High connectivity within restricted distribution range in *Pocillopora* corals

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Abstract :

Aim

Convergence, stasis and plasticity can frequently confound our understanding of species distributions in the seas. Yet delimiting species and understanding population connectivity across marine environments is mandatory for establishing appropriate management of coral reefs, which are experiencing critical declines. We test whether morphospecies from Pocillopora corals found on outer reef slopes are unique species or species complexes, in order to correctly define their respective distributions and consequently to accurately assess their population connectivity.

Location

Archipelagos and islands of the Western Indian Ocean and the Southern Pacific (New Caledonia, Tonga, French Polynesia).

Taxon

Pocillopora eydouxi/meandrina and Pocillopora verrucosa morphospecies (Scleractinia).

Methods

We analysed the 13-microsatellite genotypes of 4837 colonies from six understudied ecoregions in the southern part of the genus distribution, to first explore the genetic partitioning within morphospecies. We then characterized the spatial distribution of each delimited species and analysed patterns of genetic diversity and connectivity for each species separately.

Results

Both morphospecies are complexes of species, each found almost exclusively in the Indian or the Pacific Oceans. Moreover, some of these cryptic species are found in sympatry over their whole distribution, which sometimes was very restricted. However, within each species, genetic diversity and connectivity

were relatively high, although some populations were found differentiated for some species, while not for others.

Main conclusions

A weak connectivity was found between the Indian and Pacific Oceans, but high connectivity within both oceans, supporting the existence of a barrier impeding gene flow between both ocean basins in Pocillopora. Although constrained by the same geography and current patterns, some sympatric species present different connectivity patterns, demonstrating the importance of multi-species connectivity models to set up appropriate management plans.

SIGNIFICANCE STATEMENT

Pocillopora corals are widely distributed through the Indo-Pacific and play crucial roles in reef ecosystems functioning. Yet, some species remain understudied in terms of genetic connectivity. Here, based on 13-microsatellite genotypes, we first delimited the species within *Pocillopora* morphospecies found on outer reef slopes, to then assess the population connectivity in the Western Indian Ocean and the Southern Pacific. We revealed a weak connectivity between, but not within, both regions, with different patterns among species even in sympatry.

INTRODUCTION

Genetic connectivity, the process linking habitat patches and populations through the exchange of organisms, and, ultimately, gene flow across the marine environment, is a key driver of population dynamics, genetic structure and diversification processes of marine organisms (e.g. Bowen, Rocha, Toonen, & Karl, 2013; Cowen, Gawarkiewicz, Pineda, Thorrold, & Werner, 2007). Knowledge of seascape and population connectivity ideally forms the basis for the definition of management and conservation units (Cowen et al., 2007). However, studying population genetic connectivity is first a matter of knowing what we work on, i.e. accurately delimiting evolutionary units (e.g. Sheets, Warner, & Palumbi, 2018). Indeed, the populations among which we want to assess exchanges of alleles must be comprised of individuals that belong to a unique and same species, in order to estimate genetic distances among comparable entities (i.e. the units of connectivity). In other words, incorrectly delimiting species and misidentifying these units of connectivity make us missing the point from an ecological and evolutionary point of view, as connectivity is hidden by the differences in species proportions at each location.

Accurately estimating population genetic connectivity is particularly relevant for coral reefs. Indeed, as coral reefs face multiple threats (e.g. global warming, habitat destruction, overfishing, ocean acidification; reviewed in Wilkinson, 1999), some international initiatives were set up in order to estimate health of coral reef ecosystems and to get a better knowledge of reef functioning for their conservation and management [e.g. International Coral Reef Initiative (ICRI), Global Coral Reef Monitoring Network (GCRMN)]. Often, data at the basis of such conservation and management plans are list of species and estimation of connectivity (*via* population genetics, otolithometry or dispersal modelling) with the ultimate goal of creating networks of Marine Protected Areas (MPAs).

One of the key components of Indo-Pacific reefs are the corals from *Pocillopora* genus as its branching colonies are abundantly distributed in the whole Indo-Pacific and the Red Sea, making it the main bio-constructor in some places (e.g. Benzoni, Bianchi, & Morri, 2003). Although playing a crucial role and being the object of numerous ecological studies, a complete taxonomic revision of the genus found that under some species names (exclusively defined by morphological characteristics of the colony, i.e. morphospecies), are grouped different divergent lineages (Gélin, Postaire, Fauvelot, & Magalon, 2017; Schmidt-Roach, Miller, Lundgren, & Andreakis, 2014). For example, for *P. damicornis*, five lineages have been described and some of them can be found in sympatry at the reef scale, without being distinguishable morphologically at first glance in the field. Thus, genetic and ecological studies dealing with *P. damicornis* prior to this revision may be examined with caution. Using genetic species delimitation methods (independently of morphology), Gélin et al. (2017) defined within the *Pocillopora* genus 16 Primary Species Hypotheses (PSHs *sensu* Pante et al., 2015) and a few of these PSHs were partitioned into several Secondary Species Hypotheses (SSHs *sensu* Pante et al., 2015). Furthermore, some PSHs, and even more some SSHs, were found to be geographically restricted, while the corresponding morphospecies were thought to be widely distributed over the whole distribution range of the genus (Veron, 2000). For example, the morphospecies *P. eydouxi/meandrina* (*sensu* Schmidt-Roach et al., 2014 and corresponding to *Pocillopora* PSH09 in Gélin et al., 2017) is sub-divided in three SSHs: one is restricted to the Indian

101   Ocean, while the two others to the Pacific, and in sympatry at the reef scale (Gélin, Fauvelot, Bigot,

102   Baly, & Magalon, 2018). Examining the genetic diversity of each SSH more deeply revealed that

103   each SSH is subdivided into highly differentiated clusters, also found in sympatry at the reef scale

104   (Gélin, Fauvelot, et al., 2018). These clusters could represent distinct species, or distinct genetic

105   lineages engaged in a speciation process, but an integrative taxonomic study is needed to fully

106   conclude where to put species boundaries. Thus, distinguishing SSHs, and even less clusters, on

107   *corallum* macromorphology (i.e. on macromorphological characters such as the overall growth form

108   of the colonies, or the branches shape and thickness) seems not possible, making sampling in the field

109   quite tricky for studies that wish to work on the same species (e.g. Brener-Raffalli et al., 2018). It

110   seems crucial to identify *Pocillopora* colonies molecularly prior to experiments or analyses to know

111   what we are working on, if we want to be more precise than the genus level. Thus, this nested

112   partitioning (PSH > SSH > Cluster), reminding of Russian dolls, obliges to think about the unit on

113   which connectivity should be assessed. Whatever the causes facing this fine partitioning, the matter

114   is not how to estimate connectivity but on what. Meanwhile, we considered both SSHs and clusters

115   as our reference units and assessed genetic differentiation among populations at both levels of

116   partitioning to not miss the true image of connectivity.

117   Moreover, with exception of *P. damicornis sensu lato* that is found often in shallow water and

118   thus easily accessible by snorkeling, some species from *Pocillopora* genus remained underexplored

119   in terms of genetic connectivity as they are found in outer reef slopes (i.e. in deeper water implying

120   scuba-diving to be sampled), despite their undeniable role in reef ecosystems over the Indian and

121   Pacific Oceans. This is particularly true for the *P. eydouxi/meandrina* species complex (PSH09), for

122   which almost no data is available (this was only addressed superficially in Gélin, Fauvelot, et al.,

123   2018), for *P. verrucosa* (PSH13) with few studies available (e.g. Ridgway, Riginos, Davis, & Hoegh-

124   Guldberg, 2008; Souter, Henriksson, Olsson, & Grahn, 2009), and for some recently delimited PSHs

125   found in restricted area (e.g. PSH14 in French Polynesia and called *P. meandrina* in Magalon,

126   Adjeroud, & Veuille, 2005). In front of such a lack of knowledge, we explored the genetic diversity

127   of colonies from these three PSHs, sampled in three marine provinces located at the southern part of

128   the genus distribution range, which are largely understudied: the Western Indian Ocean, the Tropical

129   Southwestern Pacific and the South-East Polynesia, using 13 microsatellite loci. Our aim was first to

130   explore the genetic partitioning within PSH13 and PSH14, as was realized for PSH09 (Gélin,

131   Fauvelot, et al., 2018), in order to define the connectivity units (i.e. the SSHs and the clusters) and to

132   characterize their distribution range. Then, once these units were defined, the genetic structure and

133   connectivity among populations were assessed for each unit separately, at different spatial scales

134   (site < island < ecoregion < province) and partitioning levels (SSH and cluster), in both oceans. The

135   resulting connectivity patterns were then compared among them.

136

137   **MATERIALS AND METHODS**

138   **Sampling**

139   Colonies of *Pocillopora* were sampled [branch tip + photographs except for Tromelin Island and the

140   Society Islands], independently of their *corallum* macromorphology (a non-discriminant character in

141 this genus; Gélin et al., 2017; Pinzón et al., 2013; Schmidt-Roach et al., 2014), from March 2001 to
142 October 2016, in three marine provinces: the Western Indian Ocean (WIO), the Tropical
143 Southwestern Pacific (TSP) and the South-East Polynesia (SEP), extended over six ecoregions
144 (Spalding et al., 2007). The sampling followed a hierarchical scheme with several islands within a
145 province and several sites within an island (province > ecoregion > island > site; Fig. 1; see
146 Appendix S1, Table S1.1 in Supporting Information). It represented a total of 16 islands (including
147 large islands: Madagascar and New Caledonia), a hundred sampling sites, and over 9,000 *Pocillopora*
148 colonies. For a given site, colonies were usually sampled at the same depth, during one single dive,
149 so that the range of sampling for each site did not exceed some hundreds of square meters and the
150 distance between two colonies within a site varied from few centimeters to few meters, depending on
151 the density of *Pocillopora* colonies.

152

DNA extraction, microsatellite genotyping and PSH identification

154 From the sampled colonies, DNA was extracted using DNeasy Blood & Tissue kit (Qiagen^{TM}).
155 Colonies were genotyped using 13 microsatellite loci as in Gélin et al. (2017). Then, to identify
156 colonies belonging to the three PSHs studied here (PSH09, PSH13 and PSH14), all colonies were
157 assigned to one of the PSHs delimited in Gélin et al. (2017), using Bayesian assignment tests
158 performed with STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) and considering an
159 assignment probability $P \geq 0.75$, as in Gélin, Fauvelot, et al. (2018): over the ~ 9,000 sampled
160 colonies, 2,507 were assigned to PSH09, 2,162 to PSH13 and 168 to PSH14 (i.e. a total of 4,837
161 colonies; see Appendix S1, Table S1.1).

162

Identifying SSHs and clusters within PSH13 and PSH14

164 As for PSH09 (see Gélin, Fauvelot, et al., 2018), we used and compared the results of assignment
165 tests [STRUCTURE (Pritchard et al., 2000) and DAPC (Jombart, Devillard, & Balloux, 2010)],
166 Minimum Spanning Trees (MST; EDENETWORKS 2.18; Kivelä, Arnaud-Haond, & Saramäki, 2015)
167 and F_{ST} (Weir & Cockerham, 1984) to confirm or infirm the existence of the three SSHs previously
168 described for PSH13 (SSH13a, SSH13b and SSH13c; Gélin et al., 2017), and to explore the genetic
169 partitioning within PSH14. Then, each confirmed SSH was analyzed separately to determine the
170 number of clusters within each of them, as in Gélin, Fauvelot, et al. (2018).
171 As said, all further analyses were led both at the SSH and the cluster levels. A population was
172 thus defined as all the colonies assigned to a given SSH or a given cluster with an assignment
173 probability $P \geq 0.75$, sampled at the same site and the same date. Therefore, for PSH09, the admixed
174 colonies (i.e. with an assignment probability P to any cluster < 0.75) found in the previous analyses
175 from Gélin, Fauvelot, et al. (2018) were removed for further analyses at the cluster level, differing
176 slightly the number of colonies for each confirmed cluster.

177

Genetic diversity and population structure within each SSH and each cluster

179 First, considering the whole dataset (i.e. the 4,837 colonies from PSH09, PSH13 and PSH14),
180 identical Multi-Locus Genotypes (MLGs) were identified using the package 'RClone' (Bailleul,

181 Stoeckel, & Arnaud-Haond, 2016) from the software R 3.1.1 (R Core Team, 2016), to check for
182 clonal propagation.

183   For further analyses, only the populations with a number of colonies $N \geq 10$ were considered.
184 Linkage Disequilibrium (LD) was tested using ARLEQUIN 3.5 (Excoffier, Laval, & Schneider, 2005)
185 among all pairs of loci within each population with 10^3 permutation tests. Null allele frequencies and
186 other potential technical biases were assessed with MICRO-CHECKER 2.2.3 (van Oosterhout,
187 Hutchinson, Wills, & Shipley, 2004). The mean numbers of alleles and private alleles per locus and
188 per population (Na and Np, respectively) were estimated using the R package 'poppr' (Kamvar,
189 Tabima, & Grünwald, 2013). Observed (Ho) and expected (He) heterozygosities and tests for Hardy-
190 Weinberg equilibrium were computed using ARLEQUIN within all populations and over all loci.

191   Then differentiation indices between pairs of populations [F_{ST} (Weir & Cockerham, 1984) and
192 D_{est} (Jost, 2008)] were estimated with FSTAT 2.9.3 (Goudet, 2001) and the R package 'diveRsity'
193 (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013), respectively.

194   Mantel tests (Mantel, 1967) were performed using R (R Core Team, 2016) to evaluate the
195 correlation between the linearized genetic differentiation [Slatkin (1995)'s distance: $\frac{F_{ST}}{1-F_{ST}}$] and the ln
196 of the geographic distance among populations. Barrier analyses were also performed with the
197 BARRIER 2.2 program (Manni, Guérard, & Heyer, 2004), to highlight the geographic areas with
198 pronounced genetic discontinuity between populations within each connectivity unit. The
199 geographical coordinates and genetic distances [Nei (1978)'s standard genetic distances] were thus
200 connected by Delauney triangulation such that each connection had an associated distance, and
201 barriers were identified using a Monmonier (1973) maximum distance algorithm. Barriers support
202 was assessed through 1,000 distance matrices bootstrapped over loci with MICROSATELLITE
203 ANALYZER 4.05 (MSA; Dieringer & Schlötterer, 2003).

204   Finally, for each SSH and each cluster, a population-based network using the F_{ST} distance was
205 built with EDENETWORKS 2.18 (Kivelä et al., 2015). The percolation threshold (Dpe; i.e. the F_{ST}
206 threshold below which the network is fragmented; Rozenfeld et al., 2007) was calculated for each
207 network, but all networks were built at the same threshold (defined arbitrary at 0.10) to allow their
208 comparison.

209

210 **RESULTS**
211 **SSH and cluster identification and geographical distribution range**
212 *PSH13*
213 Concerning PSH13, the assignment tests and the MST performed with 2,162 individuals confirmed
214 the existence of the three SSHs previously found on a lower number of colonies ($N_{PSH13} = 297$ in
215 Gélin et al., 2017): 1,351 colonies were assigned to SSH13a, 195 to SSH13b and 616 to SSH13c
216 (Fig. 2; see Appendix S2 for more details). F_{ST} between pairs of SSHs varied from 0.148*** to
217 0.275*** (mean $F_{ST} = 0.213$; see Appendix S2, Table S2.4). To ease reading, when speaking of all
218 the SSHs of a given PSH, we will designate them by SSHXXs (e.g. SSH13s for all the SSHs of
219 PSH13).

220   For SSH13a and SSH13b, both restricted to the WIO, STRUCTURE and DAPC did not indicate
221   congruent results whatever the K value considered (see Appendix S2, Fig. S2.3). Thus, both SSHs
222   were considered to be composed of one unique cluster each. They were often found in sympatry at
223   the reef scale (30 sites over 39), but SSH13a was the most abundant, with 32 populations presenting
224    $N \geq 10$ (N varying from 1 to 90), while only four populations showed $N \geq 10$ for SSH13b (N varying
225   from 1 to 41; Table 1; Fig. 3b; see Appendix S1, Table S1.1).

226   On the contrary, SSH13c was mostly found in the TSP, within 32 populations with N varying
227   from 1 to 54 (17 populations with $N \geq 10$; Table 1; see Appendix S1, Table S1.1). For this SSH,
228   STRUCTURE and DAPC showed congruent results at $K = 2$, but not at higher K values (see
229   Appendix S2, Fig. S2.3), while the MST did not retrieve them obviously (see Appendix S2, Fig. S2.4).
230   The F_{ST} between both groups was 0.141***. Thus, SSH13c was partitioned into two clusters,
231   SSH13c-1 and SSH13c-2 ($P \geq 0.75$; $N_{SSH13c-1} = 206$; $N_{SSH13c-2} = 189$; Fig. 2a). Both clusters were
232   often found in sympatry in the TSP (18 sites over 25). Over 25 and 21 populations respectively,
233   SSH13c-1 presented eight populations with $N \geq 10$, while SSH13c-2, 11 populations (both clusters:
234   N varying from 1 to 27; Table 1; Fig. 3e; see Appendix S1, Table S1.1).

236   *PSH14*
237   For PSH14 ($N_{PSH14} = 168$), STRUCTURE and DAPC did not indicate congruent results whatever the
238   K value considered, and STRUCTURE assigned all colonies to each genetic group in similar
239   proportions, suggesting no further genetic partitioning (see Appendix S3). Therefore, PSH14 was
240   comprised of a unique SSH, so called SSH14, without any further partitioning, found exclusively
241   within the six sampled sites from the SEP (N varying from 17 to 29; Table 1; see Appendix S1,
242   Table S1.1).

244   *PSH09*
245   As already shown in Gélin, Fauvelot, et al. (2018), PSH09 is divided into three SSHs: SSH09a,
246   SSH09b and SSH09c.

247   SSH09a, which is exclusively found in the WIO ($N_{SSH09a} = 1{,}403$), is divided into three
248   divergent clusters (mean $F_{ST} = 0.103$; see Appendix S4, Table S4.5), found in sympatry in all sites
249   that were explored (Fig. 3a; see Appendix S1, Table S1.1). However, they differed consistently in
250   their abundance, SSH09a-2 being far less abundant than the two others ($N_{SSH09a-1} = 600$;
251    $N_{SSH09a-2} = 237$; $N_{SSH09a-3} = 473$). Over 38 populations (N varying from 1 to 33), SSH09a-1 and
252   SSH09a-3 presented 30 and 25 populations with $N \geq 10$ while SSH09a-2 only six populations
253   (Table 1; see Appendix S1, Table S1.1). Considering the whole SSH, 37 populations had $N \geq 10$ (N
254   varying from 9 to 68; Table 1; see Appendix S1, Table S1.1).

255   Concerning SSH09b ($N_{SSH09b} = 323$), 13 populations over 27 had $N \geq 10$ (N varying from 1 to
256   43; Table 1; see Appendix S1, Table S1.1). This SSH is divided into two clusters (SSH09b-1 and
257   SSH09b-2; $F_{ST} = 0.128$***), both found exclusively in the TSP [except some colonies of SSH09b-1
258   found in the WIO (12 over 244) and in the SEP (10 over 244)]. SSH09b-2 was always found in
259   sympatry with SSH09b-1, this latter presenting a larger distribution, even in TSP (Chesterfield

260 Islands), and being the most abundant ($N_{SSH09b-1} = 244$; $N_{SSH09b-2} = 62$) with 10 populations with
261 $N \geq 10$ over 27 (vs. 1 over 14 for SSH09b-2; N varying from 1 to 36 for both clusters; Table 1; Fig. 3c;
262 see Appendix S1, Table S1.1).

263 For SSH09c ($N_{SSH09c} = 781$), divided into three clusters (mean $F_{ST} = 0.127$; see Appendix S4,
264 Table S4.6), SSH09c-3 presented the largest distribution (WIO and TSP), while SSH09c-1 was only
265 found in Chesterfield Islands (TSP) and SSH09c-2, the most abundant cluster ($N_{SSH09c-1} = 273$;
266 $N_{SSH09c-2} = 302$; $N_{SSH09c-3} = 181$), only in New Caledonia and Loyalty Islands (TSP). These two latter
267 were found in sympatry with the former. Ten populations over 11, 14 and 25 were found with $N \geq 10$
268 for SSH09c-1, SSH09c-2 and SSH09c-3, respectively (N varying from 1 to 50 for the three clusters;
269 Table 1; Fig. 3d; see Appendix S1, Table S1.1), while 22 populations over 37 had $N \geq 10$ for SSH09c
270 (N varying from 1 to 56; Table 1; see Appendix S1, Table S1.1).

Table 1 *Pocillopora* PSH09, PSH13 and PSH14 summary statistics for each Secondary Species Hypothesis (SSH) and each cluster.

PSH SSH Cluster	N_{tot}	N	N_{pop}	N_{pop10}	N_a min	N_a max	N_p min	N_p max	H_o min	H_o max	H_e min	H_e max	F_{IS} min	F_{IS} max
PSH09														
SSH09a	1,403	9-68	38	37	4.18 ± 0.69	8.38 ± 1.19	0.00 ± 0.00	0.38 ± 0.18	0.31 ± 0.08	0.43 ± 0.10	0.48 ± 0.09	0.65 ± 0.08	0.183[NS]	0.524***
SSH09a-1	600	2-33	38	30	3.00 ± 0.59	6.85 ± 0.85	0.00 ± 0.00	0.31 ± 0.17	0.33 ± 0.06	0.43 ± 0.07	0.44 ± 0.08	0.61 ± 0.08	0.043[NS]	0.459**
SSH09a-2	237	1-14	37	6	3.75 ± 0.64	5.15 ± 0.73	0.00 ± 0.00	0.33 ± 0.19	0.33 ± 0.09	0.46 ± 0.10	0.49 ± 0.09	0.60 ± 0.08	0.113[NS]	0.340**
SSH09a-3	473	2-24	38	25	3.09 ± 0.61	6.15 ± 1.07	0.00 ± 0.00	0.18 ± 0.12	0.31 ± 0.09	0.46 ± 0.09	0.36 ± 0.08	0.61 ± 0.08	0.029[NS]	0.410***
SSH09b	323	1-43	27	13	4.08 ± 0.42	7.00 ± 1.24	0.00 ± 0.00	0.23 ± 0.17	0.38 ± 0.07	0.51 ± 0.07	0.57 ± 0.09	0.70 ± 0.06	0.231**	0.402**
SSH09b-1	244	1-36	27	10	4.08 ± 0.42	6.58 ± 1.12	0.00 ± 0.00	0.25 ± 0.13	0.38 ± 0.07	0.53 ± 0.07	0.59 ± 0.07	0.72 ± 0.08	0.195*	0.406**
SSH09b-2	62	1-22	14	1	4.77 ± 0.61		0.85 ± 0.25		0.41 ± 0.07		0.57 ± 0.07		0.317**	
SSH09c	781	1-56	37	22	3.67 ± 0.80	6.38 ± 0.63	0.00 ± 0.00	0.38 ± 0.14	0.23 ± 0.07	0.34 ± 0.07	0.40 ± 0.07	0.54 ± 0.08	0.301[NS]	0.520***
SSH09c-1	273	1-38	11	10	4.31 ± 0.63	5.54 ± 0.79	0.00 ± 0.00	0.31 ± 0.17	0.23 ± 0.07	0.32 ± 0.07	0.39 ± 0.08	0.49 ± 0.08	0.231*	0.418**
SSH09c-2	302	2-50	14	10	3.67 ± 0.80	6.38 ± 0.63	0.08 ± 0.08	0.85 ± 0.27	0.24 ± 0.07	0.34 ± 0.07	0.44 ± 0.11	0.51 ± 0.08	0.242*	0.520***
SSH09c-3	181	1-21	25	10	3.42 ± 0.66	4.92 ± 0.62	0.00 ± 0.00	0.31 ± 0.13	0.24 ± 0.08	0.36 ± 0.08	0.39 ± 0.09	0.55 ± 0.07	0.250[NS]	0.436**
PSH13														
SSH13a	1,351	1-90	38	32	5.08 ± 0.57	8.62 ± 0.63	0.00 ± 0.00	0.38 ± 0.24	0.39 ± 0.06	0.55 ± 0.09	0.62 ± 0.05	0.72 ± 0.06	0.195*	0.365**
SSH13b	195	1-41	31	4	3.91 ± 0.53	5.77 ± 0.81	0.09 ± 0.08	0.46 ± 0.18	0.31 ± 0.07	0.36 ± 0.07	0.52 ± 0.08	0.56 ± 0.08	0.333**	0.384**
SSH13c	616	1-54	32	17	4.00 ± 0.55	7.00 ± 0.58	0.00 ± 0.00	0.31 ± 0.24	0.34 ± 0.06	0.46 ± 0.06	0.57 ± 0.07	0.70 ± 0.04	0.167*	0.477***
SSH13c-1	206	1-27	25	8	3.92 ± 0.42	5.08 ± 0.54	0.00 ± 0.00	0.31 ± 0.13	0.35 ± 0.07	0.45 ± 0.07	0.53 ± 0.08	0.66 ± 0.05	0.179[NS]	0.464***
SSH13c-2	189	1-21	21	11	3.83 ± 0.32	5.38 ± 0.55	0.00 ± 0.00	0.38 ± 0.18	0.38 ± 0.05	0.48 ± 0.06	0.56 ± 0.07	0.64 ± 0.05	0.188*	0.364**
PSH14														
SSH14	168	17-34	6	6	4.54 ± 0.58	5.85 ± 0.71	0.00 ± 0.00	0.62 ± 0.27	0.36 ± 0.08	0.44 ± 0.09	0.51 ± 0.09	0.61 ± 0.08	0.203[NS]	0.279**

Statistics are summarized (minimum and maximum values) for the populations with at least 10 colonies ($N \geq 10$). See Appendix S1 for more details.

N_{tot}: total number of colonies, N: number of colonies per population, N_{pop}: total number of populations, N_{pop10}: number of populations with $N \geq 10$, N_a and N_p: mean numbers (± SE) of alleles and private alleles, respectively, H_o and H_e: mean (± SE) observed and expected heterozygosities, respectively, and F_{IS}: mean inbreeding coefficient [[NS]: non-significant ($P > 0.05$); *: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$; ***: $P < 0.001$].

Genetic connectivity among populations

Analyses of genetic structure and connectivity among populations were performed separately at the SSH level (seven SSHs: three in PSH09, three in PSH13 and one in PSH14) and at the cluster level within each SSH (13 clusters: eight within SSH09s, four within SSH13s and one within SSH14).

All MLGs were unique. Within each population, no significant LD among loci was detected over 78 tests, nor scoring errors or null alleles. At the SSH level, the number of alleles per locus and per population (Na) was high, varying from 3.67 ± 0.80 to 8.38 ± 1.19 for SSH09s, from 3.91 ± 0.53 to 8.62 ± 0.63 for SSH13s, and from 4.54 ± 0.58 to 5.85 ± 0.71 for SSH14, but was slightly lower at the cluster level (SSH09s: $3.00 \pm 0.59 < Na < 6.85 \pm 0.85$; SSH13s: $3.83 \pm 0.32 < Na < 8.62 \pm 0.63$; SSH14: $4.54 \pm 0.58 < Na < 5.85 \pm 0.71$). Nonetheless, the number of private alleles per locus and per population (Np) was relatively low within each SSH or each cluster ($0.00 \pm 0.00 \leq Np \leq 0.85 \pm 0.27$; Table 1; see Appendix S1, Tables S1.2 & S1.3). At both the SSH and cluster levels, the observed heterozygosity (Ho) was between 0.23 ± 0.07 and 0.53 ± 0.07 within PSH09, between 0.31 ± 0.07 and 0.55 ± 0.09 within PSH13 and between 0.36 ± 0.08 and 0.44 ± 0.09 within PSH14, while the expected heterozygosity (He) was between 0.36 ± 0.08 and 0.72 ± 0.08 within PSH09, between 0.52 ± 0.08 and 0.72 ± 0.06 within PSH13 and between 0.51 ± 0.09 and 0.61 ± 0.08 within PSH14 (Table 1; see Appendix S1, Tables S1.2 & S1.3). Almost all F_{IS} estimations were significantly positive ($0.03^{NS} \leq F_{IS} \leq 0.52{***}$), except for 6 populations over 72 within all SSH09s, 21 over 102 within SSH09 clusters, 1 over 53 within all SSH13s, 6 over 55 within SSH13 clusters and 1 over 6 within SSH14 that were not significantly different from zero (see Appendix S1, Tables S1.2 & S1.3).

Analysing genetic differentiation at the higher structuring level (i.e. the SSH level), different patterns were found: for SSH13b and SSH14, F_{ST} and D_{est} were low ($0.000^{NS} \leq F_{ST} \leq 0.085^{NS}$; $0.000^{NS} \leq D_{est} \leq 0.025{***}$) and not significantly different from zero (except for one D_{est} value between MOR4 and TAH1 in PSH14), suggesting no particular pattern of differentiation (see Appendix S5). On the contrary, for the other SSHs, F_{ST} and D_{est} estimations were high ($0.000^{NS} \leq F_{ST} \leq 0.267^{NS}$; $0.000^{NS} \leq D_{est} \leq 0.186{***}$; see Appendix S5), and often significantly different from zero. In particular, for SSH09a and SSH13a, both restricted to the WIO, some populations appeared differentiated from the others (REU5 and TRO1/2 for SSH09a; MAD05 and ROD2 for SSH13a). However, for the SSHs from the TSP (SSH09b, SSH09c and SSH13c), the obtained patterns of differentiation were difficult to interpret as many populations were differentiated. For SSH09b and SSH13c, populations seem relatively grouped per island or region, while for SSH09c, the observed pattern corresponded to the cluster partitioning: Chesterfield and Loyalty Islands (SSH09c-1 and SSH09c-3) vs. Grande Terre (SSH09c-2; see Appendix S5).

At the cluster level (except for SSH09b-2 removed of this analysis as only one population presented $N \geq 10$), different patterns were also found: for SSH09a-2 and SSH13c-2, F_{ST} and D_{est} were low ($0.000^{NS} \leq F_{ST} \leq 0.038^{NS}$; $0.000^{NS} \leq D_{est} \leq 0.026^{NS}$) and always not significantly different from zero (see Appendix S5). On the contrary, for the other clusters, F_{ST} were higher ($0.000^{NS} \leq F_{ST} \leq 0.194{***}$), such as D_{est} ($0.000^{NS} \leq D_{est} \leq 0.153{***}$; see Appendix S5), and some populations appeared differentiated from the others: REU5 for SSH09a-1, TRO2 for SSH09a-3,

MOR4 for SSH09b-1, CHE02 for SSH09c-1, NCA04 and NCA05 for SSH09c-2, LOY4 for SSH09c-3, and LOY4 and LOY5 for SSH13c-1 (Fig. 3; see Appendix S5). Except these populations, no particular pattern of differentiation was observed (see Appendix S5).

Mantel tests revealed IBD for four SSHs (SSH09a, SSH09b, SSH13a and SSH13c) and three clusters (SSH09a-1, SSH09b-1 and SSH09c-3) but R^2 was found high for only two of them (SSH09c-3: n = 45; R^2 = 0.386; P = 5.18.10^{-6}; SSH13c: n = 136; R^2 = 0.359; P = 7.70.10^{-15}; see Appendix S6, Fig. S6.6). Similarly, barrier analyses identified different barriers, depending on the SSH or the cluster, but significant barriers (i.e. supported by at least 75% of the bootstrapped matrices) were found in only seven groups: three in the WIO (SSH09a, SSH09a-2 and SSH13b), three in the TSP (SSH09c, SSH09c-1 and SSH13c-2) and one in the SEP (SSH14). In the WIO, for SSH09a, the barrier tends to isolate Tromelin Island, consistent with genetic differentiation indices, but for SSH09a-2 and SSH13b, as for SSH14 in the SEP, significant barriers are possibly due to the small number of populations with $N \geq 10$ (six, four and six, respectively). In the TSP, the barrier isolated the northernmost Chesterfield populations (CHE02 and CHE11) for SSH09c-1, while it tends to isolate Loyalty Islands populations in SSH09c and SSH13c-2 (see Appendix S7, Fig. S7.7 & S7.8).

Finally, population-based networks were built for each SSH and each cluster independently. The *Dpe* values were weak, varying from 0.02 for SSH09c-1 to 0.15 for SSH13c-2, except for SSH14 (*Dpe* = 0.31), and populations appeared relatively connected (see Appendix S8, Fig. S8.9 & S8.10).

DISCUSSION
Our results confirm that *Pocillopora* PSH13 splits into two SSHs in the WIO (SSH13a and SSH13b) and one SSH in the Pacific (SSH13c), while *Pocillopora* PSH14 appears as a single genetic entity (SSH14). Contrary to *Pocillopora* PSH09 for which each of the three SSHs was split into several differentiated, but sympatric, clusters, only one out of the three SSHs of PSH13 (SSH13c) was split into two clusters (SSH13c-1 and SSH13c-2). For each PSH, we did not identify any clone, implying that clonal propagation, notably through fragmentation, might be extremely rare in these taxa presenting robust *corallum* macromorphology. Moreover, for each cluster, we revealed a general genetic homogeneity among populations, suggesting high connectivity within the distribution range of the clusters, restricted to each ocean basin. Nevertheless, some populations were found differentiated for some clusters, while not for others.

1. Lack of connectivity across the Indo-Pacific
In this study, as in previous ones (Gélin et al., 2017; Gélin, Fauvelot, et al., 2018), *Pocillopora* PSH09 and PSH13 were found divided into three SSHs, each restricted to the ocean basin, reminding the genetic partitioning found in the Indo-Pacific for *P. damicornis* type *β* (now renamed *P. acuta*) species complex (PSH05). This latter was disentangled in four SSHs, two being restricted to the Pacific Ocean, while the two others to the Indian Ocean (Gélin, Pirog, Fauvelot, & Magalon, 2018). As for SSH14, it is restricted to the Pacific. Therefore, each of the four *Pocillopora* morphospecies

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357 described to be distributed over the widest range in the Indo-Pacific (i.e. *damicornis sensu lato*,
358 *eydouxi*, *meandrina* and *verrucosa*; cf. maps in Veron, 2000) are actually divided in divergent
359 lineages between both ocean basins. This contributes to question the existence of Indo-Pacific corals
360 [as in *Porites lobata* (Forsman, Wellington, Fox, & Toonen, 2015) or *Stylophora pistillata*
361 (Keshavmurthy et al., 2013)] and more widely of Indo-Pacific species [even more of cosmopolitan
362 ones, with the exception of introduced species; as in tropical algae (reviewed in Sherwood &
363 Zuccarello, 2016) or *Pontohedyle* sea slugs (Jörger, Norenburg, Wilson, & Schrödl, 2012)].

364   This suggests also a lack of connectivity between both oceans. Few studies found genetically
365 continuous populations between the Indian and the Pacific Oceans [e.g. among East Indian and West
366 Pacific populations of the starfish *Linckia laevigata* using seven allozymes (Williams & Benzie,
367 1996) or among WIO and Pacific populations of the reef fishes *Lutjanus kasmira* and *Lutjanus fulvus*
368 (Gaither, Toonen, Robertson, Planes, & Bowen, 2010) using mitochondrial cyt*b*]. Genetic
369 discontinuity is more often described [e.g. in the sea cucumber *Holothuria nobilis* (Uthicke & Benzie,
370 2003) and the parrotfish *Chlorurus sordidus* (Bay, Choat, van Herwerden, & Robertson, 2004) with
371 COI, in the starfish *Acanthaster planci* using nine allozymes (Benzie, 1999) or in the black tiger
372 prawn *Penaeus monodon* with EF1α gene (Duda Jr & Palumbi, 1999)]. However, results based on
373 different species with various dispersal abilities and using different molecular markers presenting
374 different modes of transmission and evolution, should be taken with caution. Indeed, in the bullshark
375 *Carcharhinus leucas,* the mitochondrial genes used supported an Indo-Pacific barrier to gene flow
376 between both ocean basins while microsatellites do not. Integrating information from both types of
377 markers and using Bayesian computation with a random forest procedure (ABC-RF), this discordance
378 was found to be due to a complete lack of contemporary gene flow (Pirog et al., 2019). Consequently,
379 it appears necessary to achieve multi-specific connectivity models with many markers distributed
380 over the whole genome to accurately estimate gene flow, and genomics seems promising for this.

381

382 **2. High connectivity within the Indian Ocean**
383 In this study, three SSHs (SSH09a, SSH13a and SSH13b) and the five resulted clusters (SSH09a-1,
384 SSH09a-2, SSH09a-3, SSH13a and SSH13b) were found almost restricted to the WIO. Whichever
385 the way the units of connectivity are defined (i.e. at the SSH or the cluster level), for each of these
386 SSHs and clusters, from a global point of view, a weak genetic structure was identified, suggesting
387 high genetic connectivity within the WIO. Moreover, no isolation-by-distance was found. A previous
388 study in the WIO showed weak genetic structure among populations of *P. verrucosa sensu lato* [we
389 were not able to identify the studied lineage(s) with the data available] using allozymes (Ridgway,
390 Hoegh-Guldberg, & Ayre, 2001), while weak connectivity was revealed between Mozambique and
391 South Africa using microsatellites (Ridgway et al., 2008). These contradictory results were in
392 accordance with a wider study on the East coast of Africa, in which *P. verrucosa* (ORF39, PSH13
393 *sensu* Gélin et al., 2017) populations, using microsatellites, appeared panmictic over large scales
394 (> 600 km), but differentiated over smaller scales (~ 1 km; Souter et al., 2009). This latter study
395 suggested that this pattern could be attributed to localized recruitment due to short dispersal distances
396 of brooded larvae, or site-specific selection, which may not be equally apparent when groups of

populations are compared over larger distances. Nevertheless, our results remained consistent with studies performed on other organisms [e.g. in the fish *Lutjanus fulviflamma* using 191 AFLP loci (Dorenbosch et al., 2006) or in the swordfish *Xiphias gladius* using 11 microsatellites and mitochondrial CR (Muths, Grewe, Jean, & Bourjea, 2009)].

However, for some other organisms, weak connectivity was detected in the WIO [e.g. in the shrimp *Penaeus monodon* using intron variability (Duda Jr & Palumbi, 1999), in the fish *Epinephelus merra* (Muths, Tessier, & Bourjea, 2015) with cyt*b* and microsatellites, or in the hydrozoans *Macrorhynchia phoenicea* (Postaire, Gélin, Bruggemann, Pratlong, & Magalon, 2017) and *Lytocarpia brevirostris* α (Postaire, Gélin, Bruggemann, & Magalon, 2017) using microsatellites]. In these previous studies, currents, geographic distances between populations or expanses of deep ocean waters have been proposed as barrier to dispersal. Besides short-distance exchanges of gametes, larval brooding and restricted movements of larvae may also explain the observed patterns.

Although the studied lineages are closely related and sympatric or neighbor, some populations were found differentiated for some SSHs or clusters (REU5 for SSH09a and SSH09a-1, TRO2 for SSH09a and SSH09a-3 and MAD05 and ROD2 for SSH13a; Fig. 3; see Appendix S5), while not for others. Similarly, barriers were different among clusters within the same SSH. If this differential structure is not an artifact of unequal population sizes among lineages (due to the lack of diagnostic characters to identify species in the field), it means that either SSHs and clusters do not all respond in the same way to environmental and/or geographical barriers, or they are not subject to the same constraints (i.e. they differ in their distribution at the microhabitat scale and are not strictly sympatric). For example, depth has already been suggested as a structuring factor in coral populations [e.g. in *Seriatopora hystrix* (van Oppen, Bongaerts, Underwood, Peplow, & Cooper, 2011) and in *P. damicornis sensu stricto* (van Oppen et al., 2018)]. In our study, colonies sampled on the same site were usually at the same depth, rejecting the depth hypothesis, but a differential microhabitat distribution (driven by species relationships for example) could be involved. Unfortunately, our sampling does not allow to test this hypothesis.

3. Structuring between, but not within, TSP and SEP?

Four SSHs (SSH09b, SSH09c, SSH13c and SSH14) were almost restricted to the Pacific Ocean, and mostly to the TSP (except SSH14). Only a handful of individuals sampled in Moorea (French Polynesia, SEP) were assigned to SSH09b and appeared highly differentiated from the others from the TSP, while the majority of the colonies were assigned to SSH14, restricted to this marine province. This suggests restricted gene flow between the TSP and the SEP in *Pocillopora*, as already highlighted in Forsman, Johnston, Brooks, Adam, and Toonen, (2013), where, using the mitochondrial ORF, several clades of *Pocillopora* were found unique to Moorea. This low connectivity might be due to distance acting as a barrier similar to the Eastern Pacific Barrier [i.e. the 5,000 km open ocean barrier separating the Tropical Eastern Pacific (Clipperton Atoll) from the Central Pacific (Hawaii); Combosch, Guzman, Schuhmacher, & Vollmer, 2008].

Looking deeper within the TSP, we observed different patterns of structure among SSHs and clusters. First, for the three SSHs (SSH09b, SSH09c and SSH13c), the structuring tended to group

populations by region or islands, but this signal was lost when considering the clusters. Indeed, for two clusters (SSH09b-1 and SSH13c-2), no particular pattern of differentiation among populations was found, suggesting high connectivity among the TSP populations for these clusters, as in one gastropod and four squat lobster-like species from New Caledonia (COI; Samadi, Bottan, Macpherson, De Forges, & Boisselier, 2006). Conversely, for the other clusters restricted to the TSP, we found some genetic structure among populations. First, within SSH09c-1, almost restricted to Chesterfield Islands, one population (CHE02; North of Bampton Islands; Fig. 3d) was differentiated from all others. This population was sampled between 25 m and 10 m depth, i.e. deeper than nearby populations, possibly restricting gene flow [as in *P. damicornis sensu stricto* (van Oppen et al., 2018)]. Second, within SSH09c-2, we found a population (NCA05; East coast of Grande Terre, New Caledonia) differentiated from the others, but not from the South-East coast of Grande Terre (NCA06 and NCA07; Fig. 3d). Within SSH09c-3 and SSH13c-1, two populations from Loyalty Islands [LOY4 (Lifou) and LOY5 (Ouvéa)] were also found differentiated (Fig. 3d & 3e). Considering these clusters alone, population differentiations could be explained by currents. Indeed, New Caledonia ecoregion is dominated by an East-West current, resulting from southeastern trade winds (Vega, Marchesiello, & Lefêvre, 2006). This current isolates Loyalty Islands from Grande Terre and from each other, and mainly bypasses Grande Terre from South, along the eastern barrier reef. Thus, it could transport NCA05 larvae to southern populations (NCA06 and NCA07; Fig. 3d & 3e). However, such a connectivity pattern should have been found in all clusters, especially since they are related genetic lineages. As in the WIO, differentiated populations could arise from a lack of power due to unequal population sizes, but a similar pattern of differentiation of Loyalty Islands populations was already found in *P. damicornis sensu stricto* (PSH04; Oury, Gélin, & Magalon, 2020) and in the hydrozoan *Macrorhynchia phoenicea* (Postaire, Gélin, Bruggemann, Pratlong, et al., 2017). Dispersal to adjacent populations along the reef over multiple generations (stepping-stone dispersal), currents, and expanses of open ocean were evoked to explain this pattern. Consequently, from an ecological point of view, these populations that are differentiated only within some clusters indicated different responses to a same, or relatively same, environment, having high implications in conservation.

In the same way, considering all the colonies belonging to SSH14 in French Polynesia, high connectivity was observed among the Society Islands, confirming the results from the first study using these colonies with four microsatellites (two are common with this study; Magalon et al., 2005). The same pattern was found in *P. damicornis sensu lato* with five microsatellites (same sites as herein; Adjeroud et al., 2014) and in the starfish *Acanthaster planci* with 16 microsatellites and the mitochondrial CR (same sites as herein; Yasuda, Taquet, Nagai, Yoshida, & Adjeroud, 2015). Interestingly, in the very first study (Magalon et al., 2005), the colonies were assigned to *P. meandrina* morphospecies while colonies from another study (Mayfield, Bruckner, Chen, & Chen, 2015) presenting the same ORF haplotype, sampled in French Polynesia and Cook Islands, were assigned to *P. verrucosa* morphospecies. This underlines one more time the need of a complete taxonomic revision of *Pocillopora* genus in view of the genetic data.

In conclusion, this study confirmed the three SSHs previously found in Gélin et al. (2017) under the *P. verrucosa* morphospecies (PSH13) in the Indian and Pacific Oceans. Moreover, this genetic partitioning of several morphospecies in distinct SSHs, and then in distinct clusters, each restricted to the ocean basin, (1) questioned the existence of Indo-Pacific species in *Pocillopora* and (2) lighted up the problem of sampling a sufficient number of colonies in such "completely cryptic species" that shared similar macromorphologies and at least a part of their mitochondrial DNA and that can only be revealed using nuclear markers. Such issues must be taken into account in biodiversity inventories using list of species and in ecological studies that wish to work on the same species to compare processes in different oceans (e.g. Brener-Raffalli et al., 2018), and even on a smaller scale (e.g. transplant experiments), as well as in connectivity studies to accurately assess gene flow. To circumvent these issues, one recommendation would be to identify colonies molecularly, prior to experiments. Finally, through comparisons of the connectivity patterns obtained within clusters, this study suggests that despite being relatively close genetically, and constrained by the same geographical and environmental patterns, not all clusters respond in the same way. This should be kept in mind when setting up management plans.

516

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

519

DATA ACCESSIBILITY

522

REFERENCES

Adjeroud, M., Guérécheau, A., Vidal-Dupiol, J., Flot, J.-F., Arnaud-Haond, S., & Bonhomme, F. (2014). Genetic diversity, clonality and connectivity in the scleractinian coral *Pocillopora damicornis* : A multi-scale analysis in an insular, fragmented reef system. *Marine Biology*, *161*(3), 531–541. https://doi.org/10.1007/s00227-013-2355-9

Bailleul, D., Stoeckel, S., & Arnaud-Haond, S. (2016). RClone : A package to identify MultiLocus Clonal Lineages and handle clonal data sets in R. *Methods in Ecology and Evolution*, *7*(8), 966–970. https://doi.org/10.1111/2041-210X.12550

Bay, L. K., Choat, J. H., van Herwerden, L., & Robertson, D. R. (2004). High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*) : Evidence of an unstable evolutionary past? *Marine Biology*, *144*(4), 757-767. https://doi.org/10.1007/s00227-003-1224-3

Benzie, J. A. (1999). Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution*, *53*(6), 1782–1795. https://doi.org/10.1111/j.1558-5646.1999.tb04562.x

Benzoni, F., Bianchi, C. N., & Morri, C. (2003). Coral communities of the northwestern Gulf of Aden (Yemen) : Variation in framework building related to environmental factors and biotic conditions. *Coral Reefs*, *22*(4), 475-484. https://doi.org/10.1007/s00338-003-0342-1

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology & Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Brener-Raffalli, K., Vidal-Dupiol, J., Adjeroud, M., Rey, O., Romans, P., Bonhomme, F., … Toulza, E. (2018). Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals. *PCI Ecology*, 398602. https://doi.org/10.1101/398602

Combosch, D. J., Guzman, H. M., Schuhmacher, H., & Vollmer, S. V. (2008). Interspecific hybridization and restricted trans-Pacific gene flow in the Tropical Eastern Pacific *Pocillopora*. *Molecular Ecology*, *17*(5), 1304–1312. https://doi.org/10.1111/j.1365-294X.2007.03672.x

Cowen, R. K., Gawarkiewicz, G., Pineda, J., Thorrold, S. R., & Werner, F. E. (2007). Population connectivity in marine systems an overview. *Oceanography*, *20*(3), 14–21.

Dieringer, D., & Schlötterer, C. (2003). MICROSATELLITE ANALYSER (MSA) : A platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, *3*(1), 167-169. https://doi.org/10.1046/j.1471-8286.2003.00351.x

Dorenbosch, M., Pollux, B. J. A., Pustjens, A. Z., Rajagopal, S., Nagelkerken, I., van der Velde, G., & Moon-van der Staay, S. Y. (2006). Population structure of the Dory snapper, *Lutjanus fulviflamma*, in the western Indian Ocean revealed by means of AFLP fingerprinting. *Hydrobiologia*, *568*(1), 43–53. https://doi.org/10.1007/s10750-006-0020-8

Duda Jr, T. F., & Palumbi, S. R. (1999). Population structure of the black tiger prawn, *Penaeus monodon*, among western Indian Ocean and western Pacific populations. *Marine Biology*, *134*(4), 705–710. https://doi.org/10.1007/s002270050586

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0) : An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, *1*, 47–50. https://doi.org/10.1177/117693430500100003

566 Forsman, Z., Johnston, E., Brooks, A., Adam, T., & Toonen, R. J. (2013). Genetic evidence for
567   regional isolation of *Pocillopora* corals from Moorea. *Oceanography*, *26*(3), 153-155.
568   https://doi.org/10.5670/oceanog.2013.58
569 Forsman, Z., Wellington, G. M., Fox, G. E., & Toonen, R. J. (2015). Clues to unraveling the coral
570   species problem : Distinguishing species from geographic variation in *Porites* across the
571   Pacific with molecular markers and microskeletal traits. *PeerJ*, *3*, e751.
572   https://doi.org/10.7717/peerj.751
573 Gaither, M. R., Toonen, R. J., Robertson, D. R., Planes, S., & Bowen, B. W. (2010). Genetic
574   evaluation of marine biogeographical barriers : Perspectives from two widespread Indo-
575   Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). *Journal of Biogeography*, *37*(1),
576   133-147. https://doi.org/10.1111/j.1365-2699.2009.02188.x
577 Gélin, P., Fauvelot, C., Bigot, L., Baly, J., & Magalon, H. (2018). From population connectivity to
578   the art of striping Russian dolls : The lessons from *Pocillopora* corals. *Ecology and*
579   *Evolution*, *8*(2), 1411-1426. https://doi.org/10.1002/ece3.3747
580 Gélin, P., Pirog, A., Fauvelot, C., & Magalon, H. (2018). High genetic differentiation and low
581   connectivity in the coral *Pocillopora damicornis* type *β* at different spatial scales in the
582   Southwestern Indian Ocean and the Tropical Southwestern Pacific. *Marine Biology*,
583   165-167. https://doi.org/10.1007/s00227-018-3428-6
584 Gélin, P., Postaire, B., Fauvelot, C., & Magalon, H. (2017). Reevaluating species number,
585   distribution and endemism of the coral genus *Pocillopora* Lamarck, 1816 using species
586   delimitation methods and microsatellites. *Molecular Phylogenetics and Evolution*, *109*,
587   430–446. https://doi.org/10.1016/j.ympev.2017.01.018
588 Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices
589   version 2.9.3.2, updated from Goudet 1995. (Version 2.9.3.2).
590 Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components : A
591   new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94.
592   https://doi.org/10.1186/1471-2156-11-94
593 Jörger, K. M., Norenburg, J. L., Wilson, N. G., & Schrödl, M. (2012). Barcoding against a paradox?
594   Combined molecular species delineations reveal multiple cryptic lineages in elusive
595   meiofaunal sea slugs. *BMC Evolutionary Biology*, *12*(1), 245. https://doi.org/10.1186/1471-
596   2148-12-245
597 Jost, L. O. U. (2008). G_{ST} and its relatives do not measure differentiation. *Molecular Ecology*,
598   *17*(18), 4015–4026. https://doi.org/10.1111/j.1365-294X.2008.03887.x
599 Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2013). Poppr : An R package for genetic analysis
600   of populations with clonal or partially clonal reproduction. *PeerJ PrePrints*, *4*, e281.
601   https://doi.org/10.7287/peerj.preprints.161v1
602 Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity : An R
603   package for the estimation and exploration of population genetics parameters and their
604   associated errors. *Methods in Ecology and Evolution*, *4*(8), 782-788.
605   https://doi.org/10.1111/2041-210X.12067
606 Keshavmurthy, S., Yang, S.-Y., Alamaru, A., Chuang, Y.-Y., Pichon, M., Obura, D., … Chen, C.
607   A. (2013). DNA barcoding reveals the coral "laboratory-rat", *Stylophora pistillata*
608   encompasses multiple identities. *Scientific Reports*, *3*(1), 1520.
609   https://doi.org/10.1038/srep01520
610 Kivelä, M., Arnaud-Haond, S., & Saramäki, J. (2015). EDENetworks : A user-friendly software to
611   build and analyse networks in biogeography, ecology and population genetics. *Molecular*
612   *Ecology Resources*, *15*(1), 117-122. https://doi.org/10.1111/1755-0998.12290
613 Magalon, H., Adjeroud, M., & Veuille, M. (2005). Patterns of genetic variation do not correlate
614   with geographical distance in the reef-building coral *Pocillopora meandrina* in the South
615   Pacific. *Molecular Ecology*, *14*(7), 1861-1868. https://doi.org/10.1111/j.1365-
616   294X.2005.02430.x

617 Manni, F., Guérard, E., & Heyer, E. (2004). Geographic patterns of (genetic, morphologic,
618   linguistic) variation : How barriers can be detected by using Monmonier's algorithm.
619   *Human Biology*, *76*(2), 173–190. https://doi.org/10.1353/hub.2004.0034

620 Mantel, N. (1967). The detection of disease clustering and a generalized regression approach.
621   *Cancer Research*, *27*, 209–220.

622 Mayfield, A. B., Bruckner, A. W., Chen, C.-H., & Chen, C.-S. (2015). A survey of pocilloporid
623   corals and their endosymbiotic dinoflagellate communities in the Austral and Cook Islands
624   of the South Pacific. *Platax*, *12*, 1–17.

625 Monmonier, M. S. (1973). Maximum-difference barriers : An alternative numerical regionalization
626   method. *Geographical Analysis*, *5*(3), 245–261.

627 Muths, D., Grewe, P., Jean, C., & Bourjea, J. (2009). Genetic population structure of the Swordfish
628   (*Xiphias gladius*) in the southwest Indian Ocean : Sex-biased differentiation, congruency
629   between markers and its incidence in a way of stock assessment. *Fisheries Research*, *97*(3),
630   263–269. https://doi.org/10.1016/j.fishres.2009.03.004

631 Muths, D., Tessier, E., & Bourjea, J. (2015). Genetic structure of the reef grouper *Epinephelus*
632   *merra* in the West Indian Ocean appears congruent with biogeographic and oceanographic
633   boundaries. *Marine Ecology*, *36*(3), 447–461. https://doi.org/10.1111/maec.12153

634 Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of
635   individuals. *Genetics*, *89*(3), 583–590.

636 Oury, N., Gélin, P., & Magalon, H. (2020). Cryptic species and genetic connectivity among
637   populations of the coral *Pocillopora damicornis* (Scleractinia) in the tropical southwestern
638   Pacific. *Marine Biology*, *167*, 142. https://doi.org/10.1007/s00227-020-03757-z

639 Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., … Samadi, S.
640   (2015). Species are hypotheses : Avoid connectivity assessments based on pillars of sand.
641   *Molecular Ecology*, *24*(3), 525-544. https://doi.org/10.1111/mec.13048

642 Pinzón, J. H., Sampayo, E., Cox, E., Chauka, L. J., Chen, C. A., Voolstra, C. R., & LaJeunesse, T.
643   C. (2013). Blind to morphology : Genetics identifies several widespread ecologically
644   common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*,
645   Scleractinia). *Journal of Biogeography*, *40*, 1595–1608. https://doi.org/10.1111/jbi.12110

646 Pirog, A., Ravigné, V., Fontaine, M. C., Rieux, A., Gilabert, A., Cliff, G., … Magalon, H. (2019).
647   Population structure, connectivity, and demographic history of an apex marine predator, the
648   bull shark *Carcharhinus leucas*. *Ecology and Evolution*. https://doi.org/10.1002/ece3.5597

649 Postaire, B., Gélin, P., Bruggemann, J. H., & Magalon, H. (2017). One species for one island?
650   Unexpected diversity and weak connectivity in a widely distributed tropical hydrozoan.
651   *Heredity*, *118*(4), 385-394. https://doi.org/10.1038/hdy.2016.126

652 Postaire, B., Gélin, P., Bruggemann, J. H., Pratlong, M., & Magalon, H. (2017). Population
653   differentiation or species formation across the Indian and the Pacific Oceans? An example
654   from the brooding marine hydrozoan *Macrorhynchia phoenicea*. *Ecology and Evolution*,
655   *7*(20), 8170-8186. https://doi.org/10.1002/ece3.3236

656 Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using
657   multilocus genotype data. *Genetics*, *155*(2), 945–959.

658 R Core Team. (2016). R : A language and environment for statistical computing (Version 3.3.1). R
659   Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

660 Ridgway, T., Hoegh-Guldberg, O., & Ayre, D. (2001). Panmixia in *Pocillopora verrucosa* from
661   South Africa. *Marine Biology*, *139*(1), 175–181. https://doi.org/10.1007/s002270100573

662 Ridgway, T., Riginos, C., Davis, J., & Hoegh-Guldberg, O. (2008). Genetic connectivity patterns of
663   *Pocillopora verrucosa* in southern African Marine Protected Areas. *Marine Ecology*
664   *Progress Series*, *354*, 161–168. https://doi.org/10.3354/meps07245

665 Rozenfeld, A. F., Arnaud-Haond, S., Hernández-García, E., Eguíluz, V. M., Matías, M. A., Serrão,
666   E., & Duarte, C. M. (2007). Spectrum of genetic diversity and networks of clonal organisms.
667   *Journal of The Royal Society Interface*, *4*(17), 1093-1102.
668   https://doi.org/10.1098/rsif.2007.0230

669 Samadi, S., Bottan, L., Macpherson, E., De Forges, B. R., & Boisselier, M.-C. (2006). Seamount
670   endemism questioned by the geographic distribution and population genetic structure of
671   marine invertebrates. *Marine Biology*, *149*(6), 1463–1475. https://doi.org/10.1007/s00227-
672   006-0306-4
673 Schmidt-Roach, S., Miller, K. J., Lundgren, P., & Andreakis, N. (2014). With eyes wide open : A
674   revision of species within and closely related to the *Pocillopora damicornis* species complex
675   (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the*
676   *Linnean Society*, *170*(1), 1-33. https://doi.org/10.1111/zoj.12092
677 Sheets, E. A., Warner, P. A., & Palumbi, S. R. (2018). Accurate population genetic measurements
678   require cryptic species identification in corals. *Coral Reefs*, *37*(2), 549–563.
679   https://doi.org/10.1007/s00338-018-1679-9
680 Sherwood, A. R., & Zuccarello, G. C. (2016). Phylogeography of Tropical Pacific marine algae. In
681   Z.-M. Hu & C. Fraser (Éds.), *Seaweed Phylogeography* (p. 211-226). Dordrecht: Springer.
682   https://doi.org/10.1007/978-94-017-7534-2_8
683 Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies.
684   *Genetics*, *139*(1), 457–462.
685 Souter, P., Henriksson, O., Olsson, N., & Grahn, M. (2009). Patterns of genetic structuring in the
686   coral *Pocillopora damicornis* on reefs in East Africa. *BMC Ecology*, *9*, 19.
687   https://doi.org/10.1186/1472-6785-9-19
688 Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M. A. X., …
689   Lourie, S. A. (2007). Marine ecoregions of the world : A bioregionalization of coastal and
690   shelf areas. *BioScience*, *57*(7), 573–583. https://doi.org/10.1641/b570707
691 Uthicke, S., & Benzie, J. A. H. (2003). Gene flow and population history in high dispersal marine
692   invertebrates : Mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata:
693   Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology*, *12*(10), 2635–2648.
694   https://doi.org/10.1046/j.1365-294X.2003.01954.x
695 van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER :
696   Software for identifying and correcting genotyping errors in microsatellite data. *Molecular*
697   *Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x
698 van Oppen, M. J. H., Bongaerts, P., Frade, P., Peplow, L. M., Boyd, S. E., Nim, H. T., & Bay, L. K.
699   (2018). Adaptation to reef habitats through selection on the coral animal and its associated
700   microbiome. *Molecular Ecology*, *27*(14), 2956-2971. https://doi.org/10.1111/mec.14763
701 van Oppen, M. J. H., Bongaerts, P., Underwood, J. N., Peplow, L. M., & Cooper, T. F. (2011). The
702   role of deep reefs in shallow reef recovery : An assessment of vertical connectivity in a
703   brooding coral from west and east Australia. *Molecular Ecology*, *20*(8), 1647-1660.
704   https://doi.org/10.1111/j.1365-294X.2011.05050.x
705 Vega, A., Marchesiello, P., & Lefêvre, J. (2006). *Atlas hydrodynamique de la Zone Economique*
706   *Exclusive de la Nouvelle-Calédonie*. Nouméa, Nouvelle-Calédonie: Laboratoire d'Etudes
707   Géophysiques et d'Océanographie Spatiale.
708 Veron, J. E. N. (2000). *Corals of the world*. Australia: Australian Institute of Marine Science.
709 Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population
710   structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x
711 Wilkinson, C. R. (1999). Global and local threats to coral reef functioning and existence : Review
712   and predictions. *Marine and Freshwater Research*, *50*(8), 867–878.
713   https://doi.org/10.1071/MF99121
714 Williams, S. T., & Benzie, J. A. H. (1996). Genetic uniformity of widely separated populations of
715   the coral reef starfish *Linckia laevigata* from the East Indian and West Pacific Oceans,
716   revealed by allozyme electrophoresis. *Marine Biology*, *126*(1), 99–107.
717   https://doi.org/10.1007/BF00571381
718 Yasuda, N., Taquet, C., Nagai, S., Yoshida, T., & Adjeroud, M. (2015). Genetic connectivity of the
719   coral-eating sea star *Acanthaster planci* during the severe outbreak of 2006–2009 in the

720   Society Islands, French Polynesia. *Marine Ecology*, *36*(3), 668-678.

721   https://doi.org/10.1111/maec.12175

722

SUPPORTING INFORMATION

732

BIOSKETCHES

Nicolas Oury is PhD student interested in the evolutionary history, biogeography and population connectivity in marine species, notably the scleractinian genus *Pocillopora*. This work emerged from his PhD and the one of **Pauline Gélin**, during which she used species delimitation methods to delineate molecularly species hypotheses in the genus *Pocillopora*. Both theses were supervised by **Hélène Magalon**, lecturer at Reunion Island University. All authors are interested in biogeography and population connectivity in marine environments.

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FIGURE LEDENDS

Figure 1 Sampling locations of *Pocillopora* colonies (dark and light greys indicate lands and coral reefs, respectively). Populations are numerically identified from the island code.

MAY: Mayotte, GLO: Glorioso Islands, JDN: Juan de Nova Island, BAS: Bassas da India, EUR: Europa Island, MAD: Madagascar, REU: Reunion Island, ROD: Rodrigues Island, TRO: Tromelin Island, CHE: Chesterfield Islands, NCA: Grande Terre (New Caledonia), LOY: Loyalty Islands (New Caledonia), TON: Tonga archipelago, BOR: Bora-Bora, MOR: Moorea and TAH: Tahiti.
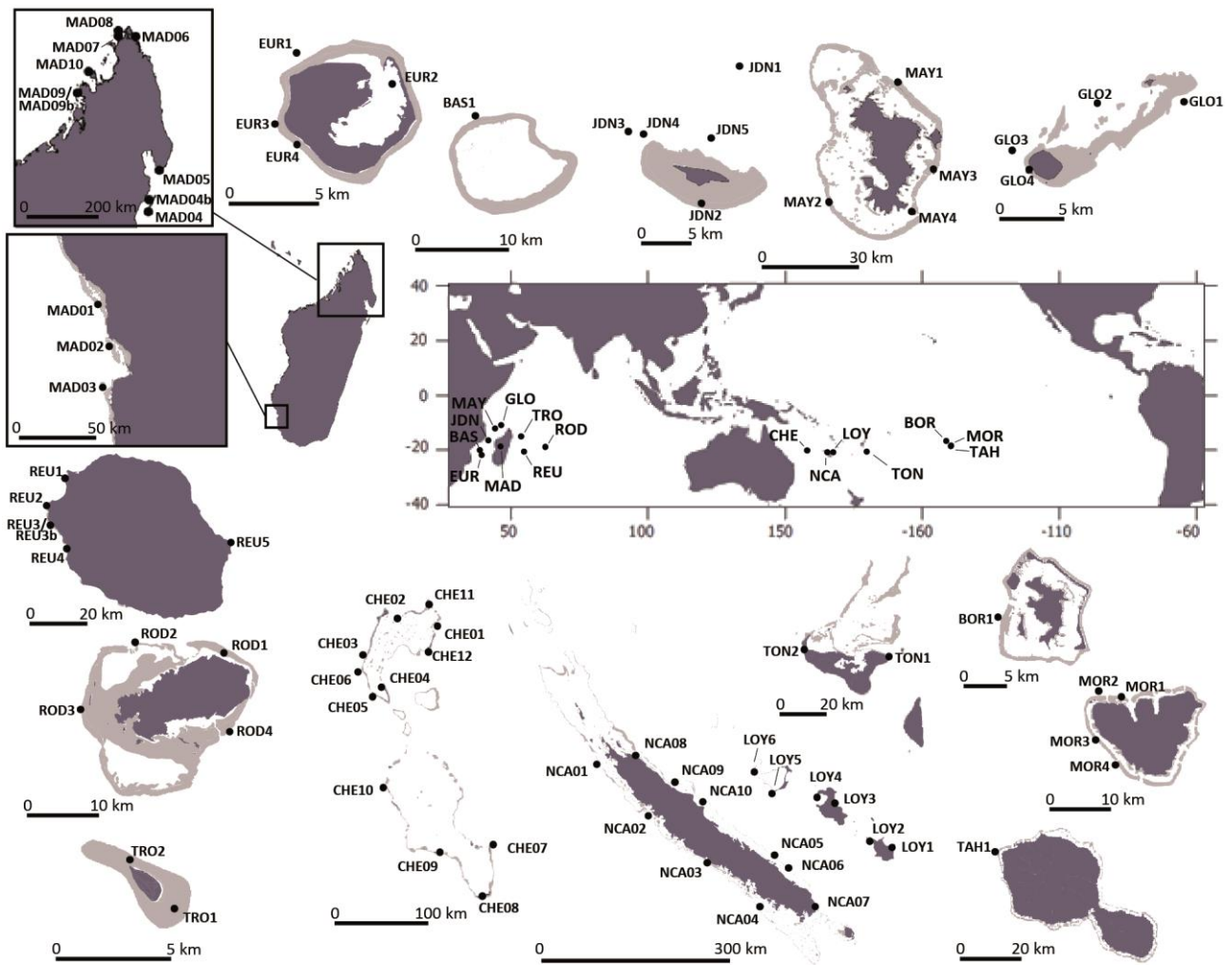
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Figure 2 *Pocillopora* PSH13 Secondary Species Hypotheses (SSHs) and clusters. (a) STRUCTURE plots at $K = 3$ for all colonies and at $K = 2$ for SSH13c, (b) DAPC assignments at $K = 3$ for all colonies and (c) Minimum Spanning Tree for all colonies. Colonies are coloured according to the SSHs identified with STRUCTURE at $K = 3$ (individual assignment probability $P \geq 0.75$).

WIO: Western Indian Ocean (MAY: Mayotte, GLO: Glorioso Islands, JDN: Juan de Nova Island, EUR: Europa Island, MAD: Madagascar, REU: Reunion Island, ROD: Rodrigues Island), TSP: Tropical Southwestern Pacific [CHE: Chesterfield Islands, NCA: Grande Terre (New Caledonia), LOY: Loyalty Islands (New Caledonia), TON: Tonga archipelago].

761

762 **Figure 3** Per site cluster distribution for each Secondary Species Hypothesis (SSH) of *Pocillopora*
763 PSH09 and PSH13 in the Western Indian Ocean: a) SSH09a, b) SSH13a and SSH13b, and in the
764 Southern Pacific: c) SSH09b, d) SSH09c and e) SSH13c. Arrows indicate the main currents [width
765 proportional to speed; sources: IFREMER (https://wwz.ifremer.fr/; Western Indian Ocean); Vega et
766 al., 2006 (New Caledonia)] and asterisks report populations differentiated from the others of the same
767 cluster (the color of the asterisk refers to the cluster).
768 MAY: Mayotte, GLO: Glorioso Islands, JDN: Juan de Nova Island, BAS: Bassas da India, EUR:
769 Europa Island, MAD: Madagascar, REU: Reunion Island, ROD: Rodrigues Island, TRO: Tromelin
770 Island, CHE: Chesterfield Islands, NCA: Grande Terre (New Caledonia) and LOY: Loyalty Islands
771 (New Caledonia), TON: Tonga archipelago and MOR: Moorea.

Figure 1 Sampling locations of *Pocillopora* colonies (dark and light greys indicate lands and coral reefs, respectively). Populations are numerically identified from the island code.
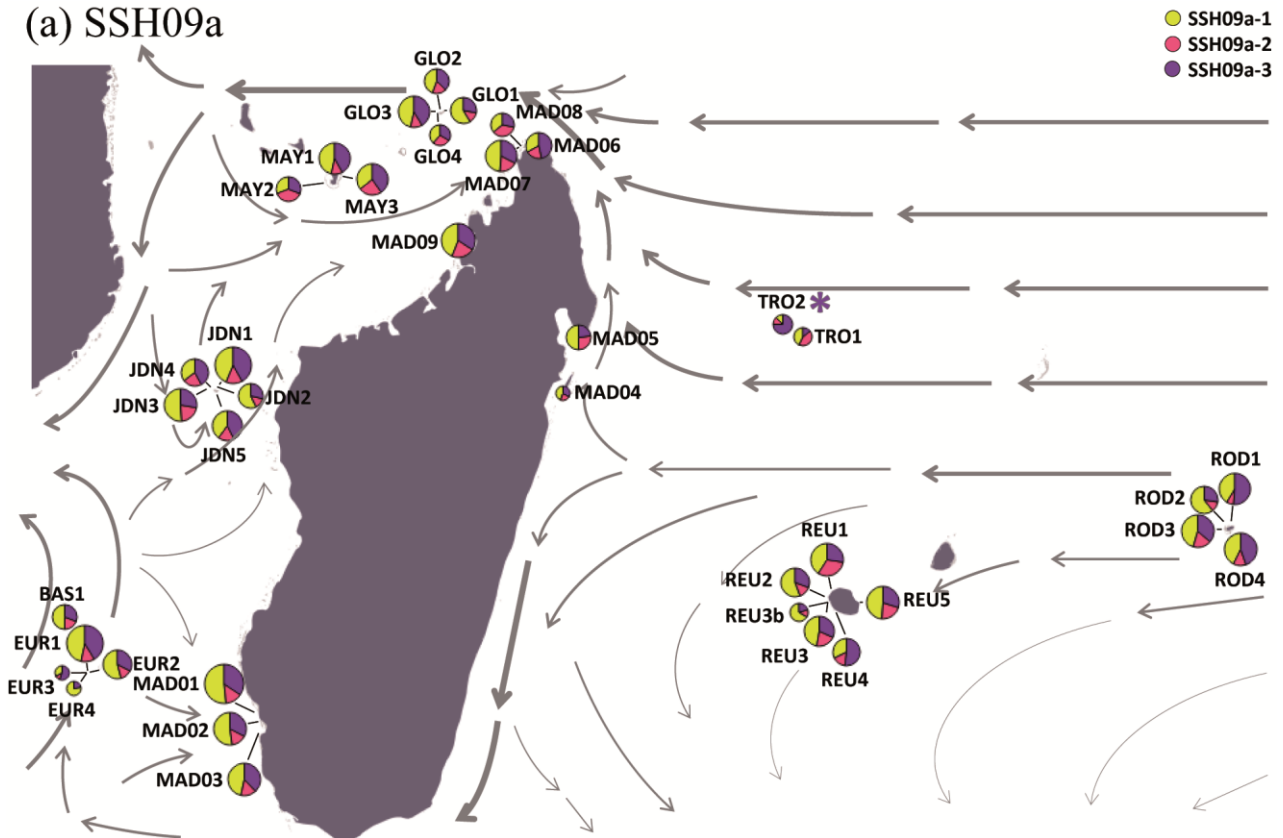
MAY: Mayotte, GLO: Glorioso Islands, JDN: Juan de Nova Island, BAS: Bassas da India, EUR: Europa Island, MAD: Madagascar, REU: Reunion Island, ROD: Rodrigues Island, TRO: Tromelin Island, CHE: Chesterfield Islands, NCA: Grande Terre (New Caledonia), LOY: Loyalty Islands (New Caledonia), TON: Tonga archipelago, BOR: Bora-Bora, MOR: Moorea and TAH: Tahiti.
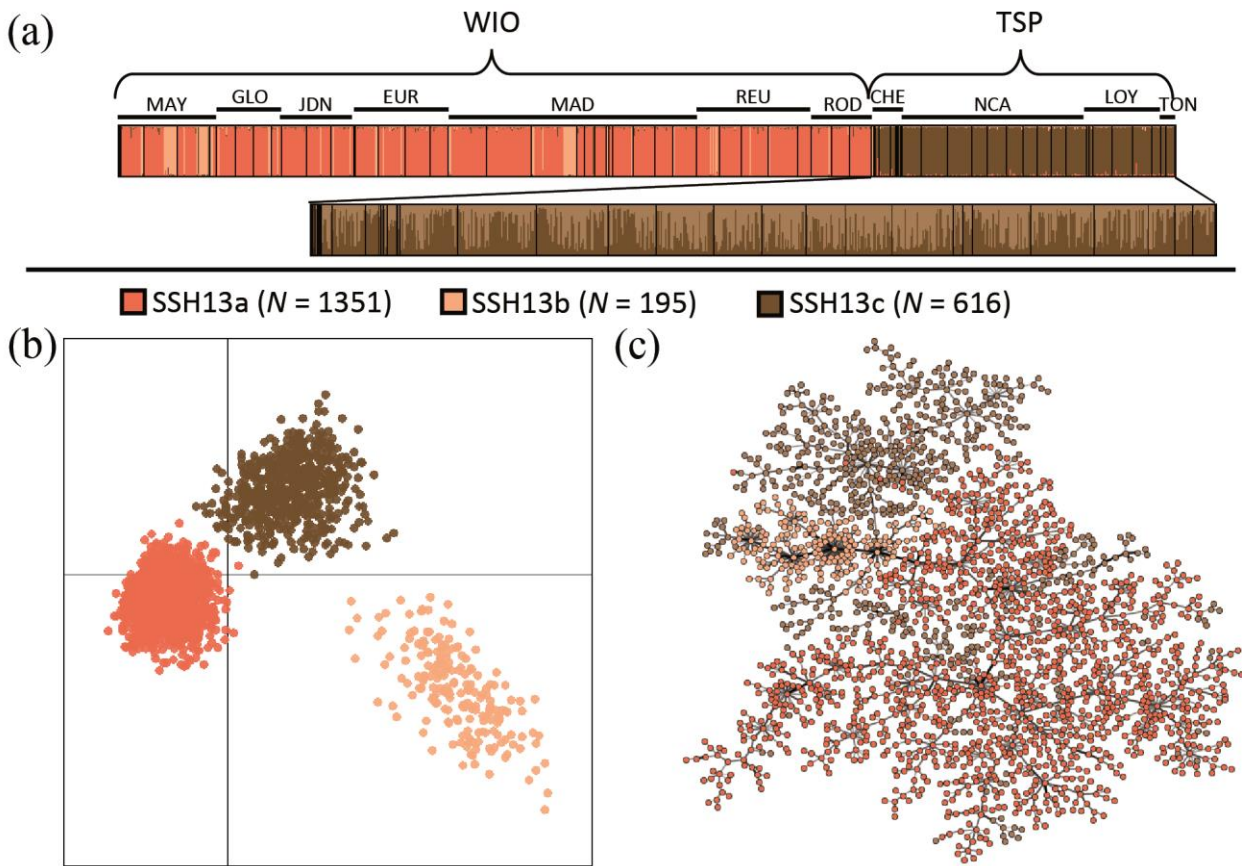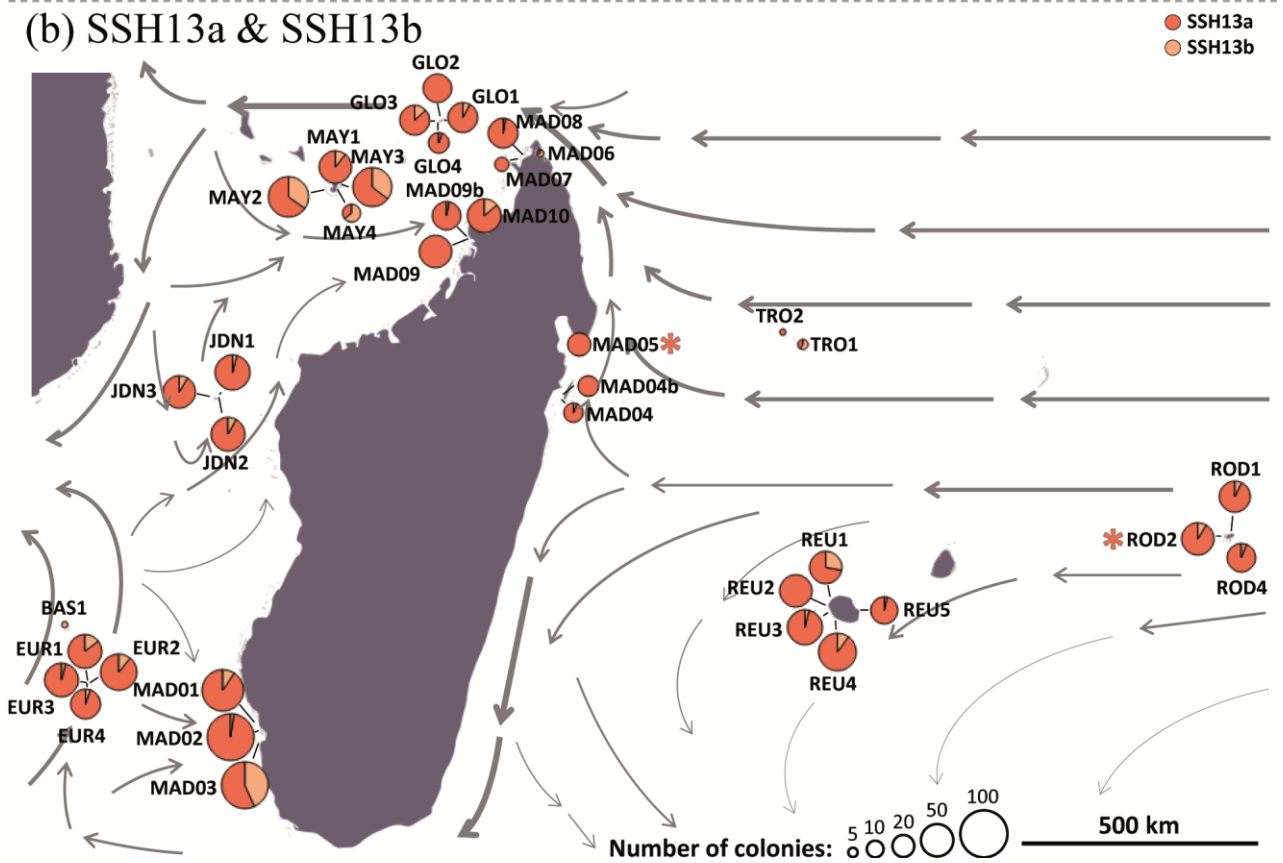
779
780 **Figure 2** *Pocillopora* PSH13 Secondary Species Hypotheses (SSHs) and clusters. (a) STRUCTURE
781 plots at $K = 3$ for all colonies and at $K = 2$ for SSH13c, (b) DAPC assignments at $K = 3$ for all colonies
782 and (c) Minimum Spanning Tree for all colonies. Colonies are coloured according to the SSHs
783 identified with STRUCTURE at $K = 3$ (individual assignment probability $P \geq 0.75$).
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786 Tropical Southwestern Pacific [CHE: Chesterfield Islands, NCA: Grande Terre (New Caledonia),
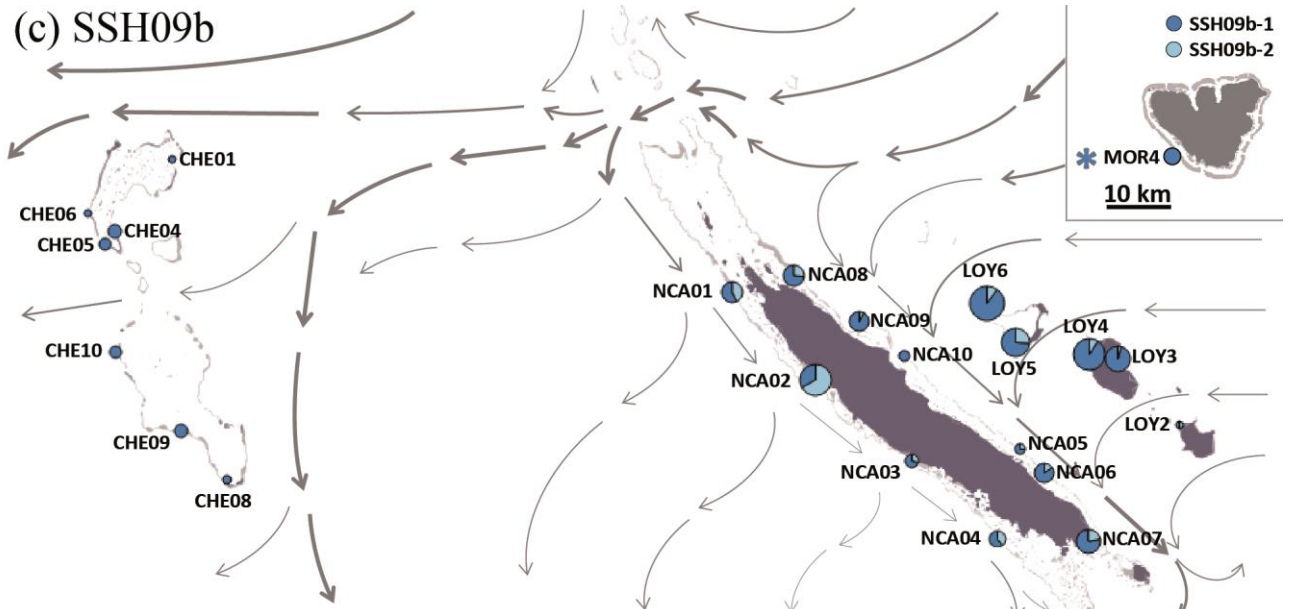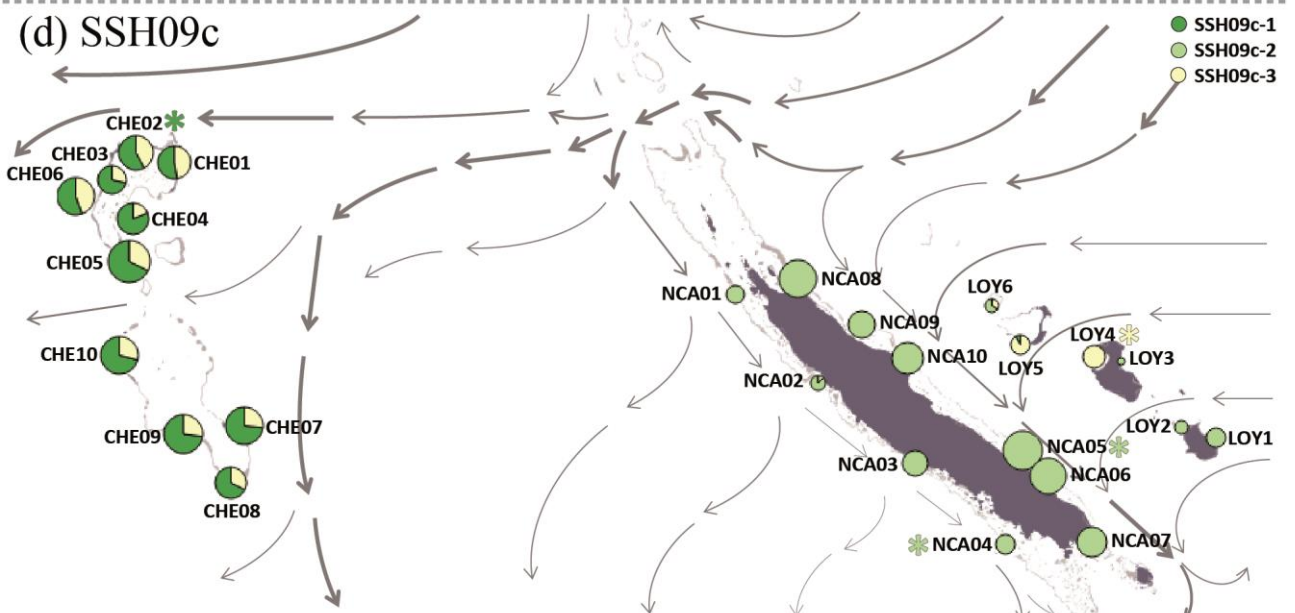787 LOY: Loyalty Islands (New Caledonia), TON: Tonga archipelago].
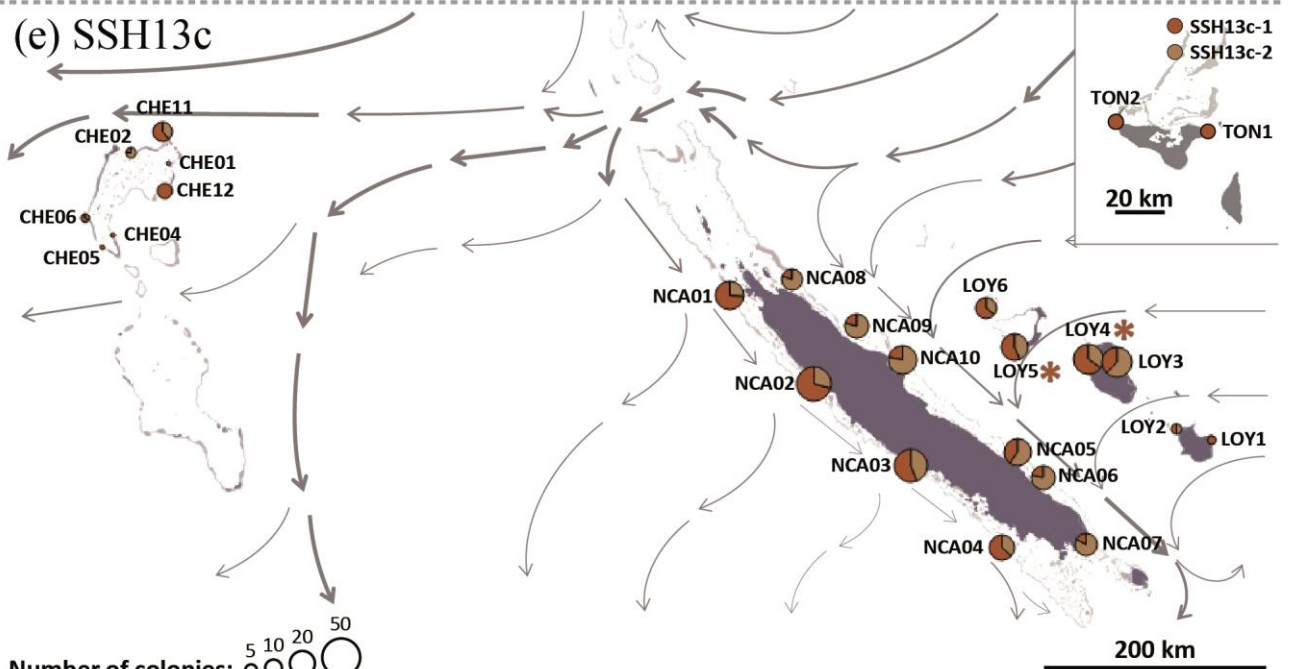
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(a) SSH09a

SSH09a-1
SSH09a-2
SSH09a-3

GLO2
GLO3
GLO1
GLO4
MAD08
MAD06
MAY1
MAY2
MAY3
MAD07
MAD09
TRO2 *
TRO1
MAD05
MAD04
JDN1
JDN4
JDN3
JDN2
JDN5
ROD1
ROD2
ROD3
ROD4
BAS1
EUR1
EUR3
EUR2
EUR4
MAD01
MAD02
MAD03
REU1
REU2
REU3b
REU3
REU4
REU5

(b) SSH13a & SSH13b

SSH13a
SSH13b

GLO2
GLO3
GLO1
MAY1
MAD08
MAY3
GLO4
MAD06
MAY2
MAD07
MAY4
MAD09b
MAD10
MAD09
TRO2
TRO1
JDN1
MAD05 *
JDN3
MAD04b
MAD04
JDN2
ROD1
BAS1
* ROD2
EUR1
EUR2
ROD4
EUR3
MAD01
EUR4
MAD02
REU1
MAD03
REU2
REU5
REU3
REU4

Number of colonies: 5 10 20 50 100

500 km

788

24

Figure 3 Per site cluster distribution for each Secondary Species Hypothesis (SSH) of *Pocillopora* PSH09 and PSH13 in the Western Indian Ocean: a) SSH09a, b) SSH13a and SSH13b, and in the Southern Pacific: c) SSH09b, d) SSH09c and e) SSH13c. Arrows indicate the main currents [width proportional to speed; sources: IFREMER (https://wwz.ifremer.fr/; Western Indian Ocean); Vega et al., 2006 (New Caledonia)] and asterisks report populations differentiated from the others of the same cluster (the color of the asterisk refers to the cluster).

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