 2013). We used a published  
210 genome of *P. damicornis* (Cunning et al. 2018) as a reference to map the reads with BWA using  
211 default parameters (Li and Durbin 2009). The mean length of RAD loci was 95 bp. At that step, the  
212 numbers of reads and percentages of missing data were very uneven among the 211 samples (see  
213 Results). As a consequence, we chose to use different assembling strategies leading to different  
214 datasets. Following preliminary assembly analyses, we removed individuals with less than 900 000  
215 reads, as their inclusion led to datasets with very low numbers of SNPs. The resulting dataset  
216 comprised 140 individuals, including 104 from Oman and 36 from French Polynesia. Then we used  
217 the module populations of Stacks to assemble three datasets: 1) one considering all 140 individuals  
218 grouped by mitochondrial lineage ("All" dataset), 2) one with only samples from Oman and  
219 grouping by mitochondrial lineage, and 3) one with only samples from French Polynesia and

220 grouping by sampling sites (apart from outgroups, all individuals from French Polynesia shared the  
221 same mitochondrial haplotype; see Results). In the populations module we removed SNPs with  
222 allele frequencies lower than 0.01, and sites which were present in less than 50% of individuals in  
223 each group (i.e. mitochondrial lineage or sampling site depending on the dataset). At that stage, the  
224 individuals of the All and Oman datasets for which we did not get any mitochondrial sequence were  
225 put in an additional group for assembly (“unknown”). We then retained the first SNP of each RAD  
226 locus with a custom script. Second, we used Tassel 5.0 (Bradbury et al. 2007) to filter the  
227 corresponding VCF files according to missing data, with two consecutive filters on missing data in  
228 each dataset. In the first case, we first retained loci present in at least 75 % of the individuals. Then  
229 we retained individuals which had genotypes for at least 75 % of the loci. This first strategy resulted  
230 in less than 25 % of missing data both for loci and individuals (hereafter strategy 75-75). In the  
231 second case, we first retained loci present in at least 95 % of the individuals, and then individuals  
232 with data for at least 75 % of the loci (hereafter strategy 95-75). These filtering led to the removal  
233 of the less informative samples or loci. The 75-75 strategy allowed to retain more loci with less  
234 individuals compared to the 95-75 strategy (Table 3). The final sampling sizes per sampling site and  
235 mitochondrial lineage for the six datasets (with the two strategies applied to the whole dataset, and  
236 separately to Oman and French Polynesia datasets) are described in Supplementary Table S1.

237

### 238 ***Phylogenetic inference and genetic differences among individuals***

239 We used two complementary approaches to study the genetic differences among individuals with  
240 RAD-sequencing data. First we performed a phylogenetic reconstruction. For that purpose, among  
241 the 140 individuals retained for the processing of RAD-sequencing data, we chose up to 10  
242 representatives of each mitochondrial lineages (those with the highest number of raw reads), and  
243 three outgroups: we thus limited potential biases due to uneven representations of lineages in the  
244 phylogenetic inference. We used the population module of Stacks to build a Phylip file, including  
245 all sequences as well as variable sites, with only loci present in all individuals, and sites with allele  
246 frequencies above 0.01. We then used IQ-TREE 2.1.1 for phylogenetic inference, with the  
247 following options: ModelFinder Plus, 1 000 bootstraps with the ultrafast bootstrap approximation,  
248 and the hill-climbing nearest neighbor interchange search (Minh et al. 2013; Hoang et al. 2018).  
249 The resulting tree was formatted using FigTree 1.4.4 (Rambaut 2012). Second we built networks of  
250 mitochondrial haplotypes with the NeighborNet option of SPLITSTREE 4.14.8 (Huson and Bryant  
251 2006). The distance matrix used to build networks was the percentage of nucleotidic divergence  
252 among individuals computed with the poppr R package (Kamvar et al. 2014). This network  
253 reconstruction was used to analyze the previously defined 75-75 and 95-95 filtered datasets, first  
254 including all samples, and then separately for the Oman and French Polynesia samples.

255

## 256 **Population genetic analyses**

257 We first estimated genetic diversity on the dataset not corrected for the presence of repeated  
258 multilocus genotypes (MLGs; see below). We used the GENEPOP R package (Rousset 2008) to  
259 compute gene diversity within individuals ( $1-Q_{intra}$ ; corresponding to observed heterozygosity)  
260 and among individuals within sampling sites or lineages ( $1-Q_{inter}$ ; corresponding to expected  
261 heterozygosity), and  $F_{IS}$  (Weir and Cockerham 1984). We used VCFTOOLS 0.1.15 (Danecek et al.  
262 2011) to compute an estimate of inbreeding coefficient,  $F$ , which compares the observed number of  
263 homozygous sites to its expectation under panmixia.

264 We tested the presence of repeated multilocus genotypes (MLGs) and multilocus lineages.  
265 Multilocus lineages (MLLs) correspond to genotypes separated by a varying number of mutations  
266 and reflecting apparent divergence among MLGs either because of sequencing or genotyping errors,  
267 or because of somatic mutations. We used the R package poppr to analyze MLGs and MLLs  
268 (Kamvar et al. 2015). The choice of thresholds to delineate MLLs was made according to two  
269 criteria: first, we used MLGs obtained with microsatellite loci (data not shown) for a subset of  
270 individuals to define an MLL threshold. We also used the distribution of genetic distances among  
271 individuals to look for lowly differentiated individuals that could belong to the same MLL. The  
272 genetic distances among individuals were measured by the percentage of nucleotide divergence  
273 computed with poppr. According to their respective levels of diversity, the retained MLL threshold  
274 was different for the different datasets (see Results).

275 One representative of each MLL was kept for clustering and  $F_{ST}$  analyses. We analyzed the genetic  
276 disequilibrium among loci by computing the modified index of association  $\bar{r}_d$  (Agapow and Burt  
277 2001) with the poppr R package. To keep reasonable computing time, we first randomly  
278 subsampled the All\_75\_75 and Oman\_75\_75 to 25 000 SNPs. Then we computed  $\bar{r}_d$  on datasets  
279 comprising randomly subsampled 200 SNPs (this number allowed enough different resampling with  
280 the smallest dataset), and with 10 000 repetitions of this subsampling. With this approach we could  
281 analyze the linkage disequilibrium in datasets with a high number of SNPs. To take into account the  
282 impact of subpopulation structure (i.e. Wahlund effect) on this analysis of linkage disequilibrium,  
283 we performed the analysis at two levels (i.e. the "strata" levels used in poppr): first at the level of  
284 the whole corresponding dataset, and second at the level of mitochondrial lineages for the All and  
285 Oman datasets, or of sampling sites for the French Polynesia and Oman datasets.

286 Genetic differentiation among populations was measured with the  $F_{ST}$  estimator of Weir and  
287 Cockerham (1984) computed with VCFTOOLS. The differentiation among individuals was  
288 visualized based on a Principal Component Analysis (PCA) performed with the R package adegenet  
289 (Jombart 2008). Missing data were replaced by the mean allele frequency as in the adegenet tutorial



290 (<https://adegenet.r-forge.r-project.org/files/tutorial-basics.pdf>). As a complementary analysis to  
291 PCA, in order to identify the main genetic groups in the dataset, we analyzed the partition in K  
292 independent units with the snmf function of the R package LEA (Frichot and François 2015). This  
293 approach performs a least squares estimates of ancestry proportions (Frichot et al. 2014). We tested  
294 K values from 1 to 10, with ten replicates for each K value.

295

## 296 **Simulations**

297 To help the interpretation of our results on individual inbreeding coefficient  $F$ , on  $\bar{r}_d$  and on  $F_{ST}$ , we  
298 performed simulations to analyze the variability of these estimates, with a focus on the impact of  
299 partial clonality. We used SLiM 3 to build genetically explicit individual-based simulations (Haller  
300 and Messer 2019). We simulated two populations, each with 100 individuals, and connected  
301 through reciprocal gene flow at a rate of  $m = 0.01$  per generation. The genetic data were modeled  
302 with 2 000 loci of 100 bp each, mutating at a rate of  $\mu = 10^{-4}$  mutation per site per generation. This  
303 high mutation rate is a way to model enough genetic diversity with a moderate number of  
304 individuals and memory usage. After an initialization phase of 5 000 generations with panmixia  
305 within each population, we performed 50 000 generations with one of the following reproductive  
306 modes: panmixia (within population), clonality at a rate of  $c = 0.1, 0.5$  or  $0.9$ , selfing at a rate of  $s =$   
307  $0.1$ , and a combination of  $0.1$  selfing rate and clonality rates of  $0.1$  or  $0.5$ . At the end of the  
308 simulations, 30 simulated individuals were sampled per population, and 30 replicates were  
309 performed for each simulation configuration. The output VCF files were analyzed with  
310 VCFTOOLS to compute the estimate of individual inbreeding coefficients  $F$  and  $F_{ST}$ . For each  
311 simulation we computed the mean, minimum and maximum values of  $F$  and  $F_{ST}$  over individuals  
312 and loci, respectively. We computed  $\bar{r}_d$  separately on each of the two population of the simulations.  
313 For computing reasons, the mean and standard deviation of  $\bar{r}_d$  were computed with 50 re-samplings  
314 of 1 000 SNPs.

315

## 316 **Results**

### 317 ***Assignment to species hypotheses according to mitochondrial sequences and microsatellite*** 318 ***genotypes:***

319 Out of the 140 individuals retained in the final RAD-sequencing dataset, we did not get any usable  
320 mitochondrial sequence for 18 individuals (three from French Polynesia and 15 from Oman). The  
321 sequences obtained in this study allowed a clear assignment of individuals to previously defined  
322 sequence groups and corresponding primary species hypotheses. The network of mitochondrial  
323 sequences is presented in Figure S2. In French Polynesia, two individuals sampled as outgroups on  
324 the basis of morphology were highly divergent from individuals characterized by RAD-Seq, and

325 corresponded to mitochondrial lineages type 1a and type 2. The high divergence of these individuals  
326 from other samples blurred the analysis of the differentiation among the other lineages with RAD-  
327 sequencing data, especially on multivariate analyses (data not shown). Giving this signal and the  
328 small sample size for these outgroups, we did not retain them in the following analyses. Apart from  
329 these two individuals, French Polynesia included only samples from mitochondrial lineage 5a (PSH  
330 5). Oman included samples from mitochondrial lineages 7a (PSH12), 3e and 3g (both in SSH 13a),  
331 and only one individual from mitochondrial lineage 5a (Supplementary Table S1). The assignments  
332 to species hypotheses were consistent with those inferred from microsatellite loci (data not shown).  
333 With microsatellites, we did not detect any repeated multilocus genotype (MLG) in French  
334 Polynesia; i.e., each individual corresponded to a unique 13 loci genotype. Among individuals from  
335 mitochondrial lineage 7a in Oman, which included over 64 individuals for which we got a complete  
336 13 loci genotype, only 53 distinct MLGs were retrieved, with one MLG repeated five times, another  
337 one four times in O2, and three MLGs repeated two times (one in O2 and two in O5). Among  
338 individuals from mitochondrial lineage 3g, one MLG was repeated two times in O5.

339

#### 340 ***RAD sequencing data***

341 The initial number of sequences obtained per individual was very uneven among samples, varying  
342 from 5 735 to 30 394 029 reads (Supplementary Table S2; Figure S3A). The mean number of reads  
343 per individual was higher for samples from Oman (mean 5 647 233) compared to French Polynesia  
344 (mean 2 309 860). The percentage of reads aligned to the *Pocillopora* genome was more regular,  
345 with a mean of 85.1 %, but it was still higher in Oman (mean 85.6%) than in French Polynesia  
346 (84.1%; Table S2; Figure S3B). The lowest percentages of alignment were obtained for the  
347 outgroup samples. This heterogeneity among samples and sampling regions motivated our different  
348 assembly strategies. Table 3 presents the characteristics of the six final datasets, with a total of 140  
349 samples distributed among the different datasets. The highest numbers of SNPs, with one SNP per  
350 RAD locus, were obtained for the 75 % filtering on loci missing data in the All (194 370 SNPs) and  
351 Oman datasets (134 307 SNPs). The separate assembly of Oman and Polynesia allowed the  
352 recovery of more SNPs than the All assembly with the 95\_75 strategy, whereas these two separate  
353 assemblies led to less SNPs than the All assembly with the 75\_75 strategy. The All\_95\_75 dataset  
354 had the highest number of individuals (132) and the lowest number of SNPs (320; Table 3).

355

#### 356 ***Genetic differences among individuals and repeated MLLs***

357 The ML tree of relationships among individuals representative of the different mitochondrial  
358 lineages is presented in Figure S4. Apart from the two outgroups from French Polynesia, the main  
359 separation corresponded to the split between samples from French Polynesia, with mitochondrial  
360 lineage 5a, and samples from Oman, with mitochondrial lineages 3e, 3g and 7a. This divergence

361 corresponded to highly divergent monophyletic groups well supported by bootstraps analyses  
362 (100%). There was no clear phylogenetic grouping by mitochondrial lineage among the samples in  
363 Oman.

364 The networks based on the percentages of differences among individuals with RAD-sequencing are  
365 presented in Figure 1 for the 95\_75 datasets which had the highest number of individuals. The  
366 corresponding histograms of the distribution of genetic distances are presented in Supplementary  
367 Material (Figure S5). The All\_95\_75 network showed a clear distinction between Oman and French  
368 Polynesia individuals, which was associated with differences in mitochondrial lineages, separating  
369 type 5a versus all other lineages. The single colony of type 5a sampled in Oman (O4\_21) grouped  
370 with other colonies from Oman in this network. In the separate analysis of Oman and French  
371 Polynesia, there was no clear sub-grouping according to sampling location or mitochondrial  
372 lineages (Figure 1). Similar results were obtained for the networks based on the 75\_75 datasets,  
373 except in French Polynesia where we observed small clusters of individuals from the same site,  
374 albeit without clear differentiation from other groups (Figure S6).

375 The histograms of pairwise differences among individuals in Oman, and to a lesser extent in French  
376 Polynesia, showed a peak of low value distances, potentially reflecting repeated MLLs (Figure S5).  
377 We used the distance observed with RAD-sequencing among individuals sharing identical  
378 microsatellite MLGs as a threshold to define MLLs in the Oman dataset. In Oman, the highest  
379 distance among individuals with repeated microsatellite MLGs reached 11% which seemed too high  
380 to define MLLs. Therefore, in this case we retained the second highest distance among  
381 microsatellite-defined MLGs to characterize MLLs. It should be noted that this did not change the  
382 main results of this study, as only a few pairwise comparisons were then removed from potential  
383 MLLs. We could not use a single threshold for all datasets because the levels of divergence differed  
384 considerably between the datasets (95\_75 vs 75\_75, and French Polynesia vs Oman), and we had no  
385 repeated microsatellite MLG to be used as a reference in some datasets. Therefore, in cases where  
386 no threshold could be defined on the basis of microsatellites, we used a threshold allowing the  
387 removal of the closest individuals, as indicated by preliminary tests and by observation of the  
388 distribution of pairwise distances among individuals. The number of individuals for each corrected  
389 dataset is given in Table 3, and the corresponding thresholds are indicated below the distribution of  
390 individual distances (Figure S5).

391

### 392 ***Heterozygosity and inbreeding coefficients***

393 The parameters of genetic diversity for the different datasets are presented in Table 4. Separate  
394 estimates of genetic diversity per lineage and site are presented as Supplementary Materials (Table  
395 S3). The *1-Qintra* and the *1-Qinter* statistics indicated a higher genetic diversity in the 75\_75

396 datasets compared to the 95\_75 ones. For a given assembly strategy, the highest levels of diversity  
397 were observed in Oman, followed by the All and the French Polynesia datasets.  
398 The  $F_{IS}$  were mainly null or positive, apart from the All\_95\_75 ( $F_{IS} = -0.15$ ) and the  
399 Polynesia\_95\_75 ( $F_{IS} = -0.21$ ) datasets, but with important variations among lineages or sites,  
400 especially in French Polynesia (Table S3). The estimates of individual inbreeding coefficient  $F$  gave  
401 highly variable and extreme values (Table 4). The distributions of the  $F$  estimates illustrate this  
402 wide dispersion, and the shift to more positive values from 95\_75 to 75\_75 datasets (Figure S7). In  
403 the All\_95\_75 dataset, the  $F$  values in Oman (from -0.278 to 0.546) were higher than in French  
404 Polynesia (from -1.306 to -0.303). There was no general signal towards higher or lower  $F$  values for  
405 individuals involved in potential MLLs (i.e. individuals involved in the closest pairwise  
406 relationships in the different datasets), apart for the French Polynesia datasets, where these  
407 individuals showed among the highest  $F$  values (Figure S7).

408

### 409 ***Linkage disequilibrium***

410 The results of the analysis of linkage disequilibrium with the  $\bar{r}_d$  index are presented as  
411 Supplementary Materials (Table S4 and Figure S8). For the analyses at the level of the whole  
412 datasets, the highest  $\bar{r}_d$  values were obtained in the All\_75\_75 dataset, followed by the French  
413 Polynesia\_95\_75 and All\_95\_75 datasets. The values obtained in French Polynesia were higher  
414 than those in Oman for the 95\_75 and the 75\_75 datasets. When the analysis was performed at the  
415 level of mitochondrial lineages or sites, the highest values were observed for the MH site in French  
416 Polynesia both for the 95\_75 and the 75\_75 datasets (Table S4, Figure S8).

417

### 418 ***F<sub>ST</sub> estimates***

419 The mean  $F_{ST}$  estimates among loci were generally lower for the datasets corrected for MLLs  
420 compared to the non-corrected datasets (Table 5), except for the comparison among mitochondrial  
421 lineages with All\_95\_75, and the comparison among populations with All\_75\_75. The mean  $F_{ST}$   
422 between French Polynesia and Oman (by grouping samples from each region) was 0.105 and 0.352  
423 for the All\_95\_75 and the All\_75\_75 non-corrected datasets, respectively. The distributions of  $F_{ST}$   
424 among loci for the Oman / French Polynesia comparison are presented in Figure S9. For this  
425 comparison, the All\_75\_75 non-corrected dataset showed an important proportion of loci with  $F_{ST}$   
426 above 0.2, and a peak at  $F_{ST} = 1$ , whereas the distribution was mainly restricted to values below 0.2  
427 for the All\_95\_75 non-corrected dataset. The  $F_{ST}$  estimates for all datasets indicated very low levels  
428 of differentiation within Oman and within Polynesia (Table 5).

429

### 430 ***Analysis of genetic structure***

431 Multivariate PCA of the All\_95\_75 and All\_75\_75 datasets separated the samples from French  
432 Polynesia and Oman, with individuals from French Polynesia (type 5a lineage) being more spread  
433 apart than those from Oman (Figure S10). The separate PCAs on the Oman and French Polynesia  
434 datasets did not reveal any clear structure according to sampling site nor mitochondrial lineage,  
435 whatever the filtering strategy. The plots of cross-entropy for all snmf analyses are presented in  
436 Figure S11. There was no clear signal for an informative K value on the basis of these cross-entropy  
437 plots, apart for the All\_95\_75 dataset where a first minimum value was observed at K = 2 and a  
438 second at K = 5, and for Polynesia\_95\_75 where a slight minimum was observed at K = 2. We then  
439 also analyzed the results with a K value corresponding to the number of mitochondrial lineages (for  
440 All and Oman) or the number of sampling sites (for French Polynesia and Oman). The  
441 corresponding barplots of coancestry coefficients for the 95\_75 datasets are given in Figure 2. For  
442 All\_95\_75, the K = 2 solution clearly separated French Polynesia (mitochondrial lineage type 5a)  
443 and Oman samples (other mitochondrial lineages, with one 5a exception). At K = 4, two additional  
444 sub-clusters were observed, one in Oman and one in French Polynesia. For the Oman\_95\_75  
445 dataset, the K = 4 solution (corresponding to the number of mitochondrial lineages) led to a major  
446 and three minor clusters. These clusters did not separate individuals neither by mitochondrial  
447 lineage nor by sampling site. This clustering was nevertheless partly linked with potential MLLs, as  
448 the purple cluster of Oman\_95\_75 grouped individuals of the MLG02 identified with microsatellites  
449 (Figures 1 and 2). The K = 5 solution for Oman (corresponding to the number of sites), did not  
450 evidence any informative clustering either, again with the separation of minor clusters mainly  
451 composed of individuals closely grouped in the network analysis (results not shown). For  
452 Polynesia\_95\_75 the K = 2 and the K = 5 solutions did not separate individuals neither by sampling  
453 site type nor by MLL. The snmf analysis of genetic structure with the 75\_75 datasets gave similar  
454 results, with a separation of Oman and French Polynesia at K = 2 for All\_75\_75, and a further  
455 distinction of a few individuals in two additional clusters in Oman at K = 4 (results not shown).

456

## 457 **Simulations**

458 The results of the analyses of individual-based simulations are detailed in the Supplementary  
459 Materials section, with a comparison with empirical data. The main results of these analyses  
460 revealed that the maximum  $F$  values tended to be higher for the configurations with the highest  
461 clonality rates (from 0.5), but were much higher for the simulations including selfing. Regarding the  
462 minimum  $F$  value, a decrease in the distribution was observed for the highest levels of clonality,  
463 with more negative values compared to other configurations. An increase in the index of linkage  
464 disequilibrium  $\bar{r}_d$  was observed with increasing clonality rate, mainly for the highest clonality rate  
465 (0.9). Regarding the average  $F_{ST}$ , without any variation neither in census size nor migration rate, the

466 resulting values were mostly similar among simulation configurations. A slight decrease and higher  
467 variance was nevertheless observed for the highest clonality rate (0.9) simulated.

468  
469

## 470 **Discussion**

### 471 ***Genomic analysis of species hypotheses***

472 When considering the All datasets, we observed a marked differentiation between French Polynesia  
473 and Oman populations, which was superimposed on a differentiation between mitochondrial lineage  
474 5a and other lineages. The mitochondrial lineage 5a corresponds to *P. acuta*, and is phylogenetically  
475 well separated from lineages 3e-3g and 7a, which correspond to *P. verrucosa* (Gélin et al. 2017b).  
476 The distinction of the corresponding species hypotheses based on mitochondrial lineages was  
477 previously confirmed with microsatellite loci (Gélin et al. 2017b), and here with RAD-sequencing.  
478 Nevertheless, our sampling scheme did not allow to test the possibility of hybridization of lineage  
479 5a with other lineages in sympatry. There was only one individual bearing mitochondrial type 5a in  
480 Oman for which we were able to get RAD-sequencing data: this individual did not separate from  
481 other individuals in Oman with different mitochondrial types. This last observation could indicate a  
482 possible introgression of mitochondrial type 5a into the 3e-3g-7a gene pool, but this should be  
483 tested by considering additional 5a individuals from Oman. We obtained six additional individuals  
484 from this lineage, hence indicating that the single 5a haplotype reported here is not likely a result of  
485 contamination; however, the RAD sequencing of these samples was not good enough to retain them  
486 and confirm a possible introgression.

487 Conversely, we did not observe a differentiation between individuals from Oman assigned to the  
488 species hypotheses corresponding to mitochondrial lineages 7 and 3e-3g (i.e., PSH12 and SSH13a  
489 in Gélin et al. 2017b, respectively). All methods of species delineation based on mitochondrial DNA  
490 used in Gélin et al. (2017b) indeed separated these two species hypotheses. Nevertheless,  
491 microsatellite data did not support this distinction (Gélin et al. 2017b; Pinzón et al. 2013). One can  
492 note that in the case of Pinzón et al. (2013) this lack of differentiation was observed in the Red Sea  
493 and in the Arabian Gulf. With a sampling in sympatry and based on our RAD-Sequencing, we also  
494 reject the distinction of PSH12 and SSH13a. The most parsimonious hypothesis here would be that  
495 these different lineages correspond to mitochondrial polymorphism present within a given species,  
496 here *P. verrucosa*, even when mitochondrial DNA in anthozoans has been shown to evolve slowly  
497 (van Oppen et al. 1999; Calderón et al. 2006; Hellberg 2006). Accordingly, one can note that 12  
498 over 16 species delineation methods based on mitochondrial ORF did not conclude to separate  
499 PSHs for the lineages 3e and 3g (Gélin et al. 2017b). Another possibility could be genetic swamping  
500 (e.g. Bog et al. 2017; Kosiński et al. 2019) following a secondary contact between different lineages  
501 in Oman. Reticulate evolution has already been proposed as a major factor shaping the current

502 diversity of scleractinian corals (van Oppen et al. 2001; Vollmer and Palumbi 2002). A more precise  
503 analysis of the genomic patterns of differentiation would be useful to study the existence, timing  
504 and direction of introgression in *Pocillopora* spp. (e.g. Nelson et al. 2020). Our sampling was also  
505 not random, with a focus on morphologically similar colonies. We excluded a few very divergent  
506 colonies and therefore we did not explore the whole diversity of *Pocillopora* in the studied areas. A  
507 more extended sampling in sympatry, random in regard of morphology, would be essential to get a  
508 better understanding of the levels of genomic differentiation and interactions among the different  
509 *Pocillopora* species.

510 In French Polynesia, neither of the datasets showed evidence of genetic structuring among sites,  
511 which were distributed in the two islands of Moorea and Tahiti. These results are consistent with  
512 previous studies on the genetic structure of *Pocillopora* (Magalon et al. 2004; Adjeroud et al. 2014).  
513 One can note that in *Pocillopora* corals the patterns of genetic structure are evidently dependent on  
514 the species hypothesis and locations considered (Oury et al. 2020b). For example, G elin et al.  
515 (2018) observed a high genetic differentiation for *P. acuta* (PSH05 in G elin et al. 2017b) at  
516 different spatial scales in the Western Indian and Tropical Southwestern Pacific Oceans. Similarly,  
517 Combosch and Vollmer (2011) have shown a significant genetic structure in *P. damicornis* at a  
518 spatial scale of a few tens of kilometers, but this was not a general result for all population  
519 comparisons. Conversely, Oury et al. (2021), Robitzch et al. (2015) and Thomas et al. (2014),  
520 observed low genetic structure along high distances in different *Pocillopora* lineages (with some  
521 exceptions reported by Oury et al., 2021).

522

### 523 ***Impact of filtering strategies on RAD-sequencing data***

524 Our RAD-sequencing analyses provided a dataset with very uneven levels of missing data. High  
525 levels of missing data, if not accounted for, can lead to incorrect conclusions regarding genetic  
526 structure (Larson et al. 2021). Missing data in RAD-sequencing can have multiple origins, including  
527 mutations in enzyme-cutting sites, technical problems associated with library preparation, uneven  
528 amplification or sequencing, or errors in the *in silico* identification of homologous sites (Eaton et al.  
529 2017; O'Leary et al. 2018). In our study, the lowest read numbers were obtained for outgroup  
530 samples, corresponding to mitochondrial lineages 1a and 2, as opposed to mitochondrial lineages  
531 5a, 7, 3e and 3g, which were the most frequent in our datasets. For example, seven of these  
532 outgroup samples had only around 20 000 reads or less and thus were not retained in our analyses.  
533 We did not observe any relationship between the number of raw reads and the number of reads  
534 mapped to a Symbiodiniaceae genome (data not shown); therefore, a potential contamination from  
535 dinoflagellate genomes cannot explain these results. Despite standard verifications, problems with  
536 DNA quantity and quality may have impacted the number of reads obtained, such as for example  
537 the presence of partially degraded DNA or PCR inhibitors (O'Leary et al. 2018).

538 Facing these difficulties, we compared different strategies for filtering missing data, which can have  
539 important consequences on the obtained results. Our study showed that some results were stable  
540 among the different filtering strategies. For example, there was a marked differentiation between  
541 samples from Oman and French Polynesia, associated with the species sampled in each region, and  
542 a lack of genetic structure among sites within each region. Conversely, the estimates of genetic  
543 diversity and structure differed among datasets. The genetic distances among individuals were much  
544 higher with more loci in both the All and Oman datasets.

545 If part of missing data are linked to mutations in the cutting sites, filtering loci according to their  
546 rates of missing data is expected to reduce the frequency of loci with high mutation rates (Huang  
547 and Knowles 2016). This agrees well with our observation of a lower diversity detected under more  
548 stringent filtering. Missing data can also lead to allele dropout, which corresponds to the non-  
549 observation of a SNP linked to a mutated restriction site. For the retained variable loci, an  
550 overestimation of heterozygosity can be expected with allele dropout, particularly if the dropout  
551 concerns more ancestral allelic states and therefore leads to an increase in minimum allele  
552 frequencies (Gautier et al. 2013). Consistent with this expectation, our analysis showed an increase  
553 in heterozygosity for the 75\_75 compared to the 95\_75 datasets. Finally, allele dropout can  
554 overestimate  $F_{ST}$  (Gautier et al. 2013), which seems consistent with our results, with higher  $F_{ST}$  for  
555 the 75\_75 compared to the 97\_75 All datasets. Regarding linkage disequilibrium, the increase in  $\bar{r}_d$   
556 observed for All\_75\_75 compared to All\_95\_75 can result from a combination of higher Wahlund  
557 effect (with the inclusion of more differentiated loci), and of a higher number of physically linked  
558 loci. An important question is whether the variance of polymorphism among loci is high enough to  
559 explain the observed differences through the aforementioned effects.

560

### 561 **Signals of clonality with RAD-sequencing data**

562 Populations of *Pocillopora* corals, notably in *P. acuta* (mitochondrial lineage 5) and *P. damicornis*  
563 (mitochondrial lineage 4), can show different levels of clonal reproduction (Pinzón et al. 2012;  
564 Torda et al. 2013; Adjeroud et al. 2014), with sometimes different ramets of the same genet  
565 separated by several kilometers (Gélin et al. 2017a, 2018). Clonal reproduction in these species can  
566 happen through fragmentation of individuals, polyp bail-out or asexual production of larvae  
567 (Highsmith 1982; Gélin et al. 2017a; Oury et al. 2019). Clonal reproduction can lead to  
568 heterozygote excess compared to panmixia (Balloux et al. 2003; Reichel et al. 2016), and to shifts  
569 in the distribution of  $F_{IS}$  among loci towards negative values for the highest rates of clonality  
570 (Stoeckel and Masson 2014; Reynes et al. 2021). Results from this study show the effect of  
571 clonality in the studied populations. First, samples corresponding to MLGs detected with  
572 microsatellites were indeed grouped with reduced distance in networks based on RAD-sequencing.  
573 Second, the distribution of pairwise differences showed a peak of low divergence, which can be an



574 indication of repeated MLLs. Third, the distribution of estimates of the inbreeding coefficient  $F$   
575 showed some individuals with negative values, corresponding to highly heterozygous individuals,  
576 especially in French Polynesia. Our results point to the first observation of clonality (either with  
577 microsatellites or RAD-sequencing loci) in populations with mitochondrial lineages 7a and 3g  
578 (corresponding to *P. verrucosa*). This observation of clonality could be explained by the fact that  
579 these individuals were sampled in shallow water, with a *P. acuta*-like corallum macromorphology  
580 (i.e. thin branches, highly breakable). In such environment, waves and swell might favor  
581 fragmentation. Testing this hypothesis would require studying this lineage in different environments  
582 with contrasted levels of energy. In all cases, estimating the rate of clonality in these populations  
583 would require a dedicated sampling.

584 We have also observed individuals with very low  $F$  values, indicating high rates of heterozygous  
585 loci. This was observed in our simulations only with the highest clonal rates which are not  
586 compatible with the low frequency of repeated MLLs observed here. Other effects could explain  
587 these observations, such as the presence of brooded larvae, or the intra-colonial genetic diversity  
588 linked to chimerism or mosaicism (Oury et al. 2020a). Hybrids between divergent lineages could  
589 also create such high heterozygosity, but it could not be detected here if the parental lineages were  
590 not analyzed.

591 From a methodological point of view, our simulations provide new avenues in the study of clonality  
592 with RAD-sequencing data. Our simulations showed a discernible effect of clonality on the  
593 distribution of  $F$  and  $\bar{r}_d$  for the highest clonality level tested here (0.9). This is in line with previous  
594 studies demonstrating an effect of clonality only for extreme rates of clonality (Balloux et al. 2003).  
595 Our simulations may be limited in the exploration of the impact of clonality for several reasons.  
596 First, we did not explore the impact of sampling scheme on the estimates of genetic diversity and  
597 genetic structure. Second, *Pocillopora* corals show overlapping generations, and a given clone may  
598 persist over several generations, a scenario that was not specifically implemented in our simulation  
599 framework. Third, selective effects can lead to the expansion of one clone (see G elin et al. 2017a,  
600 and references therein) and thus modify the distribution of clones. These simulations were also not  
601 used here to estimate clonal rates, which would require dedicated approaches, such as Approximate  
602 Bayesian Computation (Csill ery et al. 2010).

603 Regarding genetic structure, our simulations showed a slight decrease of  $F_{ST}$  only for a clonal rate of  
604 0.9. This agrees well with theoretical expectations (Balloux et al. 2003). The correction of datasets  
605 for repetitions of clonal lineages changed the estimated  $F_{ST}$  values but this did not change our main  
606 conclusions. Our results showed an important differentiation for the All datasets, and low to no  
607 genetic structure in Oman and French Polynesia. In some cases such correction for clonal diversity  
608 can lead to very different conclusions, from genetic differentiation without correction to near  
609 panmixia in *Pocillopora* in French Polynesia (Adjeroud et al. 2014). Therefore, one should not rely

610 on a single analysis strategy (e.g., using all individuals or single genotypes), but consider different  
611 sampling and filtering strategies (De Meeûs et al. 2006).  
612  
613

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641

642 **Data availability:**

643 Raw sequences are available in Genbank under BioProject ID PRJNA689941 and SRA accession  
644 number SRA PRJNA689941.

645 The mitochondrial ORF sequences, microsatellite genotypes and SliM scripts are available in  
646 Zenodo: <https://zenodo.org/record/4748346>. The scripts used for SLiM simulations are also  
647 available at: <https://gitlab.osupytheas.fr/aurelle/slim-simulations>.

648 The scripts used for the analyses of RAD-sequencing data are available at:

649 [https://gitlab.osupytheas.fr/aurelle/rad\\_pocillopora.git](https://gitlab.osupytheas.fr/aurelle/rad_pocillopora.git)

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652 **Table and figure captions :**

653 **Table 1:** Correspondence between the nomenclature of mitochondrial lineages used in the  
654 manuscript (indicated as ORF haplotypes numbers), and primary and secondary species hypotheses  
655 (PSH and SSH respectively) in G lin et al. (2017), the types of Pinz n et al. (2013) and Schmidt-  
656 Roach et al. (2012, 2013, 2014), and the potential nominal species associated to these types. Note  
657 that the correspondence between mitochondrial lineages and SSH indicated here for our samples is  
658 based on the complementary analysis of microsatellite genotypes: it may therefore be different for  
659 other samples. See table 1 of Johnston et al., 2017 as well.

660 **Table 2** Characteristics of sampling sites, including region, site, GPS location, and sampling depth  
661 and date. The thermal regime gives a qualitative indication of temperature variability at the  
662 corresponding sampling site. See Supplementary Table S1 for the final sampling sizes used  
663 depending on datasets and mitochondrial lineages,

664 **Table 3** Numbers of SNPs and individuals for the different datasets. The locus threshold  
665 corresponds to the minimum proportion of available data among individuals to retain a locus. The  
666 individual threshold corresponds to the minimum proportion of available data among loci to retain  
667 an individual. The mean depth indicates the mean depth per individual averaged over all individuals  
668 in the dataset. The last column indicates the number of retained individuals after correction for the  
669 presence of Multiple Multilocus Lineages (MLLs; see text for details). *All* corresponds to dataset  
670 including all samples (French Polynesia and Oman).

671 **Table 4** Estimates of gene diversity within (*1-Qintra*) and among individuals (*1-Qinter*), and of  $F_{IS}$   
672 averaged over samples. For the All datasets, the average was estimated over Oman and French  
673 Polynesia samples. For the Oman and French Polynesia datasets, average was done over  
674 corresponding sampling locations. The last three columns provide indicators of the distribution of  
675 inbreeding coefficient (mean, minimum, and maximum) computed over all individuals for each  
676 dataset.

677 **Table 5** Mean  $F_{ST}$  estimates across loci for the datasets without and with corrections for repeated  
678 MLLs. The "pop" comparison estimates  $F_{ST}$  between sampling sites; for the All dataset, it

679 corresponds to the French Polynesia / Oman differentiation. For the "ORF" comparison, this  
680 estimate compares samples of individuals grouped according to their ORF haplotypes (individuals  
681 without ORF sequence were not taken into account).

682 **Fig. 1** Network based on the percentage of differences among individuals for the All, Oman and  
683 French Polynesia 95\_75 datasets. The colors indicate the corresponding species hypothesis  
684 according to the sequence of mitochondrial ORF. The red ellipses indicate groupings of individuals  
685 corresponding to the same MLG according to microsatellite data.

686 **Fig. 2** Plots of coancestry coefficients inferred with the LEA R package for the 95\_75 datasets. For  
687 the All and French Polynesia datasets, the cross-entropy gave a first minimum value at  $K = 2$ . Plots  
688 of coancestry coefficients considering the number of mitochondrial lineages in the dataset gave a  
689 minimum value of  $K = 4$  for All and Oman, and  $K = 5$  for the number of populations in French  
690 Polynesia. Each bar corresponds to one individual and the colors in the bars correspond to the  
691 different ancestry groups inferred by this method. The colors of the dots under barplots indicate the  
692 mitochondrial lineage for a subset of individuals.

693 **Table 1:** Correspondence between the nomenclature of mitochondrial lineages used in the  
694 manuscript (indicated as ORF haplotypes numbers), and primary and secondary species hypotheses  
695 (PSH and SSH respectively) in Gélín et al. (2017), the types of Pinzón et al. (2013) and Schmidt-  
696 Roach et al. (2012, 2013, 2014), and the potential nominal species associated to these types. Note  
697 that the correspondence between mitochondrial lineages and SSH indicated here for our samples is  
698 based on the complementary analysis of microsatellite genotypes: it may therefore be different for  
699 other samples. See table 1 of Johnston et al., 2017 as well.

700

ORF	PSH Gélín et al. (2017b)	SSH Gélín et al. (2017b)	Type Pinzón et al. (2013)	Type Schmidt-Roach et al. (2012, 2013, 2014)	Nominal species
27	9	9c	1a	e/m	<i>P. grandis</i> / <i>meandrina</i>
1	1	1	2	-	<i>P. sp. B</i>
18	5	to be defined	5a	β	<i>P. acuta</i>
34	12	12	7a	-	
36	13	13a	3e	-	<i>P. verrucosa</i>
43	13	13a	3g	-	<i>P. verrucosa</i>

701 **Table 2** Characteristics of sampling sites, including region, site, GPS location, and sampling depth  
702 and date. The thermal regime gives a qualitative indication of temperature variability at the  
703 corresponding sampling site. See Supplementary Table S1 for the final sampling sizes used  
704 depending on datasets and mitochondrial lineages,

705

Region	Site	GPS	Code	Sampling date	Depth (m)	Thermal regime
French Polynesia	Moorea Haapiti	17°32'39.27 S 149°53'37.40 W	MH	03/2014	0.5-2	Low variations
French Polynesia	Moorea Tiahura	17°29'17.41 S 149°53'45.58 W	MT	03/2014	0.5-2	Low variations
French Polynesia	Moorea Vaiare	17°31'24.10 S 149°46'33.85 W	MV	03/2014	0.5-2	Low variations
French Polynesia	Tahiti Faratea	17°43'17.61 S 149°18'11.78 W	TF	03/2014	0.5-2	Low variations
French Polynesia	Tahiti Vairao	17°48'20.90 S 149°17'43.13 W	TV	03/2014	0.5-2	Low variations
French Polynesia	Tahiti Tautira	17°45'12.11 S 149° 9'26.68 W	TT	03/2014	0.5-2	Low variations*
Oman	Bandar Al Khayral 1	23°30'54.25 N 58°45'15.70 E	O1	06/2014	2-8	High variations
Oman	Bandar Al Khayral 21	23°31'26.66 N 58°44'2.18 E	O2	06/2014	2-8	High variations
Oman	Bandar Al Khayral 3	23°31'8.90 N 58°45'29.40 E	O3	06/2014	> 12	High variations but less than O1, O2 and O4
Oman	Muscat	23°37'28.61 N 58°36'1.39 E	O4	06/2014	2-8	High variations
Oman	Daymaniat	23°51'25.12 N 58° 6'3.43 E	O5	06/2014	2-8	High variations but less than O1, O2 and O4

706 \* see Brener-Raffalli et al. (2022) for further details on thermal regime in Oman.

707 **Table 3** Numbers of SNPs and individuals for the different datasets. The locus threshold  
708 corresponds to the minimum proportion of available data among individuals to retain a locus. The  
709 individual threshold corresponds to the minimum proportion of available data among loci to retain  
710 an individual. The mean depth indicates the mean depth per individual averaged over all individuals  
711 in the dataset. The last column indicates the number of retained individuals after correction for the  
712 presence of Multiple Multilocus Lineages (MLLs; see text for details). All corresponds to dataset  
713 including all samples (French Polynesia and Oman).

714

Dataset	Populations	Locus threshold	Individual threshold	Mean depth	SNPs	Individuals before MLL correction	Individuals after MLL correction
All_95_75	All	0.95	0.75	389	320	132	100
All_75_75	All	0.75	0.75	66.9	194370	98	78
Oman_95_75	Oman	0.95	0.75	155	1711	99	82
Oman_75_75	Oman	0.75	0.75	72.7	134307	77	62
Polynesia_95_75	Polynesia	0.95	0.75	433.3	558	31	29
Polynesia_75_75	Polynesia	0.75	0.75	204.2	3285	25	18



715 **Table 4** Estimates of gene diversity within ( $1-Q_{intra}$ ) and among individuals ( $1-Q_{inter}$ ), and of  $F_{IS}$   
716 averaged over samples. For the All datasets, the average was estimated over Oman and French  
717 Polynesia samples. For the Oman and French Polynesia datasets, average was done over  
718 corresponding sampling locations. The last three columns provide indicators of the distribution of  
719 inbreeding coefficient (mean, minimum, and maximum) computed over all individuals for each  
720 dataset.

721

Dataset	$1-Q_{intra}$	$1-Q_{inter}$	$F_{IS}$	Mean $F$	Min $F$	Max $F$
All_95_75	0.07	0.06	-0.15	-0.024	-1.306	0.546
All_75_75	0.12	0.15	0.21	0.396	0.043	0.732
Oman_95_75	0.16	0.16	0.00	-0.009	-0.311	0.602
Oman_75_75	0.20	0.24	0.14	0.138	-0.062	0.539
Polynesia_95_75	0.04	0.04	-0.21	0.095	-2.733	0.539
Polynesia_75_75	0.05	0.05	0.02	0.292	-0.838	0.489

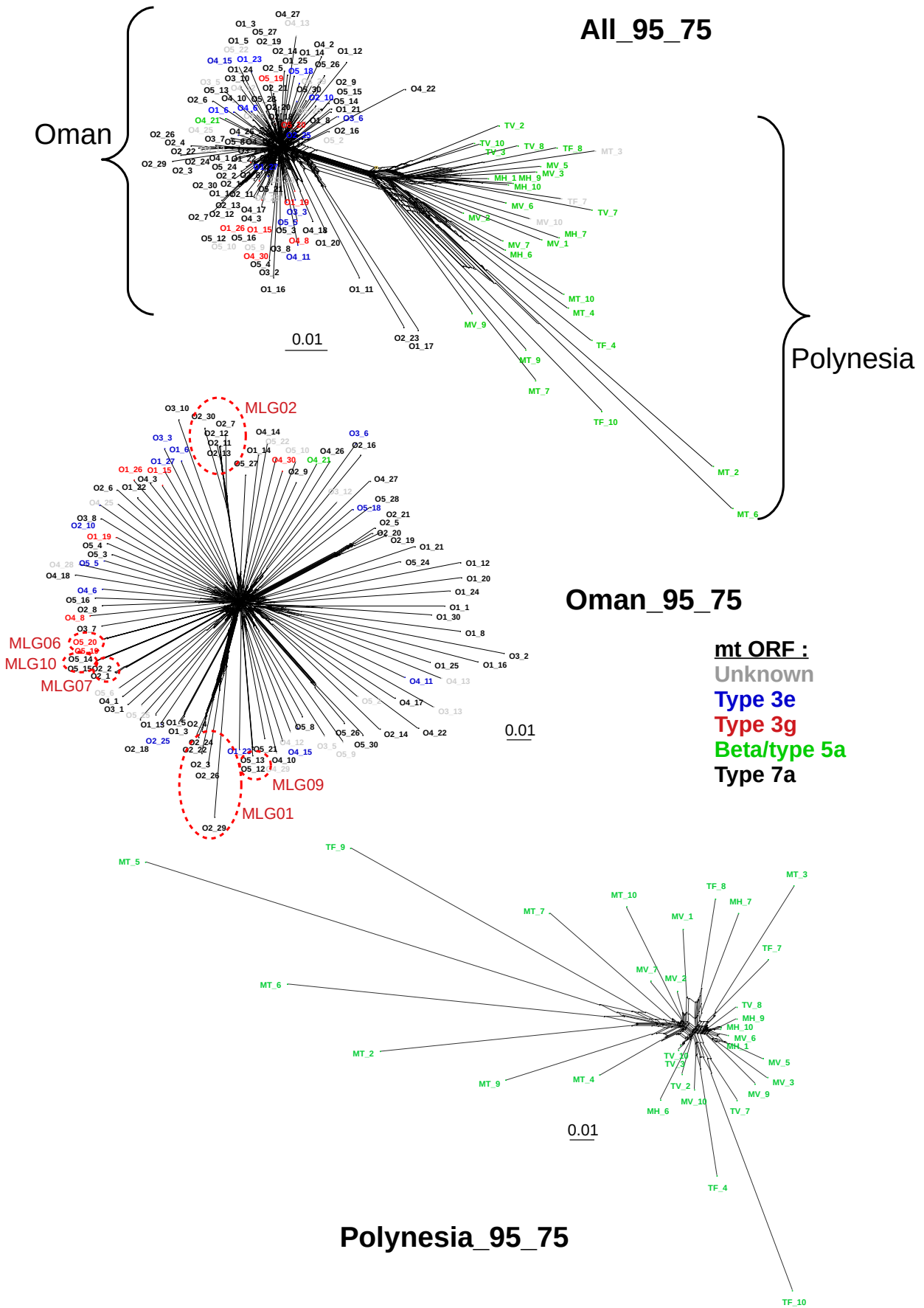
722

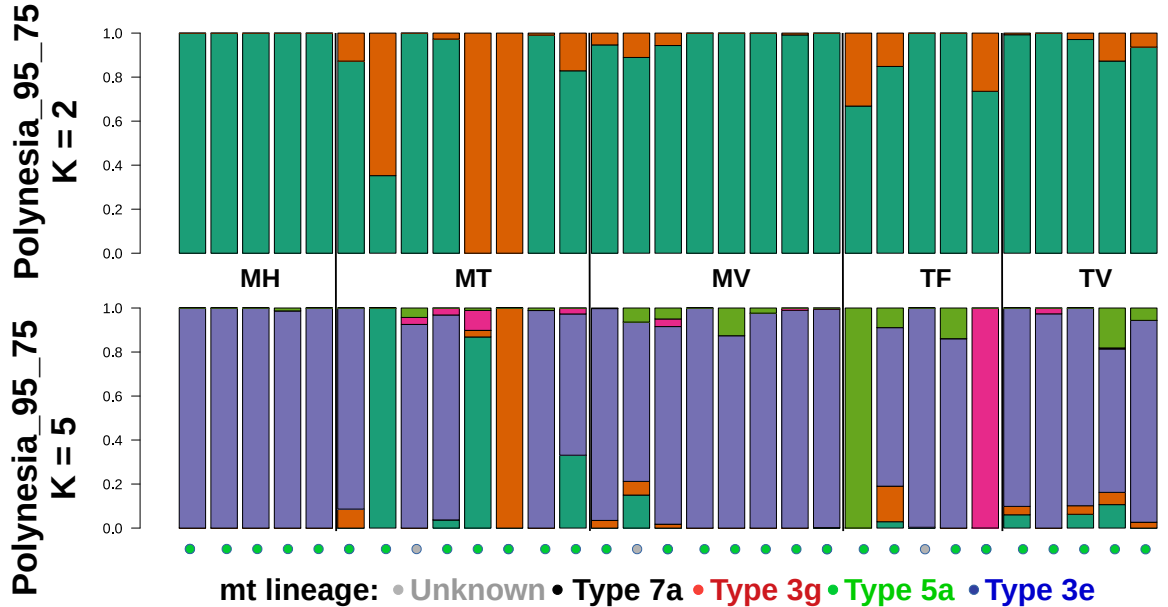
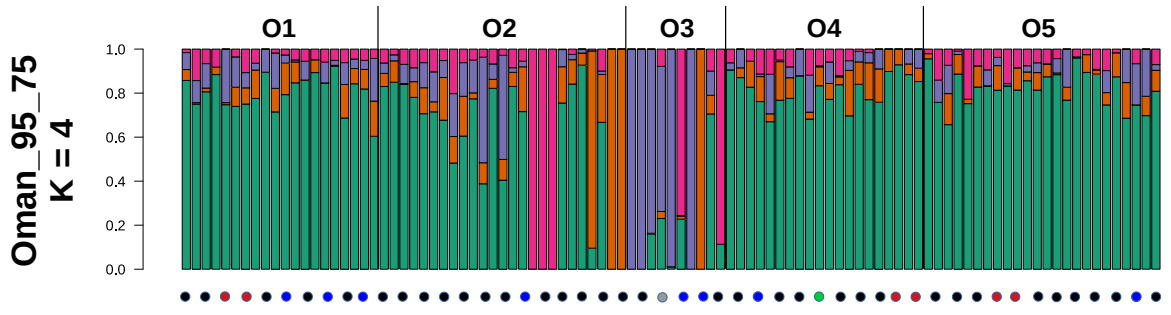
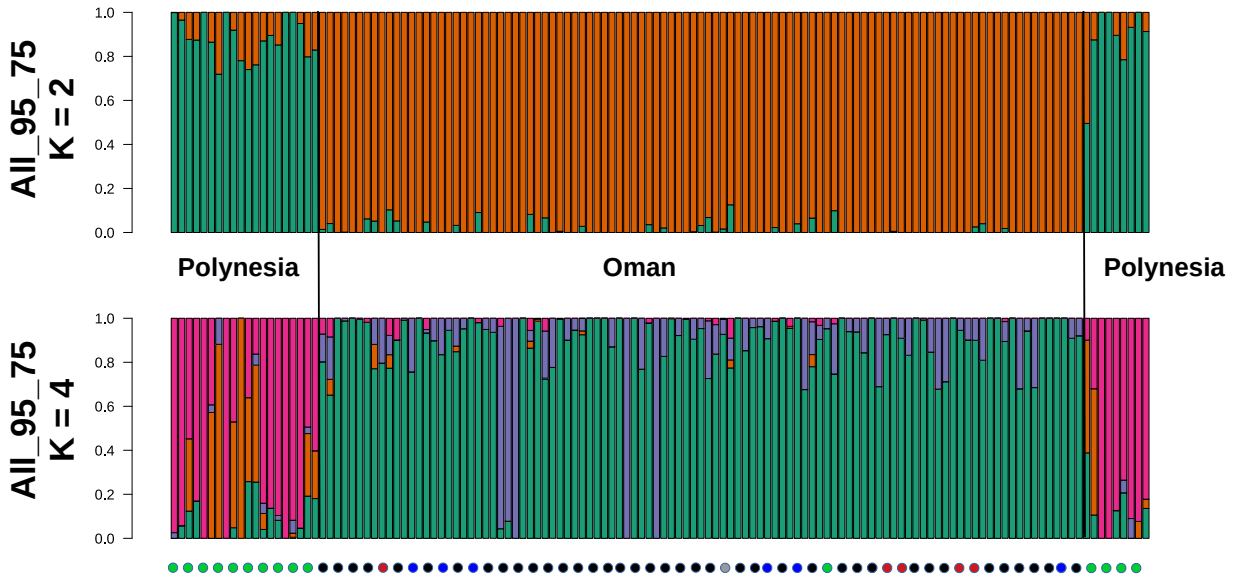
723 **Table 5** Mean  $F_{ST}$  estimates across loci for the datasets without and with corrections for repeated  
724 MLLs. The "pop" comparison estimates  $F_{ST}$  between sampling sites; for the All dataset, it  
725 corresponds to the French Polynesia / Oman differentiation. For the "ORF" comparison, this  
726 estimate compares samples of individuals grouped according to their ORF haplotypes (individuals  
727 without ORF sequence were not taken into account).

728

Dataset	$F_{ST}$ all individuals	$F_{ST}$ corrected for MLLs
All_95_75 pop	0.105	0.088
All_95_75 ORF	0.178	0.186
All_75_75 pop	0.352	0.355
All_75_75 ORF	0.361	0.312
Oman_95_75 pop	0.011	0.001
Oman_95_75 ORF	0.003	-0.003
Oman_75_75 pop	0.016	0.002
Oman_75_75 ORF	0.005	-0.002
Polynesia_95_75 pop	0.000	-0.007
Polynesia_75_75 pop	0.015	-0.024

729





**Table S1:** samples sizes per species hypothesis (on the basis of mitochondrial ORF) and sampling site for the different datasets. See main text for details on sampling sites and G elin et al. (2017) for species hypotheses. For French Polynesia, all mitochondrial sequences corresponded to type 5a. Note that some individuals were shared among datasets.

Dataset	Region	Site	type 5a	type 7a	type 3g	type 3e	unknown
All_95_75	Oman	O1		16	3	3	
All_95_75	Oman	O2		24		2	
All_95_75	Oman	O3		6		2	2
All_95_75	Oman	O4	1	13	2	4	1
All_95_75	Oman	O5		19	2	3	
All_95_75	French Polynesia	MH	5				
All_95_75	French Polynesia	MT	6				1
All_95_75	French Polynesia	MV	7				1
All_95_75	French Polynesia	TF	3				1
All_95_75	French Polynesia	TV	5				
<b>All_95_75</b>		<b>Total</b>	<b>27</b>	<b>78</b>	<b>7</b>	<b>14</b>	<b>6</b>
All_75_75	Oman	O1		5	3	3	
All_75_75	Oman	O2		19		2	
All_75_75	Oman	O3		5			2
All_75_75	Oman	O4	1	11	2	3	
All_75_75	Oman	O5		19	2	3	
All_75_75	Polynesia	MH	4				
All_75_75	Polynesia	MV	6				1
All_75_75	Polynesia	TF	1				1
All_75_75	Polynesia	TV	5				
<b>All_75_75</b>		<b>Total</b>	<b>17</b>	<b>59</b>	<b>7</b>	<b>11</b>	<b>4</b>
Oman_95_75	Oman	O1		14	3	3	
Oman_95_75	Oman	O2		23		2	
Oman_95_75	Oman	O3		7		2	1
Oman_95_75	Oman	O4	1	13	2	4	
Oman_95_75	Oman	O5		19	2	3	
<b>Oman_95_75</b>		<b>Total</b>	<b>1</b>	<b>76</b>	<b>7</b>	<b>14</b>	<b>1</b>
Oman_75_75	Oman	O1		5	3	3	
Oman_75_75	Oman	O2		18		2	
Oman_75_75	Oman	O3		5			1
Oman_75_75	Oman	O4	1	10	2	3	
Oman_75_75	Oman	O5		19	2	3	
<b>Oman_75_75</b>		<b>Total</b>	<b>1</b>	<b>57</b>	<b>7</b>	<b>11</b>	<b>1</b>
Polynesia_95_75	French	MH	5				

Polynesia				
Polynesia_95_75	French Polynesia	MT	7	1
Polynesia_95_75	French Polynesia	MV	7	1
Polynesia_95_75	French Polynesia	TF	4	1
Polynesia_95_75	French Polynesia	TV	5	
<b>Polynesia_95_75</b>		<b>Total</b>	<b>28</b>	<b>3</b>
<b>Polynesia_75_75</b>	French Polynesia	MH	5	
<b>Polynesia_75_75</b>	French Polynesia	MT	6	1
<b>Polynesia_75_75</b>	French Polynesia	MV	7	1
<b>Polynesia_75_75</b>	French Polynesia	TV	5	
<b>Polynesia_75_75</b>		<b>Total</b>	<b>23</b>	<b>2</b>

**Table S2 :** statistics on read numbers and alignment after initial filtering on sequence quality : mean, minimum, maximum and standard deviation (s.d.) of the initial numbers of reads, and of the percentage of reads mapped on genome par individual.

Samples	Number of individuals	Mean reads	Min reads	Max reads	s.d.	Mean mapped	Min mapped	Max mapped	s.d.
All	211	4540048	5735	30394029	5169991	85.1	70.6	86.7	1.9
Oman	141	5647233	5735	30394029	5669214	85.6	78.3	86.7	1.1
French Polynesia	70	2309860	20495	11800706	2930211	84.1	70.6	86.2	2.7

**Table S3** : estimates of gene diversity within ( $1-Q_{intra}$ ) and among individuals ( $1-Q_{inter}$ ), and of  $F_{IS}$  over all loci. For each dataset the estimates are given first for the whole dataset and per population (here sampling site) and per ORF haplotype if at least two individuals with the corresponding ORF haplotype have been analysed. The results are given for datasets not corrected for repeated MLLs. For French Polynesia, all individuals for which we got a mitochondrial ORF sequence corresponded to beta/type5a.

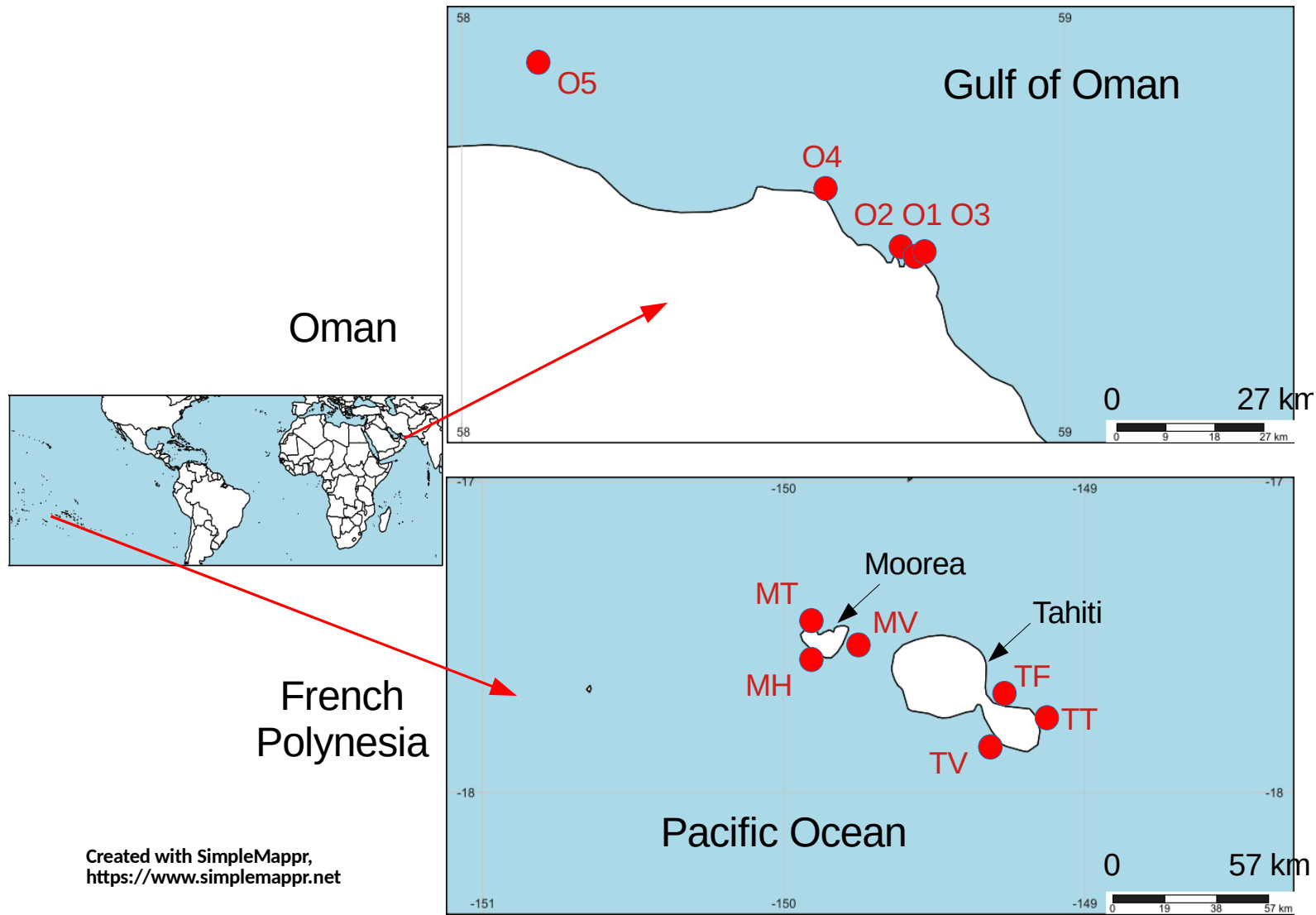
<b>Dataset</b>	<b>Population / ORF</b>	<b><math>1-Q_{intra}</math></b>	<b><math>1-Q_{inter}</math></b>	<b><math>F_{IS}</math></b>
<b>All 95 75</b>	All	0.07	0.06	-0.15
	Population			
	French Polynesia	0.09	0.09	-0.04
	Oman	0.05	0.04	-0.27
	ORF			
	type5a	0.09	0.09	-0.01
	7a	0.05	0.04	-0.27
	3g	0.04	0.03	-0.42
	3e	0.05	0.04	-0.28
	<b>All 75 75</b>	All	0.12	0.15
Population				
French Polynesia		0.13	0.17	0.27
Oman		0.11	0.13	0.15
ORF				
type5a		0.13	0.19	0.33
7a		0.11	0.13	0.14
3g		0.12	0.13	0.06
3e		0.11	0.13	0.13
<b>Oman 95 75</b>		All	0.16	0.16
	Population			
	O1	0.15	0.16	0.07
	O2	0.16	0.15	-0.04
	O3	0.15	0.16	0.11
	O4	0.16	0.16	0.01
	O5	0.19	0.16	-0.16
	ORF			
	7a	0.16	0.16	0
	3g	0.18	0.15	-0.18
3e	0.16	0.16	-0.05	
<b>Oman 75 75</b>	All	0.20	0.24	0.14
	Population			
	O1	0.22	0.24	0.08
	O2	0.19	0.23	0.15
	O3	0.18	0.24	0.24
	O4	0.20	0.24	0.18
	O5	0.23	0.24	0.05
	ORF			
	7a	0.21	0.24	0.13
	3g	0.22	0.24	0.07
3e	0.21	0.24	0.13	
<b>Polynesia 95 75</b>	All	0.04	0.04	-0.21
	Population			
	MH	0.04	0.02	-0.52
	MT	0.05	0.06	0.14
	MV	0.04	0.03	-0.34
	TF	0.05	0.06	0.09
	TV	0.03	0.02	-0.44
<b>Polynesia 75 75</b>	All	0.05	0.05	0.02
	Population			
	MH	0.04	0.04	-0.15
	MT	0.05	0.08	0.39
	TV	0.05	0.04	-0.17



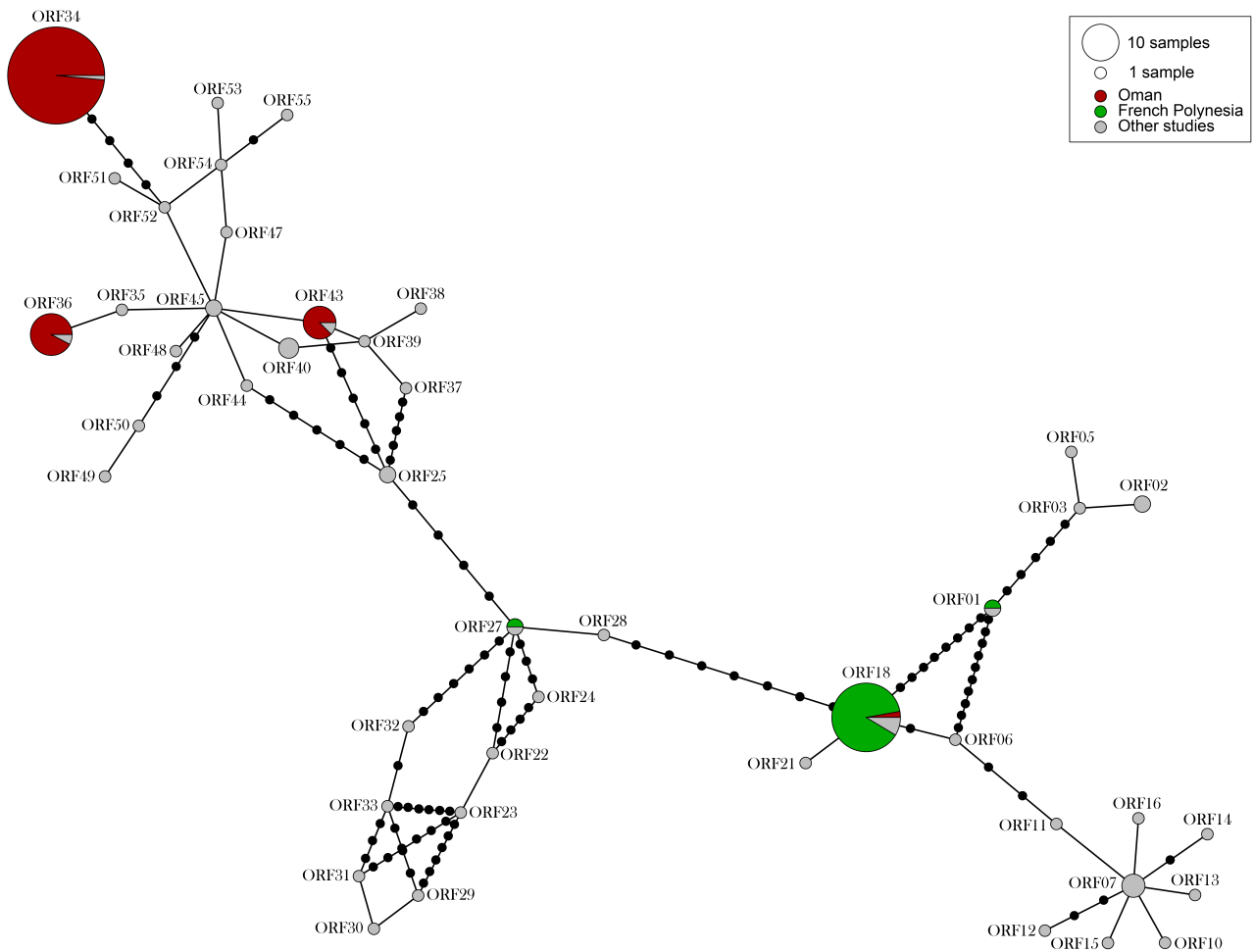
**Table S4** : estimate of the index association  $\bar{r}_d$  to study linkage disequilibrium in the different datasets. The first column gives the mean and the second the standard deviation computed over 10 000 replicates of 320 SNPs. The "global" results correspond to analyses done at the level of the whole corresponding dataset. The "strata" results correspond to analyses done at the level of mitochondrial lineages for the All and Oman datasets, and at the level of sampling site for the French Polynesia datasets (i.e. the "strata" levels used in the poppr R package). Note that the analysis was not done for 5a in Oman where only one individual was analysis.

<b>Dataset</b>	<b>mean</b>	<b>s.d.</b>
<b>Global</b>		
All_95_75	0.033	0.005
All_75_75	0.099	0.019
Oman_95_75	0.002	0.001
Oman_75_75	0.004	0.001
Polynesia_95_75	0.035	0.007
Polynesia_75_75	0.017	0.007
<b>Strata All_95_75</b>		
3e	0.003	0.006
3g	0.025	0.025
5a	0.019	0.004
7a	0.005	0.002
<b>Strata All_75_75</b>		
3e	0	0.003
3g	0.049	0.009
5a	0.083	0.017
7a	0.007	0.002
<b>Strata Oman_95_75</b>		
3e	0.003	0.003
3g	0.002	0.006
7a	0.003	0.001
<b>Strata Oman_75_75</b>		
3e	0	0.002
3g	0.05	0.006
7a	0.007	0.001
<b>Strata Polyn_95_75</b>		
MH	0.278	0.154
MT	0.008	0.009
MV	0.014	0.024
TF	0.086	0.043
TV	0.116	0.101
<b>Strata Polyn_75_75</b>		
MH	0.235	0.072
MT	0.01	0.011
MV	0.008	0.014
TV	0.108	0.065

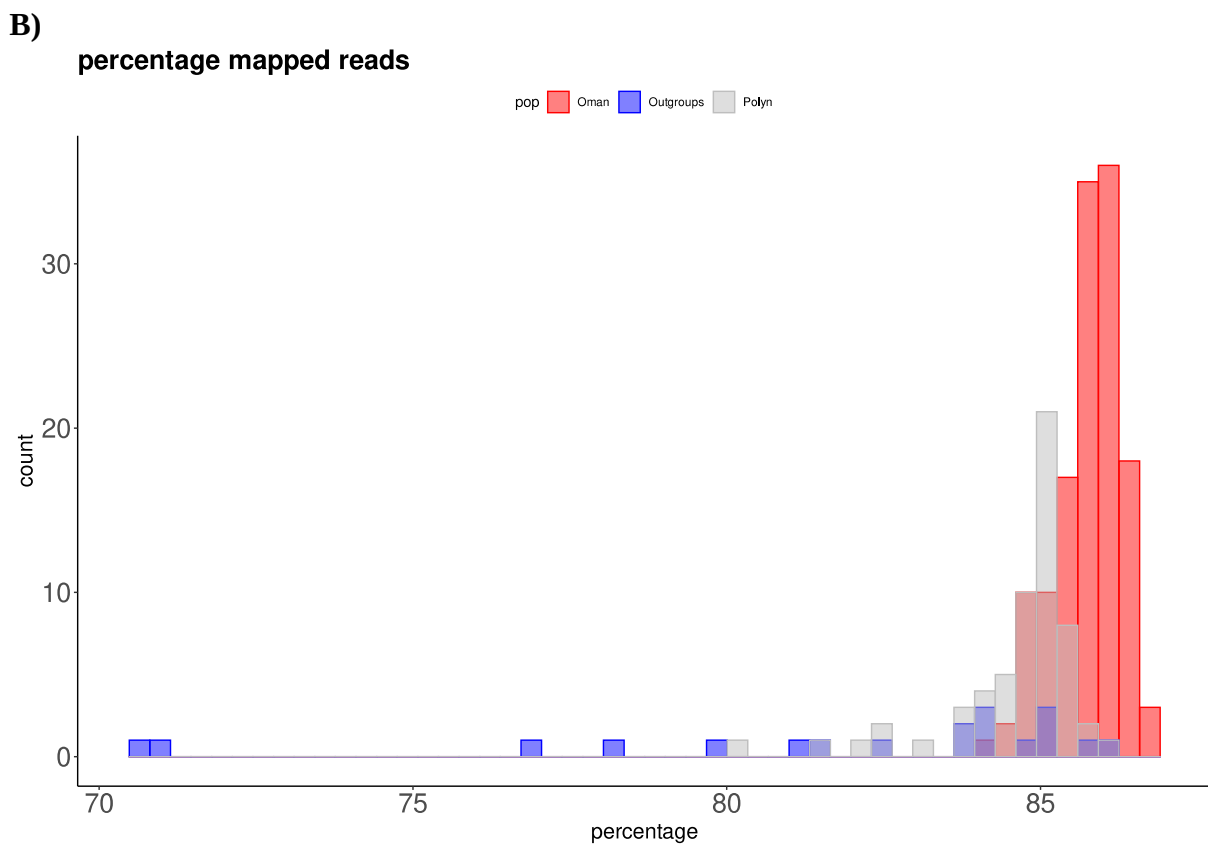
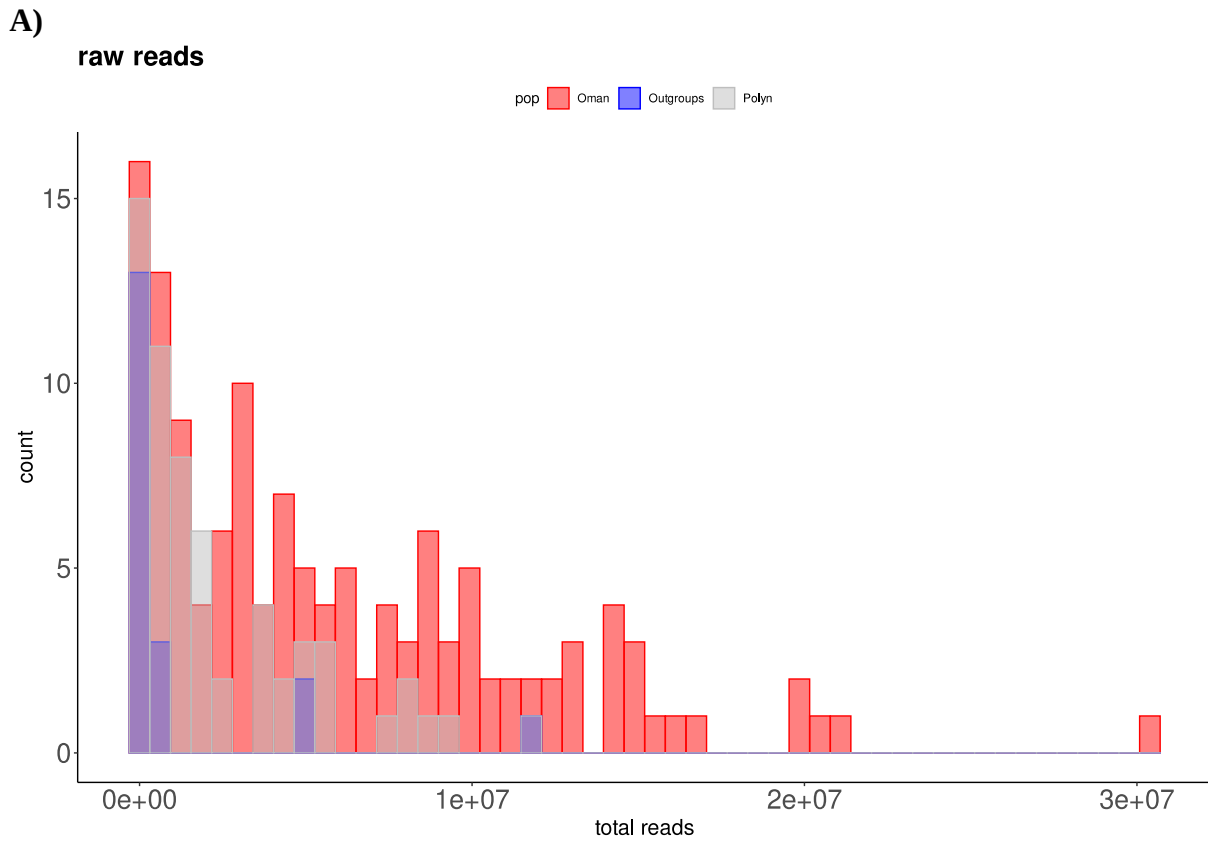
**Figure S1** : map of sampling points. See main text for details on sampling sites



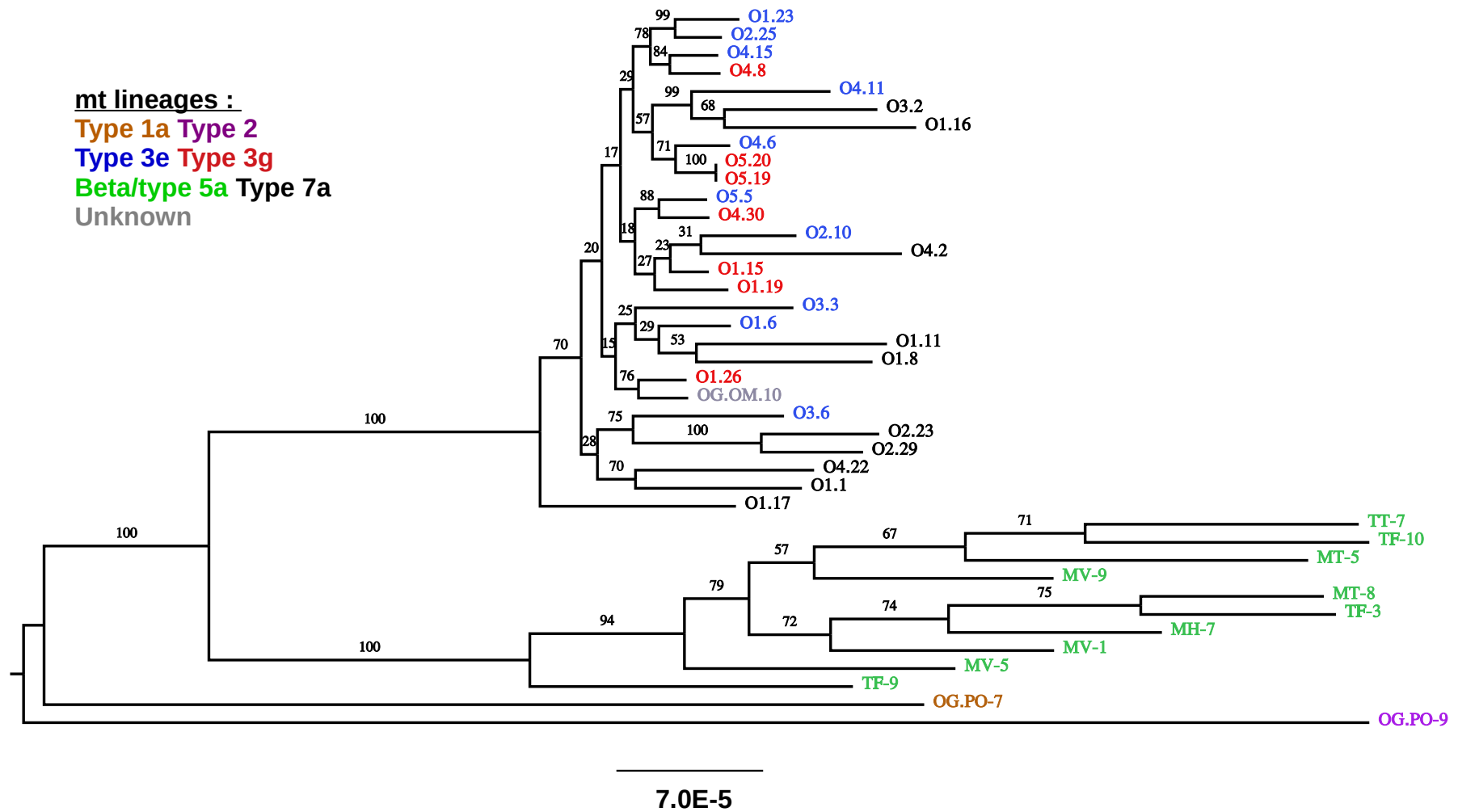
**Figure S2** : network of mitochondrial ORF sequences. The colors indicate the origin of sequences (Oman, French Polynesia or other studies). Sequences from previous studies can be found in Gélín et al. (2017b).



**Figure S3:** assembly statistics for RAD-Seq data. A) distribution of the number of raw reads, with color according to groups: red: Oman, grey: French Polynesia, blue: outgroups; B) distribution of the percentage of reads mapped to the *Pocillopora* genome for the different samples

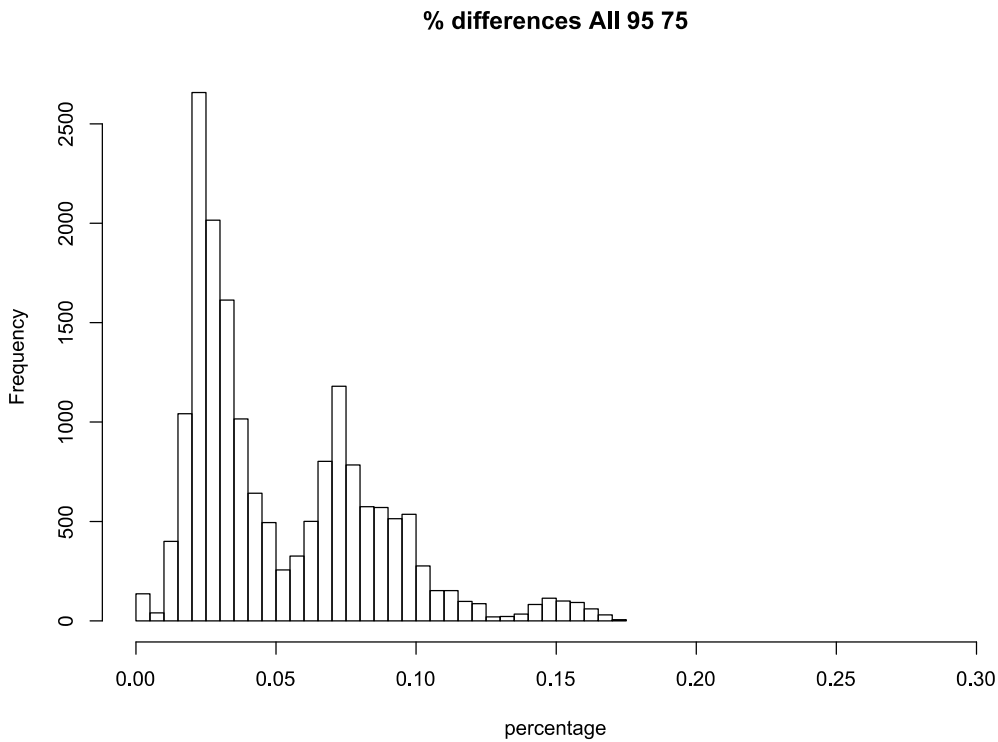


**Figure S4:** maximum-likelihood phylogenetic inference from RAD-Sequencing on the basis of 80 596 RAD loci, with 1 000 ultrafast bootstraps. The tree has been rooted at mid-point. The model retained by IQ-TREE was the TN+F+R5. The percentages of bootstraps are indicated at the left of the corresponding nodes. The colors indicate the mitochondrial lineage for each sample.



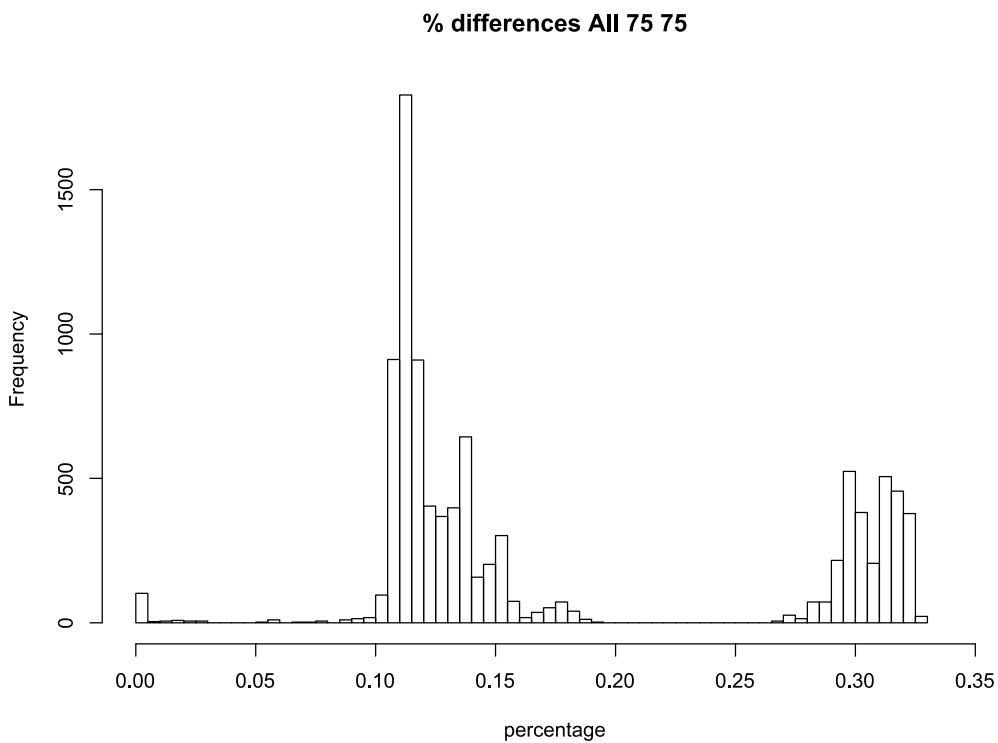
**Figure S5 :** histograms of genetic distance among individuals for the different datasets. The distances correspond to the proportion of differences among loci computed with poppr. We indicate below each histogram the retained threshold to remove repeated MLLs.

**A) All\_95\_75**



MLL threshold 0.0157

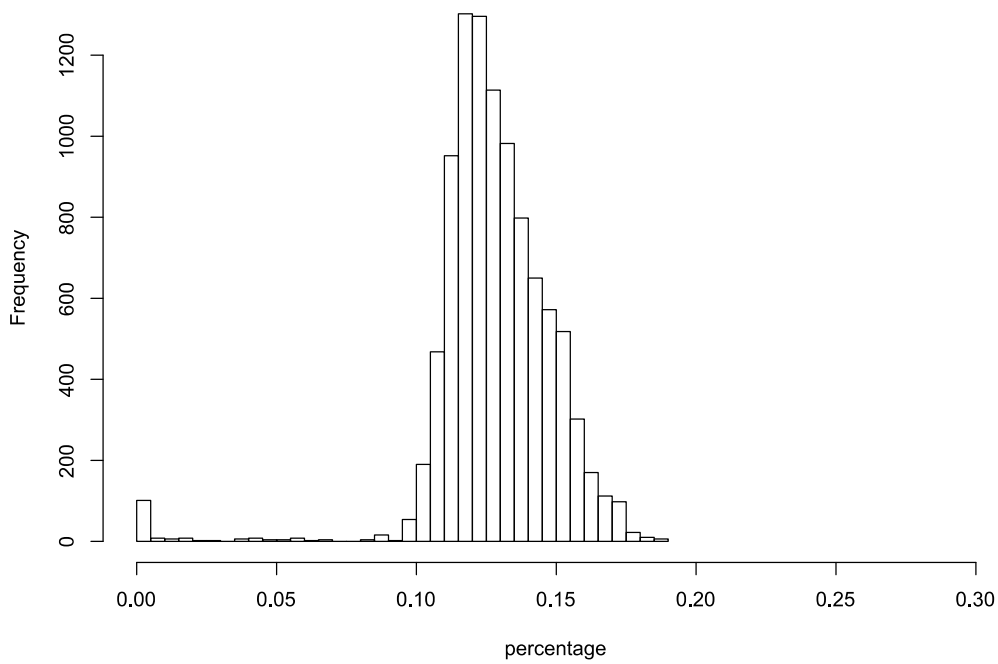
**B) All\_75\_75**



MLL threshold 0.06

### C) Oman\_95\_75

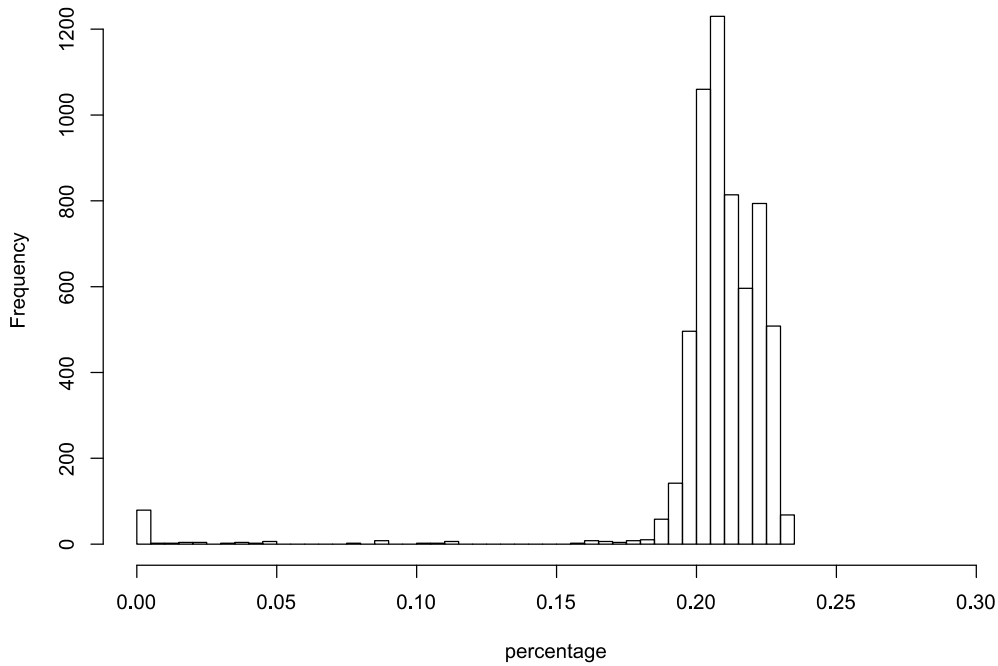
**% differences Oman 95 75**



MLL threshold 0.0685

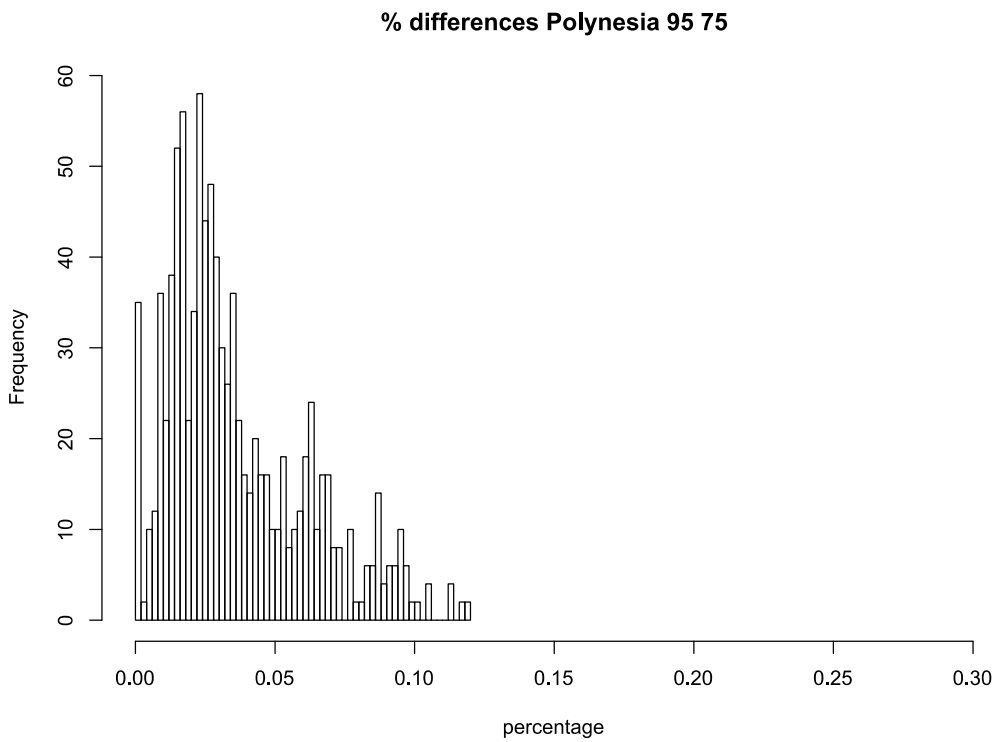
### D) Oman\_75\_75

**% differences Oman 75 75**



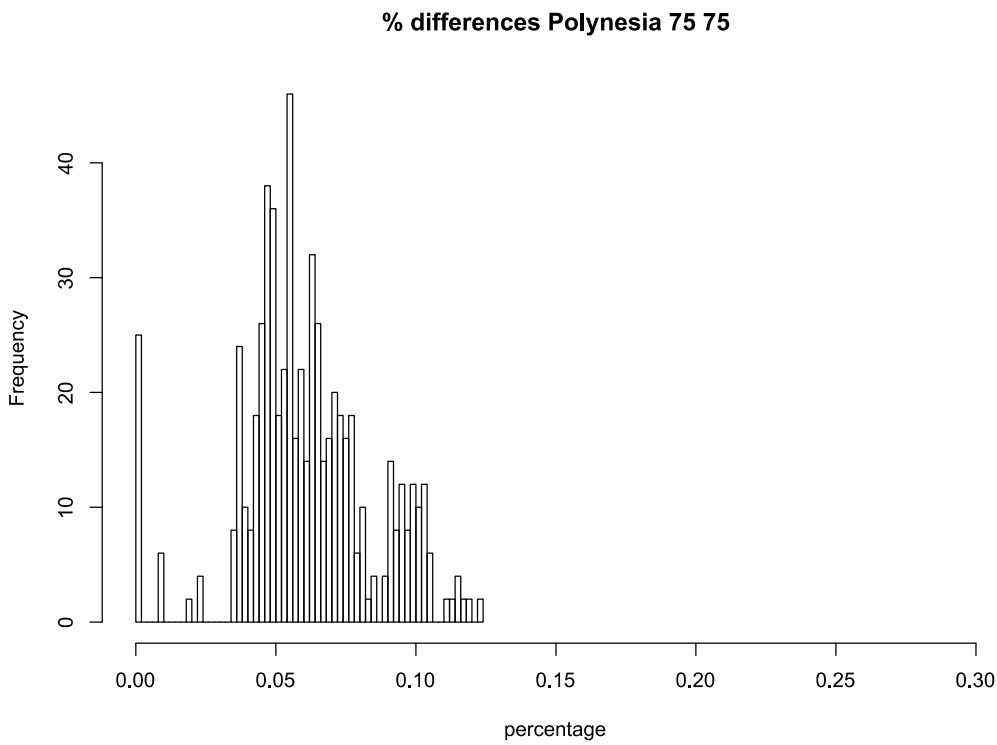
MLL threshold 0.09

### E) Polynesia\_95\_75



MLL threshold 0.002

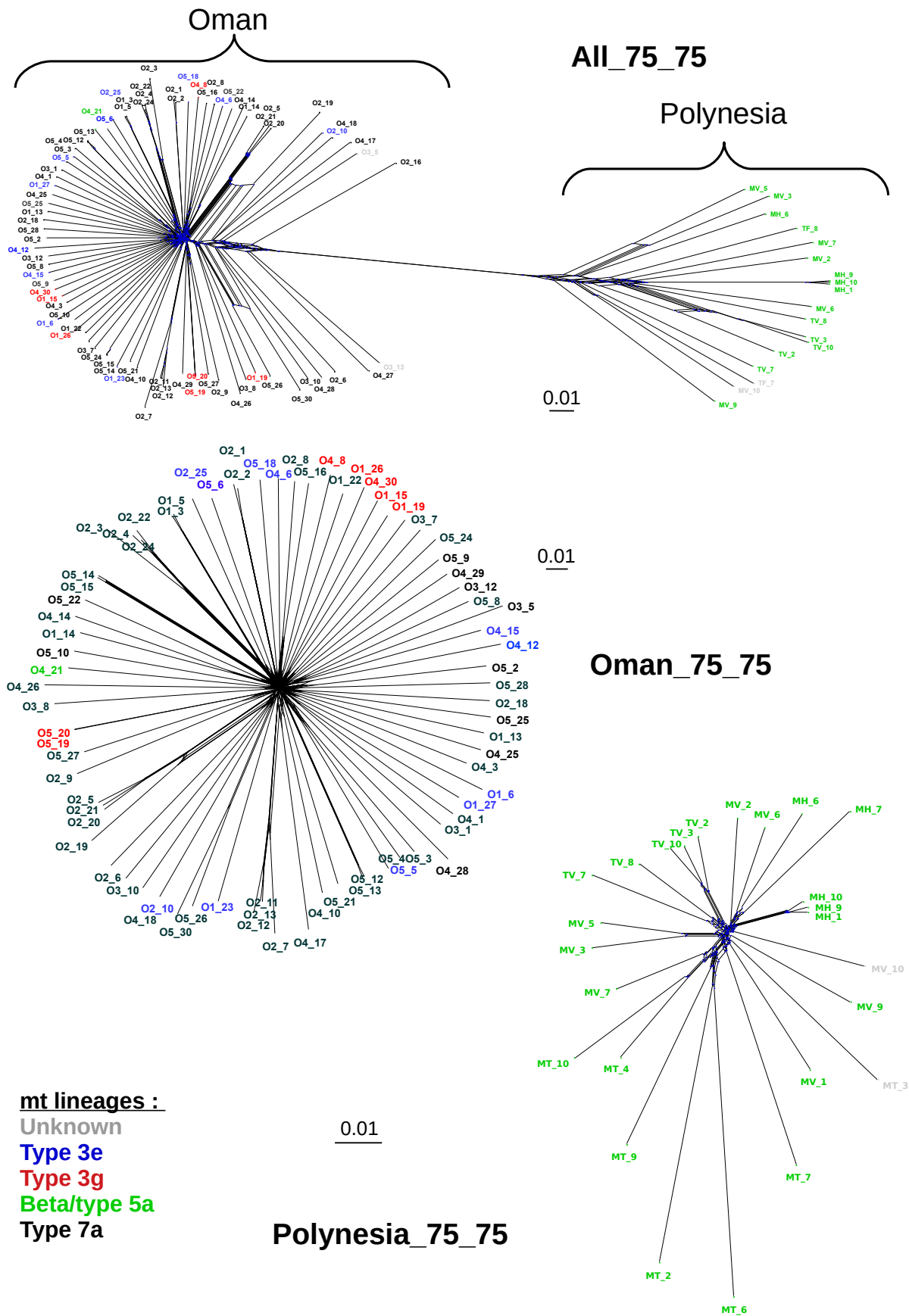
### F) Polynesia\_75\_75



MLL threshold 0.04

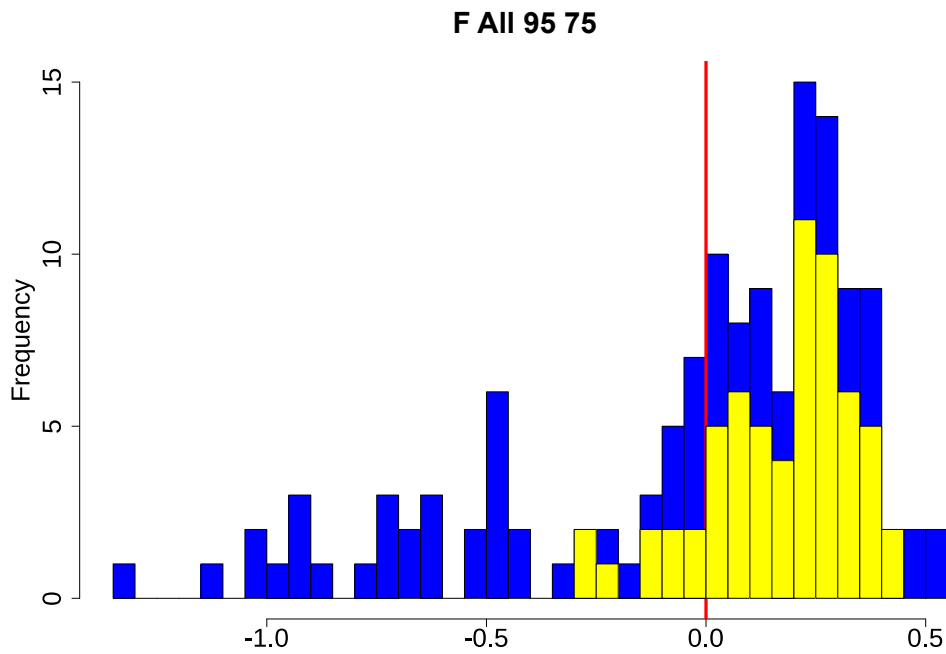


**Figure S6:** networks based on the percentage of difference among individuals for the All, Oman and Polynesia 75\_75 datasets. The colors indicate the corresponding mitochondrial lineage.

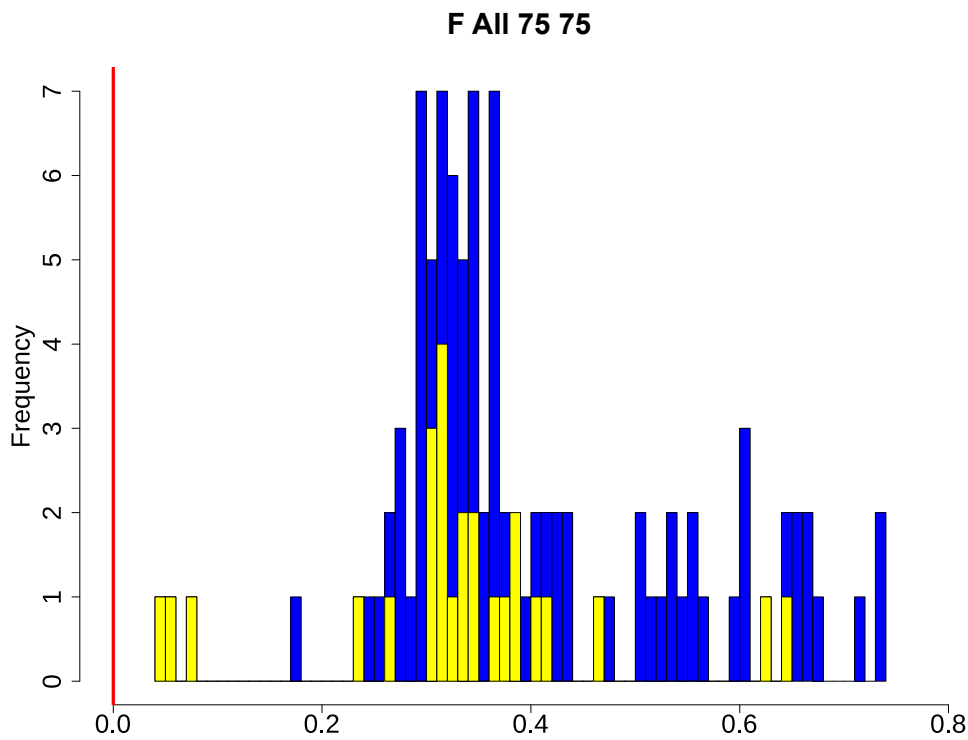


**Figure S7** : histograms of individual inbreeding coefficients  $F$  estimated with VCFtools for the different datasets. The blue histograms correspond to all individuals, and the yellow histograms correspond to individuals potentially involved in MLLs with the previously defined distance thresholds. For visibility the x axis is different among figures. A red line at  $x = 0$  is given for comparison.

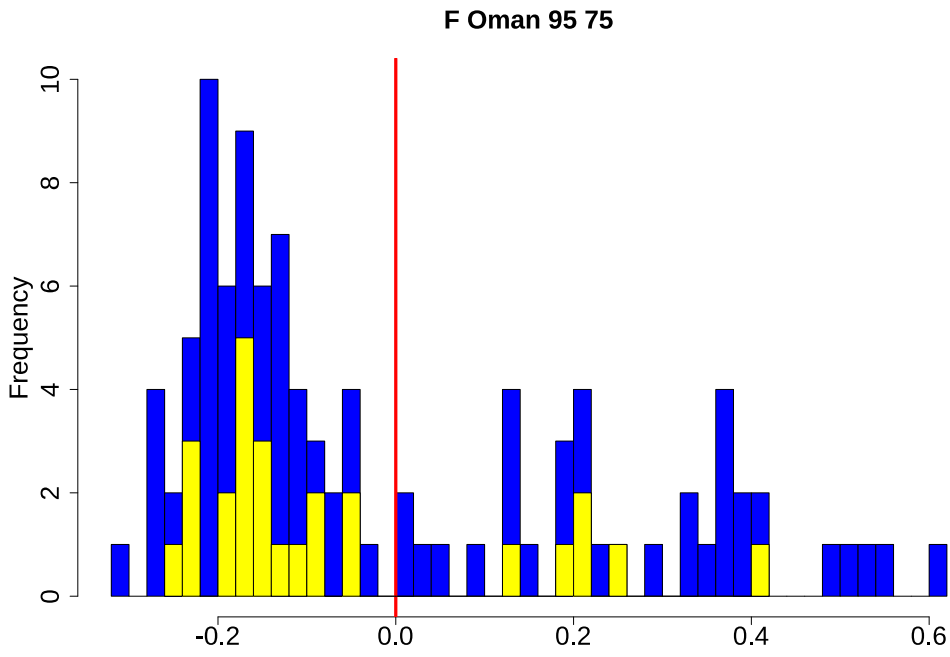
**All\_95\_75**



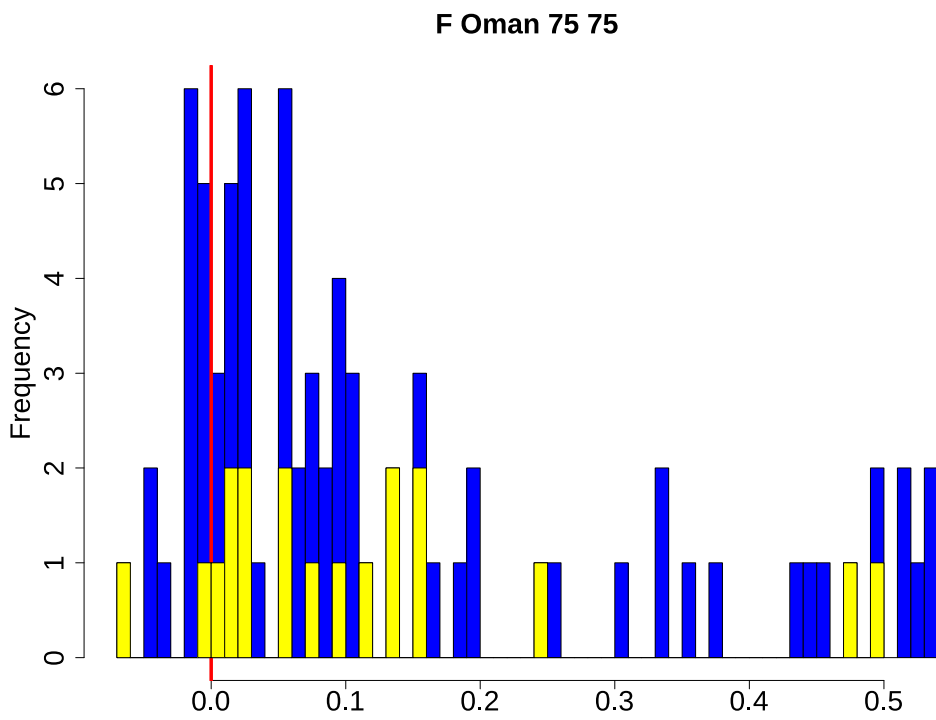
**All\_75\_75**



### Oman\_95\_75

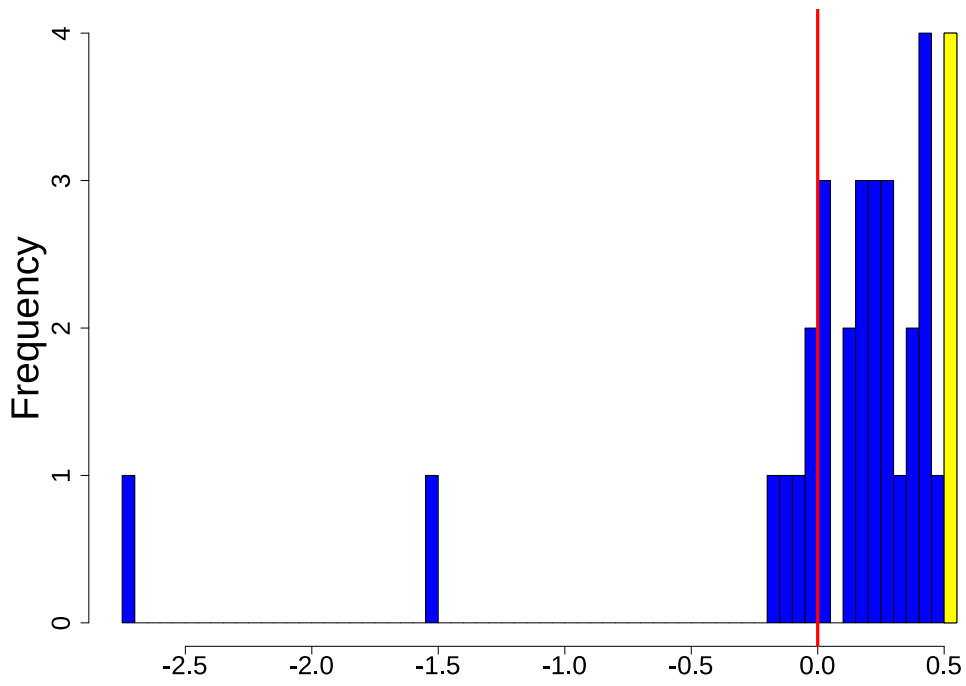


### Oman\_75\_75



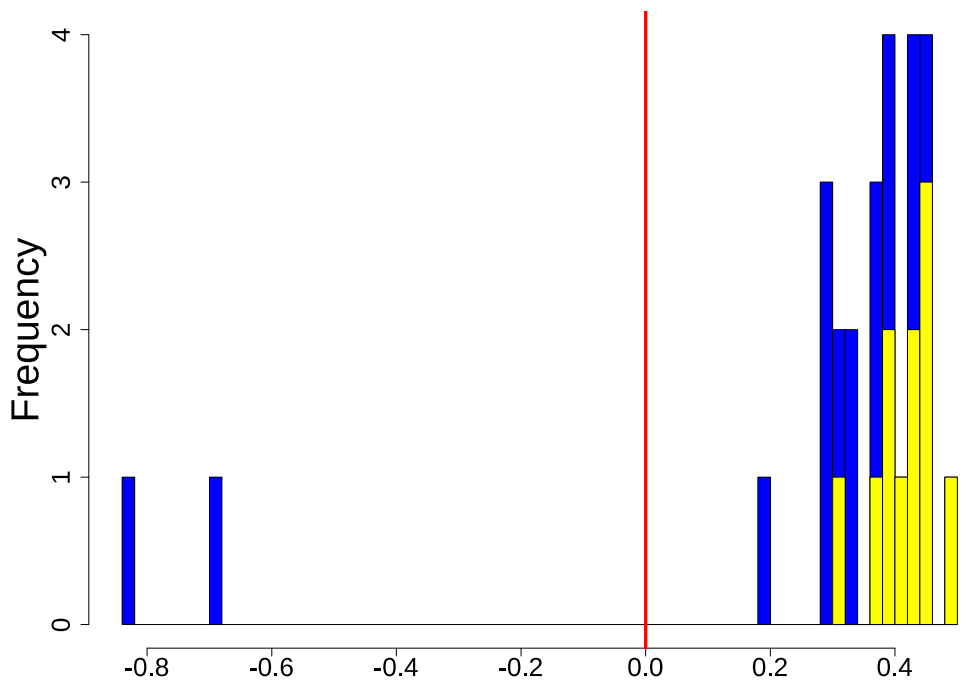
Polynesia\_95\_75

F Polyn 95 75

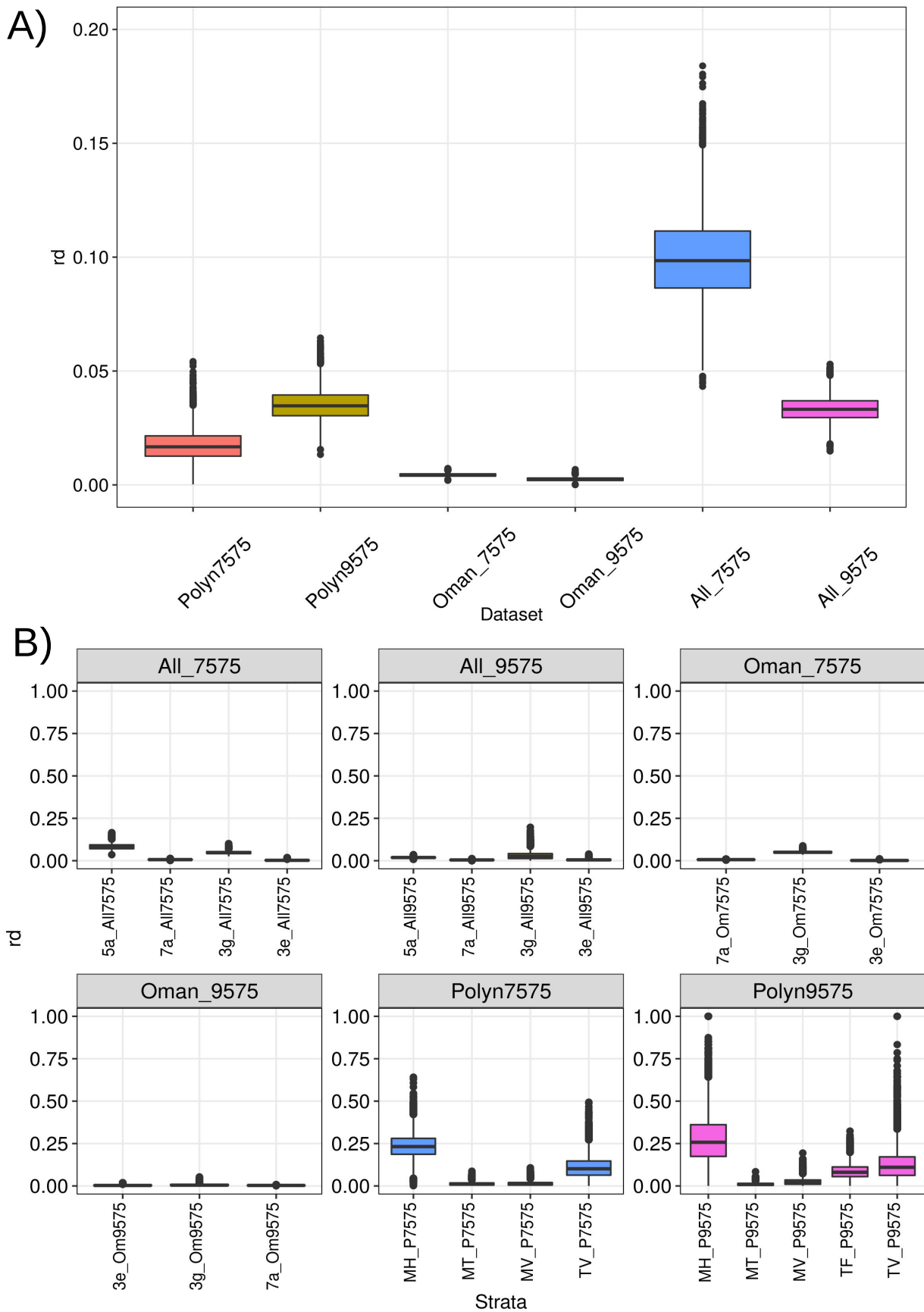


Polynesia\_75\_75

F Polyn 75 75

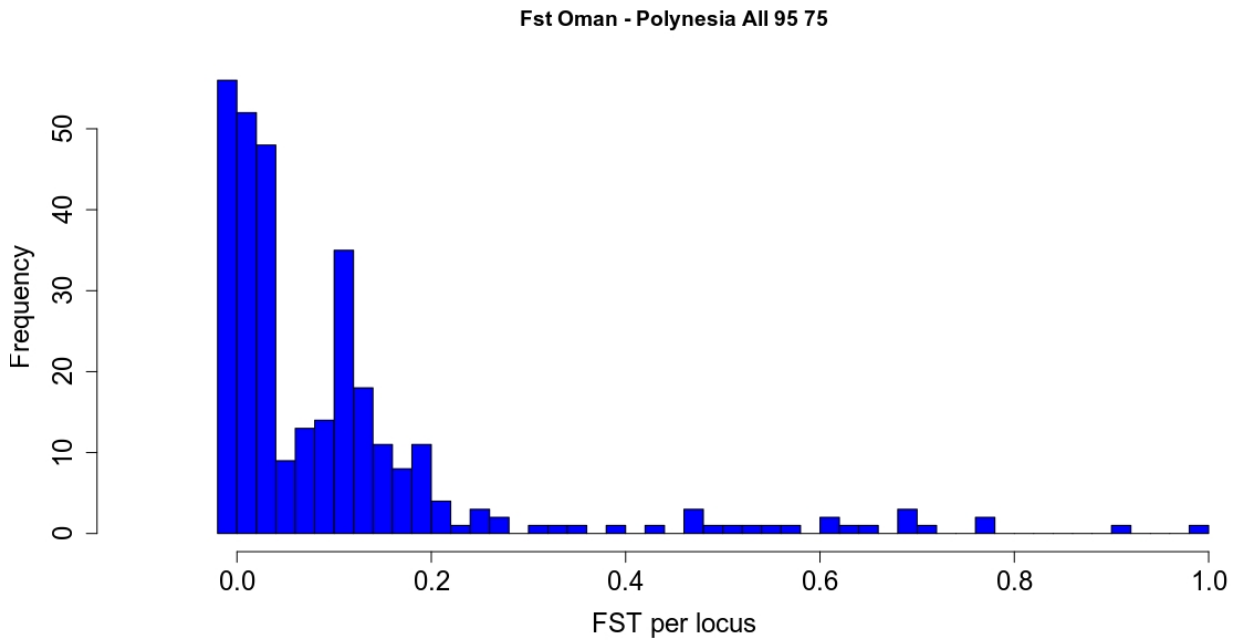


**Figure S8** : distribution of the estimates of the index association  $\bar{r}_d$  to study linkage disequilibrium in the different datasets : A) on the whole datasets, B) per mitochondrial lineages or per site. See the main text and the legend of Table S4 for details.

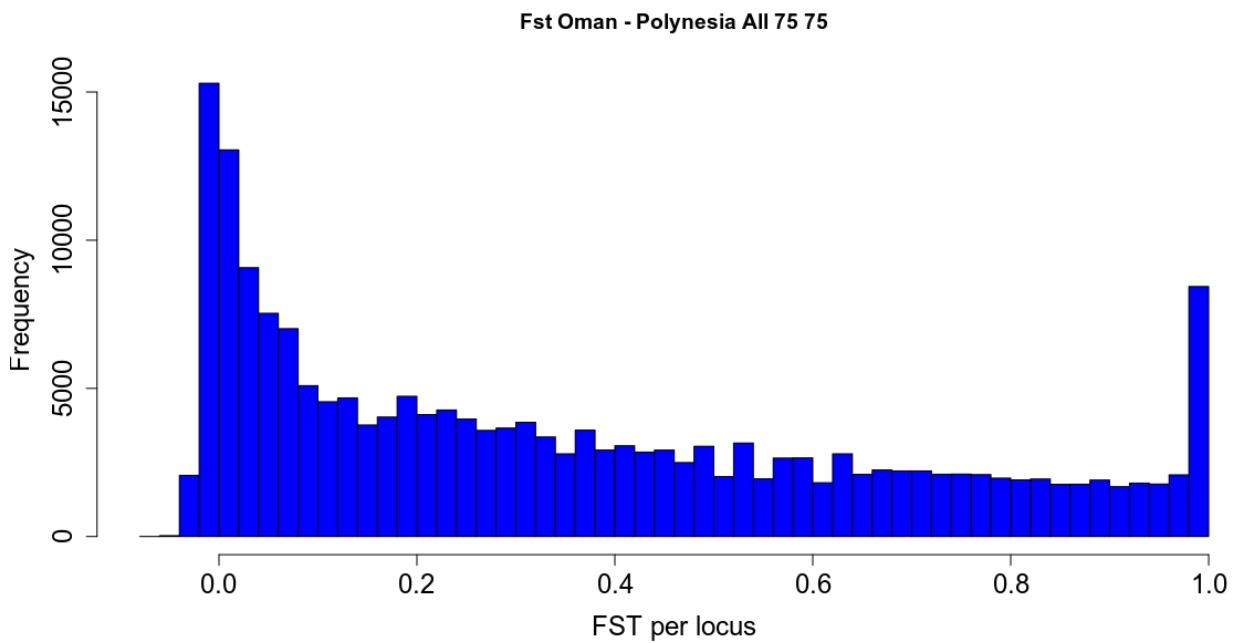


**Figure S9:** distribution of  $F_{ST}$  over all loci for the comparison between Oman and French Polynesia for A) the All\_95\_75 dataset (mean  $F_{ST}$  estimate: 0.105) and B) the All\_75\_75 dataset (mean  $F_{ST}$  estimate: 0.352).

**A)**

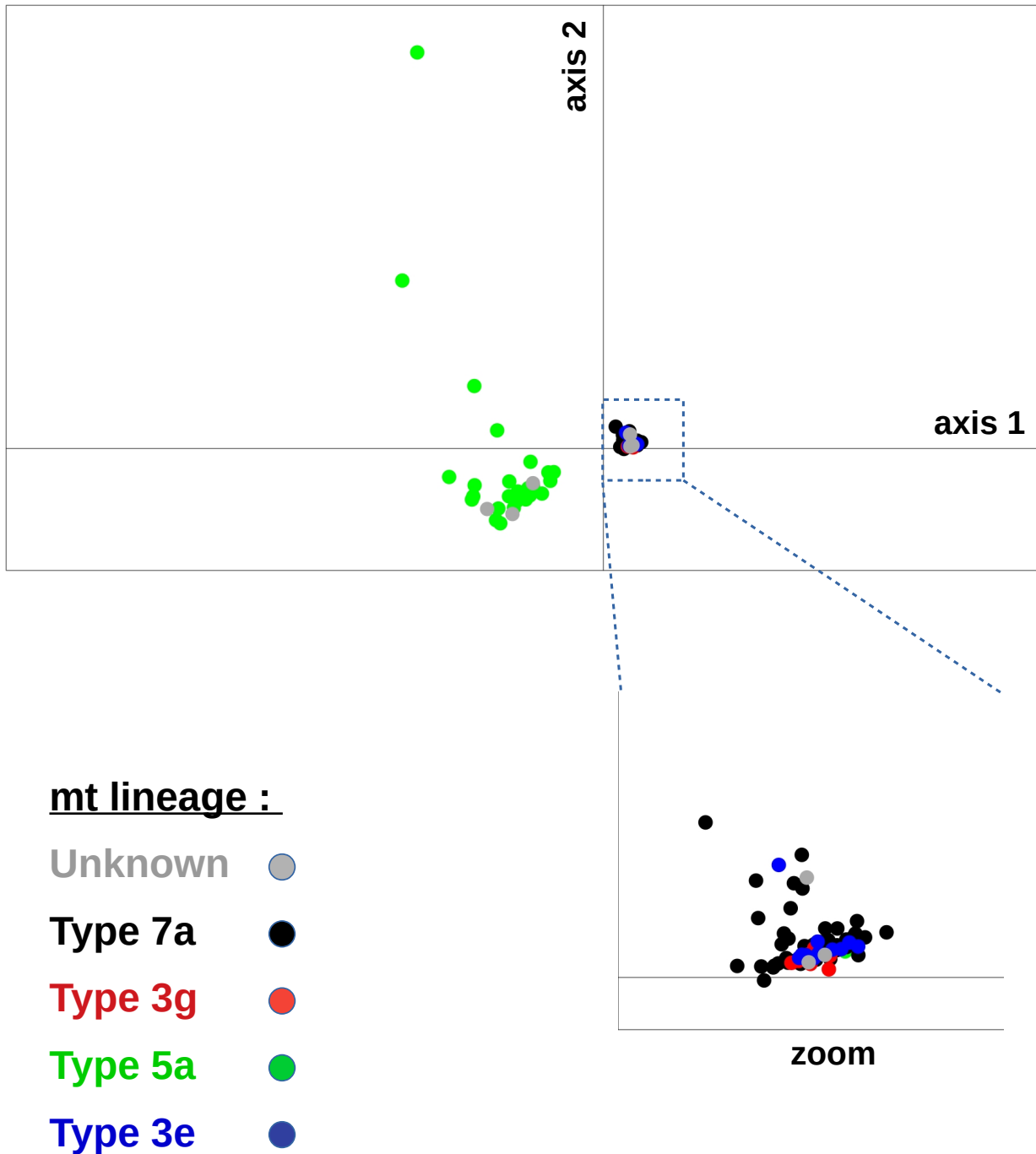


**B)**

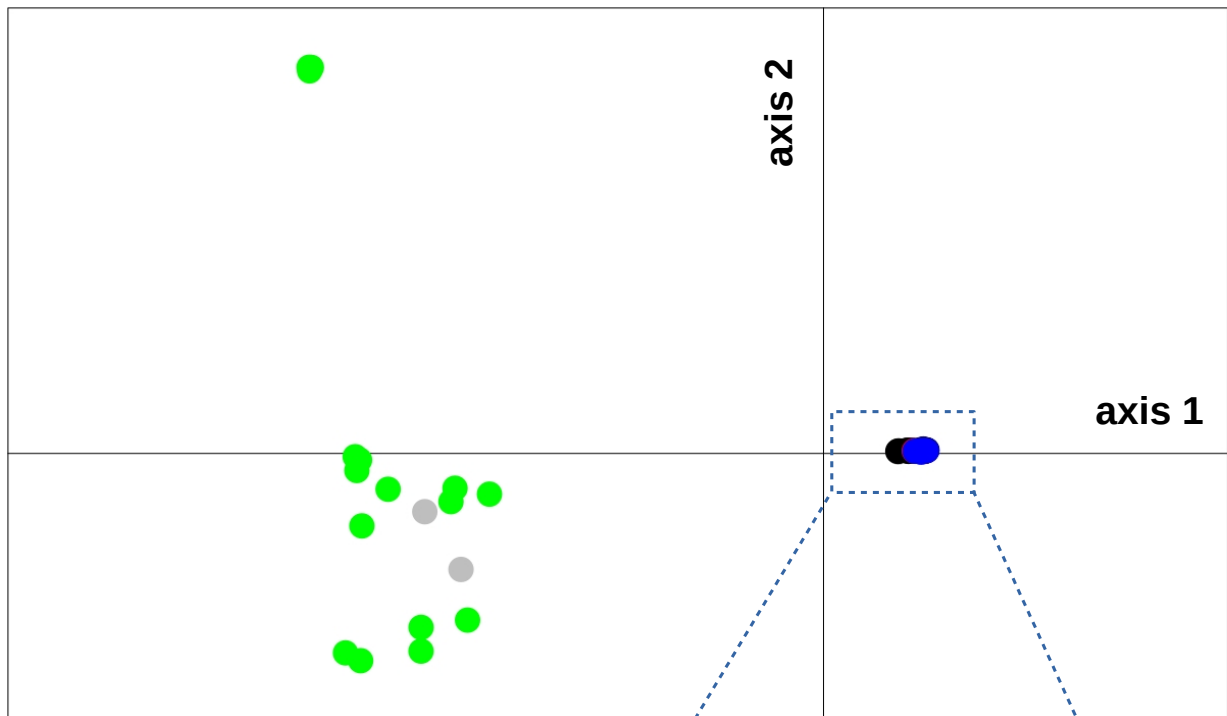


**Figure S10:** plot of individuals on the PCA axes 1 and 2 for A) the All\_95\_75, and B) the All\_75\_75 datasets. For All\_95\_75, the percentage of inertia was 9.2 for axis 1 and 5.6 for axis 2. For All\_75\_75, the percentage of inertia was 25.1 for axis 1 and 3.9 for axis 2. The individual dots are colored according to their ORF mitochondrial lineage, and a focus on the central part is given under each plot.

### A) All\_95\_75



## B) All\_75\_75



### mt lineage :

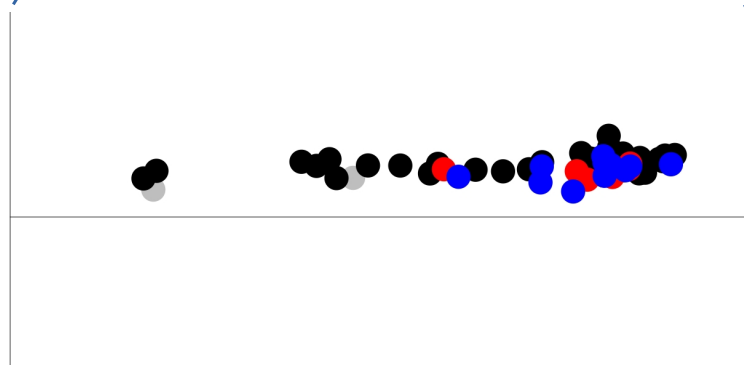
Unknown ●

Type 7a ●

Type 3g ●

Type 5a ●

Type 3e ●

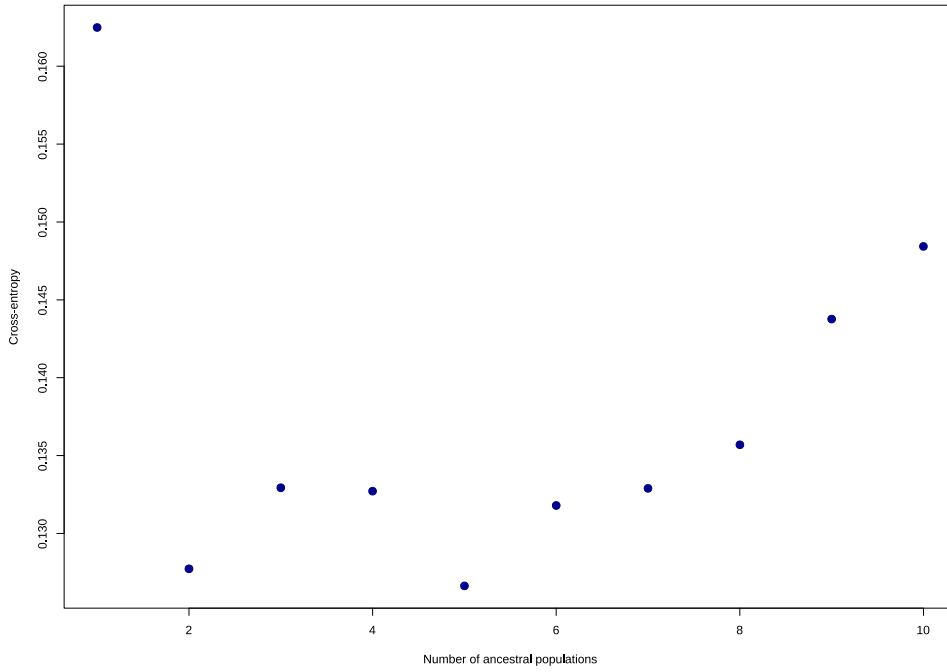


zoom

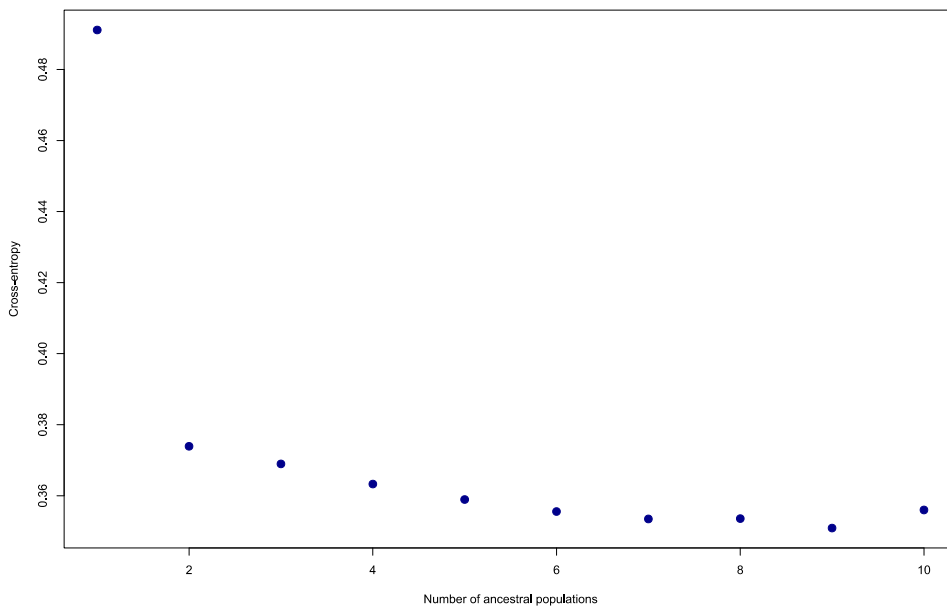


**Figure S11:** cross-entropy plots according to the number of clusters K for the snmf analyses on the different datasets: A) All\_95\_75, B) All\_75\_75, C) Oman\_95\_75, D) Oman\_75\_75, E) Polynesia\_95\_75, F) Polynesia\_75\_75.

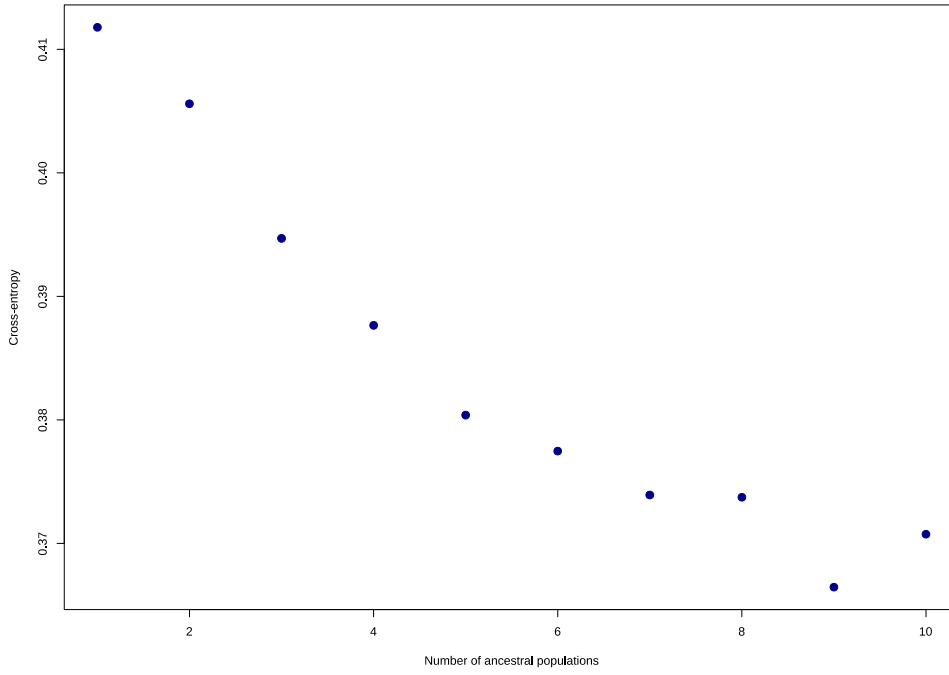
**A)**



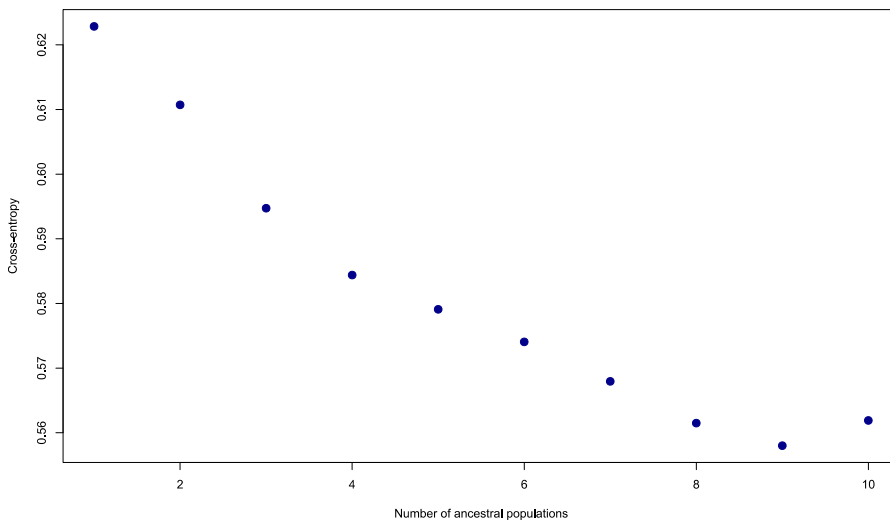
**B)**



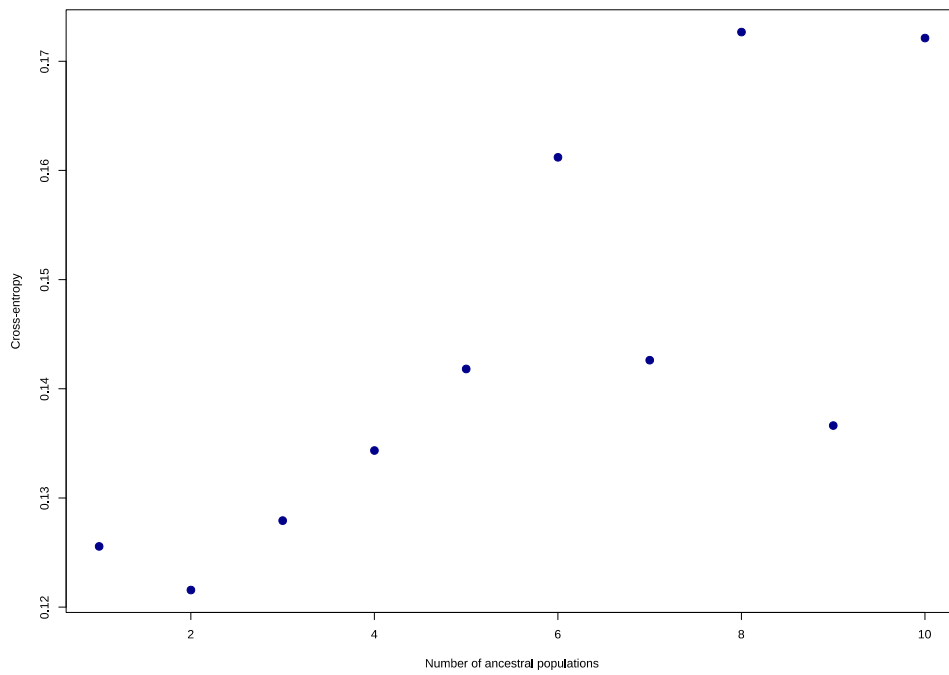
C)



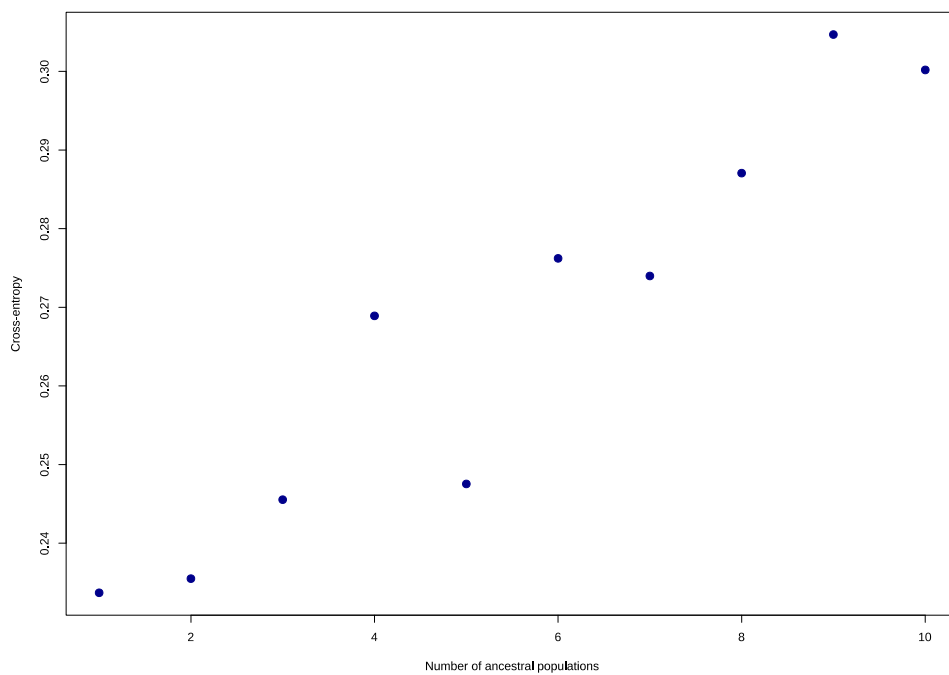
D)



**E)**



**F)**



### **Results of individual-based simulations :**

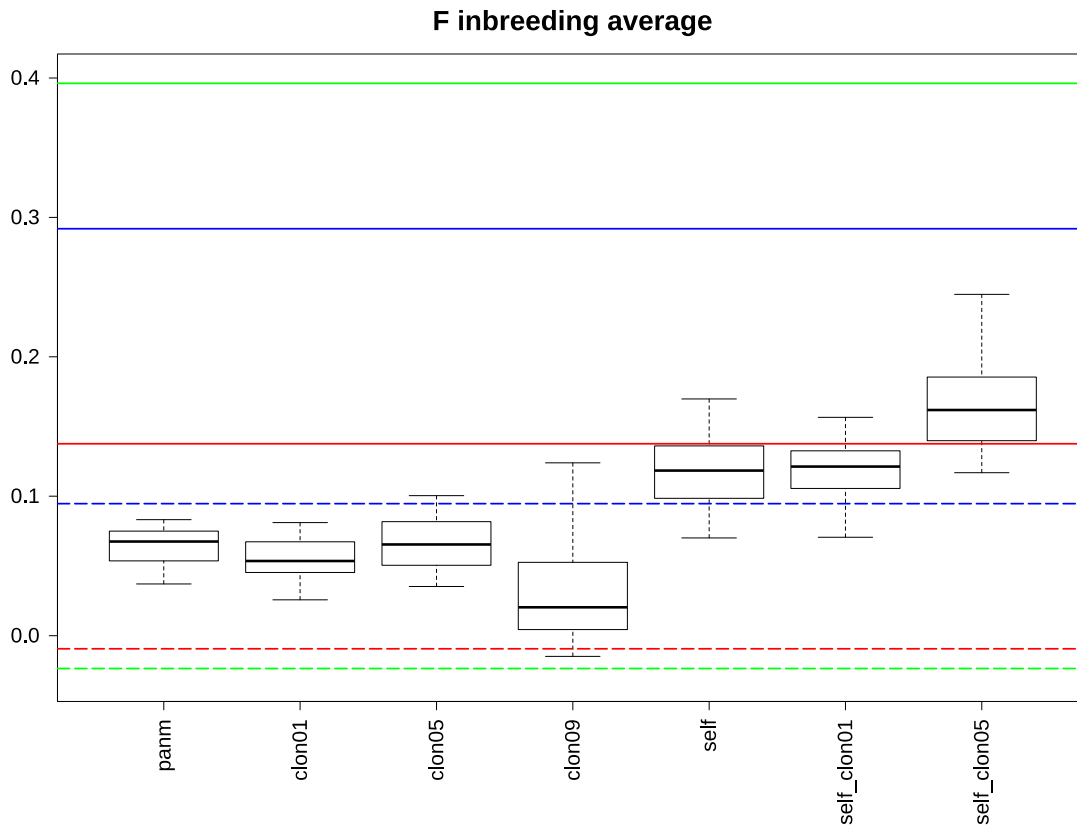
In this part we detail the results obtained for the analyses of individual-based simulations. We first present the figures, before developing the presentation of the results, by comparison with empirical data.

#### **Figures:**

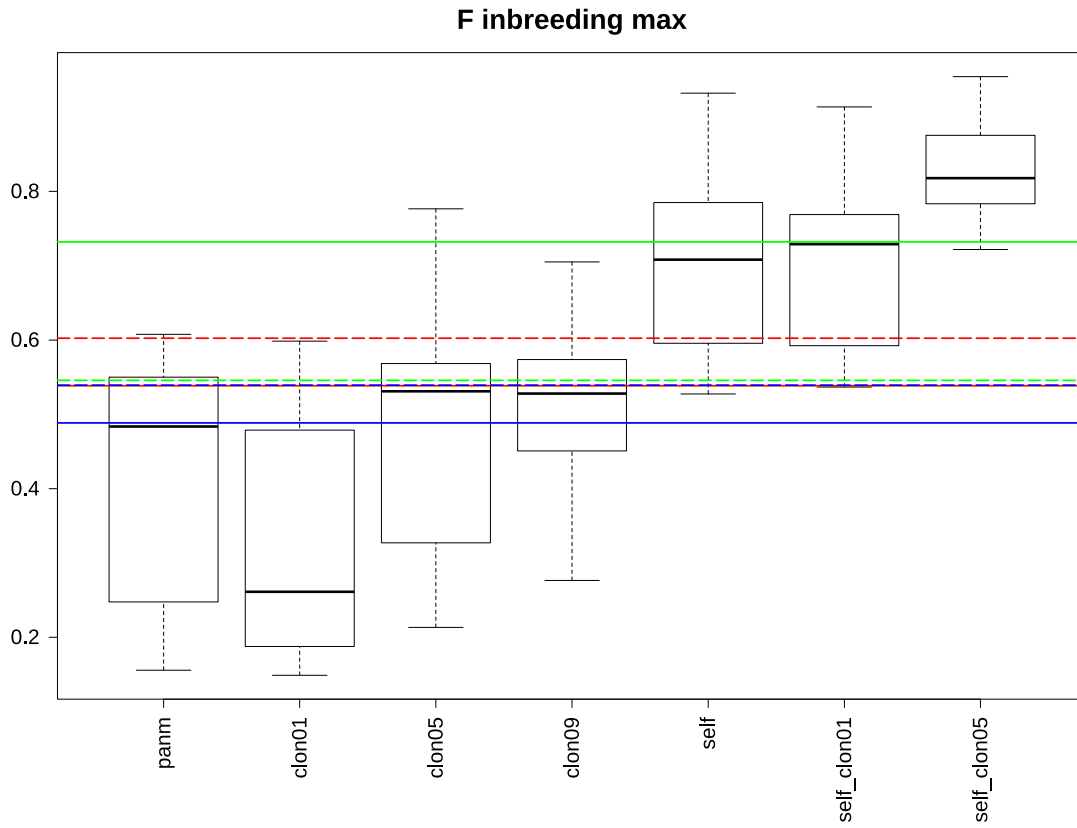
##### **Distribution of $F$ estimates :**

The figures below show the distribution of the average (A), maximum (B) and minimum (C) of the  $F$  parameter over individuals for different simulation configurations, with 30 simulations each. These distributions are compared to observed average, maximum and minimum values displayed as horizontal lines for the different datasets (see legends). Notation of the simulation configurations : panm : panmixia ; clon01, clon05, and clon09 : clonality present at rates 0.1, 0.5 and 0.9 respectively ; self\_clon01 and self\_clon05 : selfing present at rate 0.1, and clonality present at rates 0.1 and 0.5 respectively.

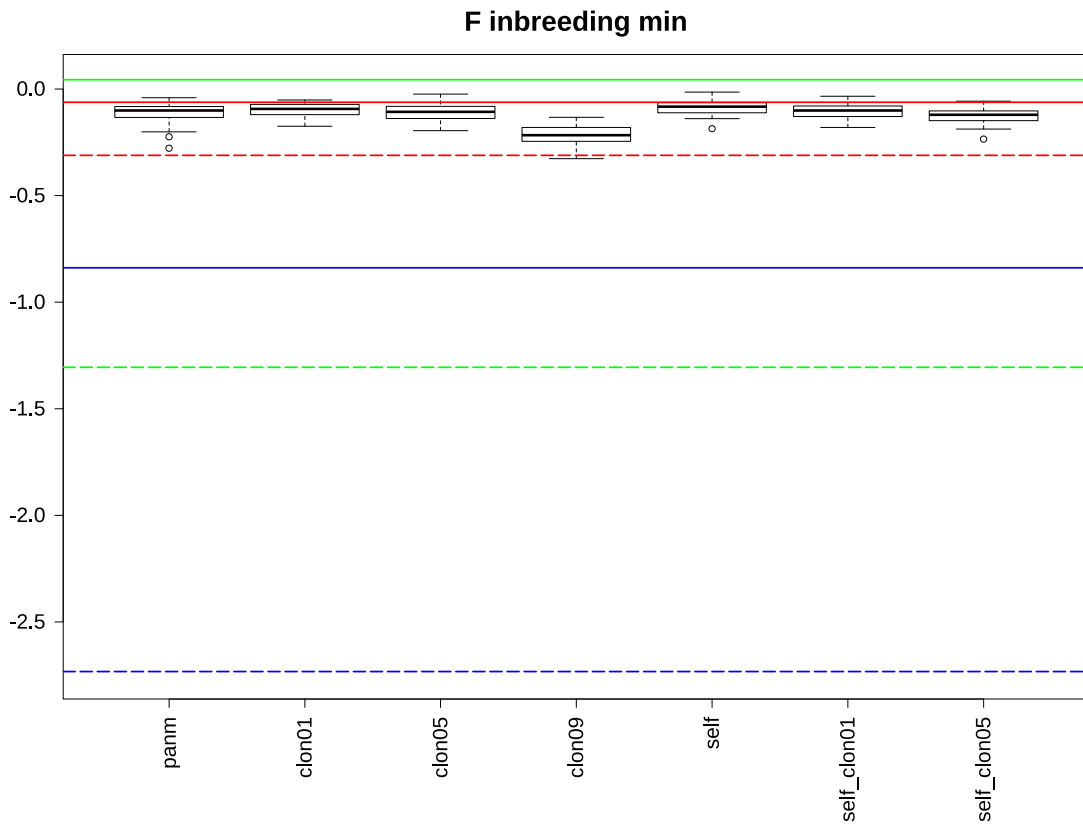
**A)** average of  $F$  parameter over simulation replicates ; horizontal lines give observed values for the different datasets : green : all, red : Oman, blue : French Polynesia, continuous line : 75\_75, dotted line : 95\_75.



**B)** maximum of  $F$  parameter over simulation replicates ; horizontal lines give observed values for the different datasets : green : all, red : Oman, blue : French Polynesia, continuous line : 75\_75, dotted line : 95\_75.



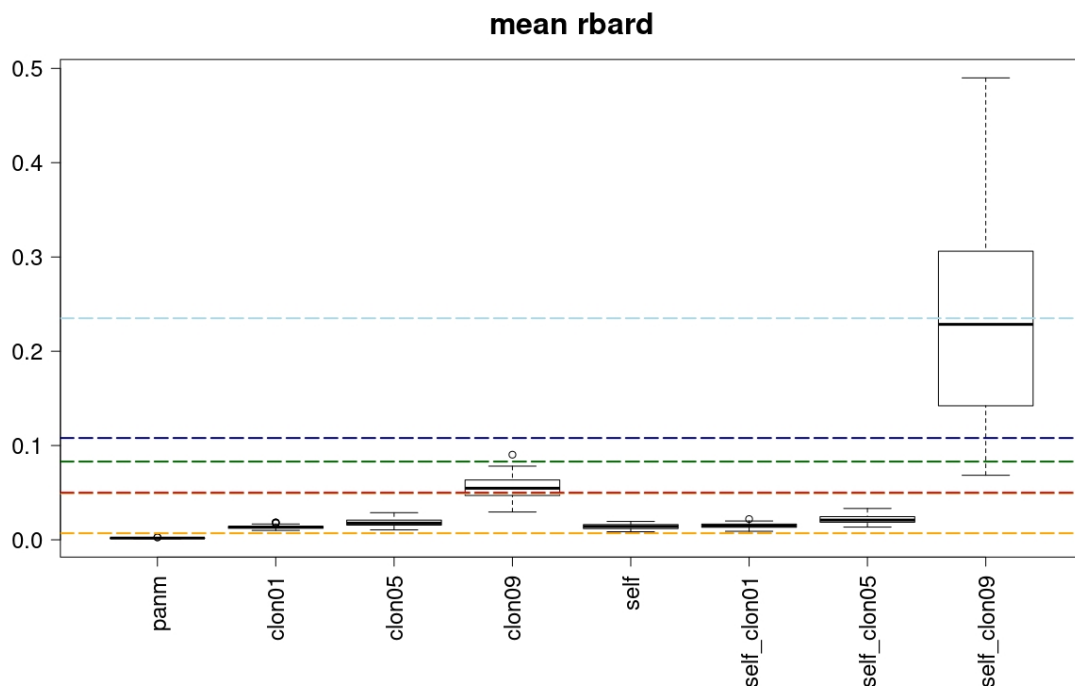
C) minimum of  $F$  parameter over simulation replicates ; horizontal lines give observed values for the different datasets : green : all ; red : Oman ; blue : French Polynesia ; continuous line : 75\_75 ; dotted line : 95\_75.



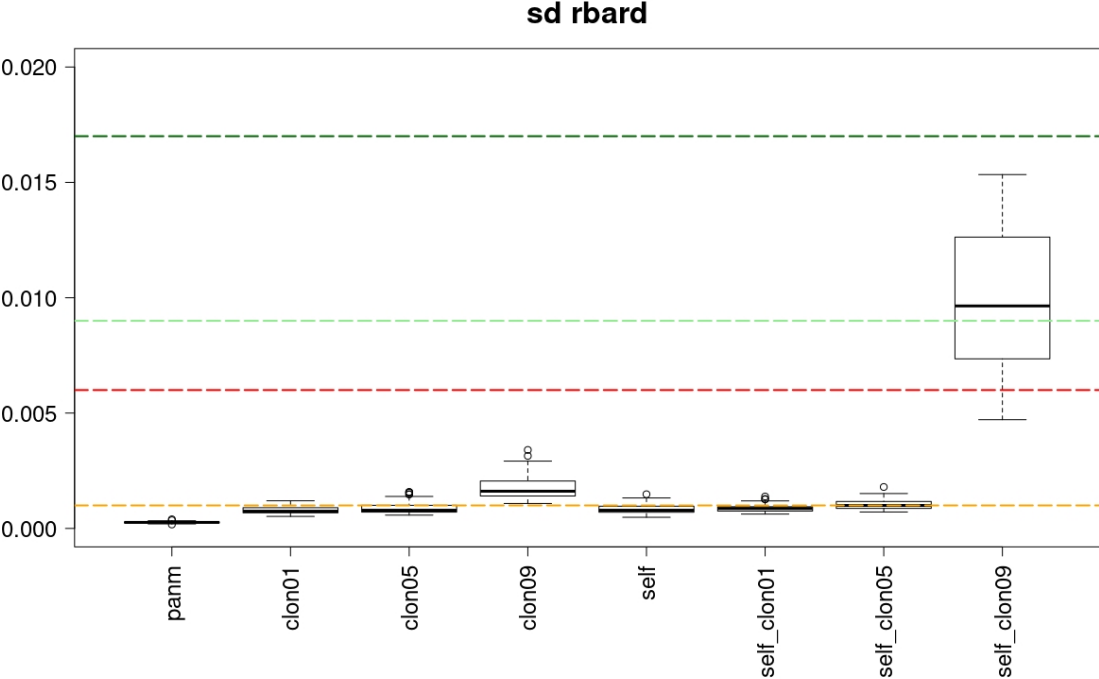
### Analysis of linkage disequilibrium :

The figures below show the distribution of the mean (A) and standard deviation (B) of  $\bar{r}_d$  over simulations. Note that  $\bar{r}_d$  was here computed separately for the two simulated populations in each simulation, with mean and standard deviations computed across subsampling replicates in each population. These distributions are compared to samples with the two highest observed mean values obtained in the 75\_75 datasets at the level of populations or mitochondrial lineages : All\_75\_75 : 3g (light green) and 5a (dark green) ; Oman\_75\_75 : 3g (red) and 7a (orange) ; Polynesia\_75\_75 : MH (light blue) and TV (dark blue) ; close values

**A) mean of  $\bar{r}_d$**  ; the lines for All\_75\_75 3g (light green) and Oman\_75\_75 3g overlap (red).



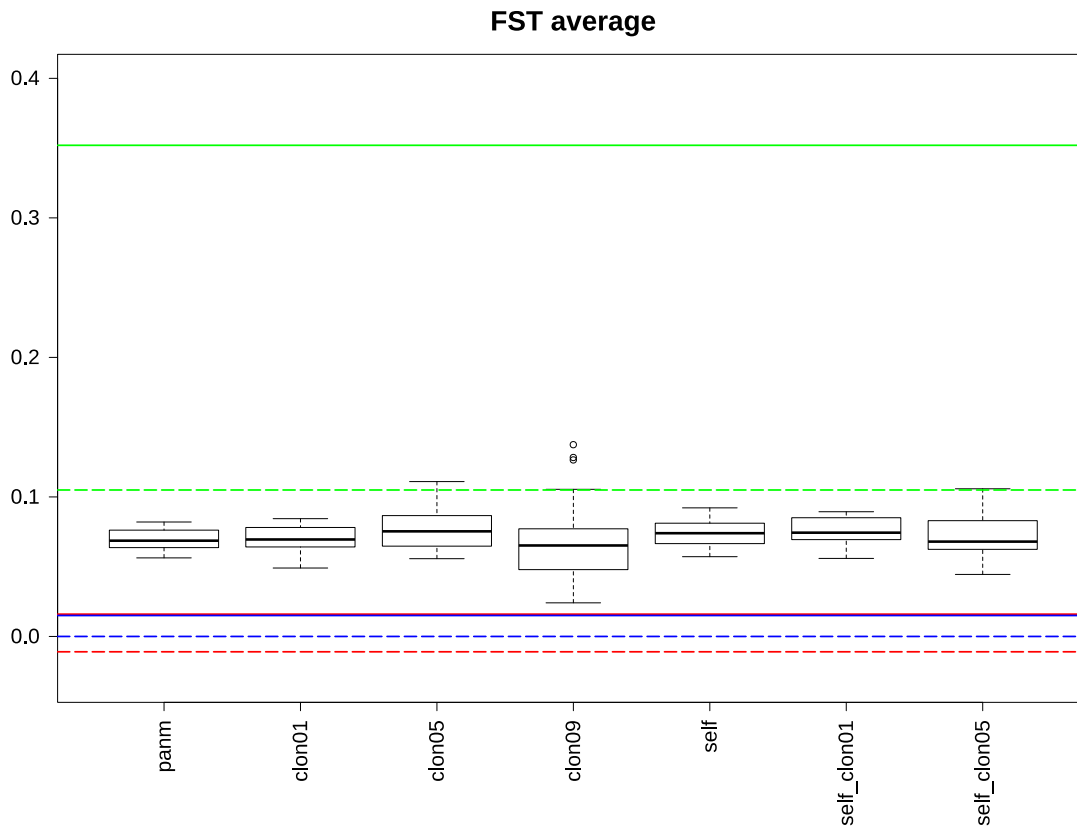
**B)** standard deviation of  $\bar{r}_d$  ; for clarity we did not plot the lines for Polynesia\_75\_75 MH (s.d. = 0.072) and TV (s.d. = 0.065).





### Distribution of $F_{ST}$ estimate :

The figures below show the distribution of the  $F_{ST}$  (average over loci) between the two populations over 30 simulations, for the different simulation configurations. These distributions are compared to observed average, maximum and minimum values displayed as horizontal lines for the different datasets : green : all, red : Oman, blue : French Polynesia, continuous line : 75\_75, dotted line : 95\_75.



### Discussion of the results of individual-based simulations:

The average and  $F$  values were higher for the simulations including selfing. The maximum  $F$  values were highly variable and tended to be higher for the configurations with the highest clonality rates (from 0.5), but were much higher for the simulations including selfing. Regarding the minimum  $F$  value, a decrease in the distribution was observed for the highest levels of clonality compared to other configurations. When comparing these results with observed data, one should note that for the All dataset, the  $F$  estimates were based on the pool of Oman and French Polynesia samples, whereas for the simulations we analysed the two simulated populations separately. The average  $F$  obtained in the All\_75\_75 and Polynesia\_75\_75 was higher than all values obtained with simulations. Regarding the maximum  $F$  values, the highest values observed in All\_75\_75 and Oman\_95\_75 appeared only compatible with simulations integrating selfing. For the minimum  $F$  values, no configuration allowed to recover such highly negative  $F$  as those observed in French Polynesia (both datasets), nor in All\_95\_75.

We analysed the linkage disequilibrium within each of the two simulated populations with the  $\bar{r}_d$  index. An increase in  $\bar{r}_d$  was observed with increasing clonality rate, mainly for the highest clonality rate tested here (0.9). A much higher increase both in mean and standard deviation was observed for a combination of selfing and a 0.9 clonality rate. We compared these values to the highest observed values obtained with the 75\_75 datasets. Apart from one value in Oman, these mean observed values were similar or higher to those obtained with simulations at clonal rates of 0.9. The standard deviation for the observed estimates were usually very high, and in most cases these values were only approached by simulations with selfing and a 0.9 clonal rate.

Regarding the average  $F_{ST}$ , without any variation neither in census size nor migration rate, the resulting values were mostly similar among simulation configurations. A slight decrease and higher variance was nevertheless observed for the highest clonality rate (0.9). The observed  $F_{ST}$  for the Oman and French Polynesia datasets were lower than those obtained in all simulations, while the  $F_{ST}$  for the All datasets were higher than those obtained in almost all simulations, except a for a few simulations performed with the highest clonality rates.