Cryptic species and genetic connectivity among populations of the coral Pocillopora damicornis (Scleractinia) in the tropical southwestern Pacific

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Abstract
Studying population genetic connectivity (i.e., identifying gene flow among populations and understanding their impacts on the genetic structure and diversity of populations) is first a matter of knowing what we work on, that is, accurately delimiting evolutionary units. Here, we focused on *Pocillopora damicornis* sensu stricto (or *Pocillopora* PSH04 sensu Gélin et al. in Mol Phylogenet Evol 109:430–446. http://dx.doi.org/10.1016/j.ympev.2017.01.018, 2017). From 458 colonies sampled within the tropical southwestern Pacific [Chesterfield Islands and New Caledonia (Grande Terre and Loyalty Islands)], Bayesian assignments and network analyses were conducted with 11-microsatellite loci to first evaluate the genetic partitioning of the colonies in distinct Secondary Species Hypotheses (SSHs), then in distinct clusters. Population genetic connectivity was then assessed for each cluster separately. *Pocillopora* PSH04 was partitioned into two highly differentiated SSHs (SSH04a and SSH04b), regularly found in sympatry. Furthermore, SSH04a was subdivided into two clusters (SSH04a-1 and SSH04a-2). This pattern of genetic structuring seems not related to clonality, but rather to the establishment of reproductive barriers. Nevertheless, considering each cluster separately, the populations appeared highly differentiated, suggesting relatively weak gene flow. This low connectivity among populations, coupled with the existence of cryptic species, brings new insights to the connectivity pattern of this understudied Pacific region.

Introduction

Population connectivity (i.e., the process linking habitats and populations geographically separated) is a powerful force that maintains the genetic cohesion of a species over its distribution range, via the homogenisation of genetic variations among its populations (Mayr 1963). In marine environments, population connectivity occurs through exchanges of individuals or propagules (e.g., gametes, eggs, or larvae), synonym of a transfer of alleles (effective dispersal; Cowen et al. 2003; Cowen and Sponaugle 2009). Population genetic connectivity, therefore, represents a key element of population dynamics, genetic structuring, and diversification processes of marine organisms (Palumbi 1992; Paulay and Meyer 2002; Cowen et al. 2003; Bowen et al. 2013). Thus, understanding genetic connectivity among populations appears mandatory to define effective management and conservation units (Mills and Allendorf 1996; Mönkkönen and Reunanen 1999; Drechsler et al. 2003).

Studying genetic connectivity among populations of scleractinian corals is particularly crucial in the context of declining coral reefs (Wilkinson 2008). Indeed, most of the physical structure and the primary production of coral reef ecosystems are supplied directly or indirectly by scleractinian corals (e.g., Hatcher 1990). Thus, better understanding the processes that govern coral ecosystems, and those that promote the resilience and recovery of coral populations, such as connectivity (Hughes et al. 2011), is fundamental.

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However, scleractinians adopt several reproduction strategies (reviewed in Harrison 2011), influencing the dispersive capabilities of the propagules, and so the connectivity. In particular, they can reproduce asexually through different processes [e.g., by fragmentation (Highsmith 1982) or production of asexual larvae (Stoddart 1983)], leading to the production of genetically identical colonies. In the presence of clonal or partially clonal populations, traditional estimators of genetic structure and connectivity among populations might be biased (Balloux et al. 2003; Halkett et al. 2005), as the Hardy–Weinberg equilibrium tends to be modified. This renders more complex genetic connectivity studies, but must be taken into account to faithfully assess the genetic structure of populations.

Nevertheless, studying population genetic connectivity is first a matter of knowing what we work on, that is, accurately delimiting the evolutionary units. Indeed, incorrectly delimiting species and misidentifying these units of connectivity lead to biased estimation of connectivity among populations, as belonging to distinct species or regrouping individuals from distinct sympatric species. This seems trivial; however, with the recent development and democratisation of genetic tools, discoveries of highly divergent clusters among populations reveal the possible presence of cryptic species, that the sole use of traditional taxonomic (often morphological) characters may have not highlighted (reviewed in, e.g., Knowlton 1993; Bickford et al. 2007; Fišer et al. 2018). As an illustration, within scleractinian corals, the morphospecies *Stylophora pistillata*, previously thought to be distributed in the whole Indo-Pacific and the Red Sea (Veron 2000), appears as a complex of species with narrower distribution range (Keshavmurthy et al. 2013), as the coral *Porites lobata* (Forsman et al. 2015). Two novel species of *Leptastrea*, formally considered as *L. pruinosa*, were also recently delimited and described in the Indo-Pacific (Arrigoni et al. 2020).

The widespread Indo-Pacific coral genus *Pocillopora* particularly illustrates this issue, as phenotypic plasticity encrypts the morphospecies boundaries and complicates the definition of valid taxonomic units (see Todd 2008). Thus, in the last decade, several molecular approaches have contributed to the investigation of *Pocillopora* species boundaries (summarized in Gélin et al. 2017a). For example, the morphospecies *P. damicornis* (Linnaeus 1758; referred as *P. damicornis* sensu lato hereafter to avoid any ambiguity) was disentangled in five genetic lineages: *P. damicornis* types α, β, δ, γ, and ε (Schmidt-Roach et al. 2012a), a posteriori defined as five distinct species and named *P. damicornis* (Linnaeus 1758), *P. acuta* (Lamarck 1816), *P. aliciae* (Schmidt-Roach, Miller and Andreakis 2013), *P. verrucosa* (Ellis and Solander 1786), and *P. brevicornis* (Lamarck 1816), respectively (Schmidt-Roach et al. 2014). Besides, using species delimitation methods based on genetic data, Gélin et al. (2017a) defined within the *Pocillopora* genus 16 Primary Species Hypotheses (PSHs sensu Pante et al. 2015), with some of them partitioned into several Secondary Species Hypotheses (SSHs sensu Pante et al. 2015), themselves partitioned into several divergent genetic clusters, often found in sympatry at the site scale. In this latter study, lineages α (PSH04 therein), δ (PSH03), and ε (PSH10) appeared as unique entities with no further genetic partitioning into SSH, but this needs to be confirmed by completing the sampling and further genetic analyses. However lineage β (PSH05) was found to be subdivided into different SSHs and clusters, revealing possible cryptic species in this lineage (Gélin et al. 2017a, b, 2018b), such as for lineage γ, whose ORF haplotypes were shared between PSH13 and PSH16 (Gélin et al. 2017a) and which, therefore, needs to have its limits refined.

Here, we focused on *P. damicornis* type α sensu Schmidt-Roach et al. (2012a) or PSH04 sensu Gélin et al. [2017a; also corresponding to *Pocillopora* type 4 sensu Pinzón et al. (2013) and *Pocillopora* Clade Ib sensu Marti-Puig et al. (2014); referred as *P. damicornis* sensu stricto hereafter to avoid confusing it with the morphospecies (i.e., *P. damicornis s.l.*)]. *Pocillopora damicornis s.s.* is common and found in lagoons and shallow waters from the East and West coasts of Australia (Pinzón et al. 2013; Torda et al. 2013a, b; Thomas et al. 2014; Schmidt-Roach et al. 2014), and the northwestern subtropical and southwestern tropical regions of the Pacific Ocean (Pinzón et al. 2013; Gélin et al. 2017a). Colonies are also present, but rare, in the southwestern Indian Ocean (two colonies over thousands sampled; Gélin et al. 2017a) and in the central and northeastern Pacific (Pinzón et al. 2013). It is a broadcast spawner (Schmidt-Roach et al. 2012b), and is also able to propagate asexually (Torda et al. 2013a, b; Thomas et al. 2014) through fragmentation or release of asexually produced larvae (Schmidt-Roach et al. 2012a). The previous studies (Richmond 1987; Harii et al. 2002) reported a pelagic larval stage with zooxanthellae ranging from few hours to ~ 100 days, suggesting high dispersal abilities. However, this must be taken cautiously, as inferred from *P. damicornis s.l.* (probably *P. acuta*).

For now, a few studies clearly investigated genetic connectivity in this redefined species as the previous studies dealt with *P. damicornis s.l.* and it was not always possible to identify a posteriori the studied lineage(s). Besides, a relatively small part of its distribution range is covered. Thus, in the Great Barrier Reef (GBR) region, strong genetic similarities at large spatial scales (approximately 1,000 km) were observed among populations of *P. damicornis s.s.* However, populations relatively close geographically were more differentiated (Torda et al. 2013b). On the contrary, in western Australia (Ningaloo Reef and Muiron Islands), populations of *P. damicornis s.s.* geographically close were poorly differentiated, while the differentiation among populations

increased with distance (Thomas et al. 2014). In both studies, clonality was important [13.7% of the colonies shared their multi-locus genotype (MLG) with at least one other colony in Torda et al. (2013b); 84 different MLGs were identified among 162 colonies in Thomas et al. (2014)].

In front of such a lack of knowledge for *P. damicornis s.s.*, and regarding the sampling locations were we found it, we inferred the genetic connectivity among populations from an ecoregion (sensu Spalding et al. 2007) located in the southern part of the distribution range of the genus, which is largely understudied: New Caledonia. This ecoregion includes several archipelagos comprising small islands (Chesterfield and Loyalty Islands) and one main island (Grande Terre). Some are inhabited (Grande Terre and Loyalty Islands), with growing disturbances on marine environment (e.g., coastal anthropisation, tourism, and mining), while some others are uninhabited (Chesterfield Islands), and thus relatively poorly influenced by human activities. Besides, this area is protected by one of the largest Marine Protected Areas (MPAs): the Natural Park of the Coral Sea (1.3×10^6 km^2). This ecoregion thus represents an interesting patched framework to study population connectivity in habitats differently impacted by human activities, but a few studies focused on this particular area (e.g., Planes et al. 1998; Postaire et al. 2017; Gélin et al. 2018b), especially in models with a life history similar to *Pocillopora*.

In this study, we first explored the genetic partitioning within *Pocillopora* PSH04, using assignment and network analyses based on 11-microsatellite genotypes. Then, once all units of connectivity (i.e., the clusters) were defined, the genetic diversity and the connectivity among populations from New Caledonia ecoregion were assessed for each unit separately.

Materials and methods

Sampling design

Pocillopora colonies were collected between March 2001 and October 2016 within three marine provinces (sensu Spalding et al. 2007): the western Indian Ocean, the tropical southwestern Pacific, and the southeastern Polynesia, representing six ecoregions (sensu Spalding et al. 2007), 16 localities (Fig. 1), and over hundred sampling sites. Different habitats (reef slope, fringing reef, flat reef, or lagoon) were sampled, at various depths (from sea surface to 30 m depth), to maximise colonies genetic diversity. On each site, colonies were haphazardly sampled (branch tip + photograph) by snorkelling or scuba diving, without any a priori on morphology (a non-discriminant character in this genus; Pinzón et al. 2013; Schmidt-Roach et al. 2014; Gélin et al. 2017a), except in southeastern Polynesia, where the sampling focused on colonies presenting *P. verrucosa* macromorphology (but which could be *P. damicornis s.s.*). Samples were fixed in 90% ethanol at lab and stored at room temperature.

DNA extraction, microsatellite genotyping, and species identification

From the sampled colonies, DNA was extracted using DNeasy Blood & Tissue kit (Qiagen^{TM}). Samples were genotyped using 13 microsatellite loci, as in Gélin et al. (2017a; Online Resource 1). Then, colonies belonging to *Pocillopora* PSH04 were identified a posteriori of sampling and a priori of analyses using assignment tests performed with STRUCTURE 2.3.4 (Pritchard et al. 2000), by compiling all the genotypes with the 975 ones from Gélin et al. (2017a), corresponding to colonies from various PSHs already identified and whose ORF has been sequenced. Five iterations of STRUCTURE were run at $K = 12$ (this value was found to retrieve the main PSHs in Gélin et al. 2017a), with the same parameters as in Gélin et al. (2017a). Colonies assigned to the cluster corresponding to *Pocillopora* PSH04 (ORF07-17) with a mean probability over the five runs of at least 0.75 (i.e., the mean assignment probability to include all colonies with PSH04 ORF haplotypes but none with other haplotypes) were retained and constitute the dataset for this study (Online Resource 2).

Global diversity and clonality

For each locus, the number of alleles (Na) and the percentage of missing data (%NA) were estimated. Then, the occurrence of identical multi-locus genotypes (MLGs) among the colonies was assessed with GENCLONE 2.0 (Arnaud-Haond and Belkhir 2007). The probability of obtaining the same MLG twice or more from distinct random reproductive events was further estimated using P_{SEX} (F_{IS}) (Arnaud-Haond et al. 2007). However, as GENCLONE considers missing data as different alleles (Arnaud-Haond and Belkhir 2007), the number of different MLGs in a population (N_{MLG}) could be overestimated. Thus, the genotypes of the colonies were also compared manually by considering missing data as potentially identical alleles. This latter method provides an underestimation of N_{MLG}, but combined with GENCLONE method, both methods provide a range containing the true value of N_{MLG}. The clonal richness R (Dorken and Eckert 2001) was then calculated over all colonies and for each method, with the formula: $R = \frac{(N_{MLG}-1)}{(N-1)}$. Potential multi-locus lineages (MLLs) were identified using GENOTYPE (Meirmans and van Tienderen 2004), considering missing data as potentially identical alleles. Pairwise genetic distances based on mutational steps under both the Infinite Allele Model (IAM) and the Stepwise Mutational Model

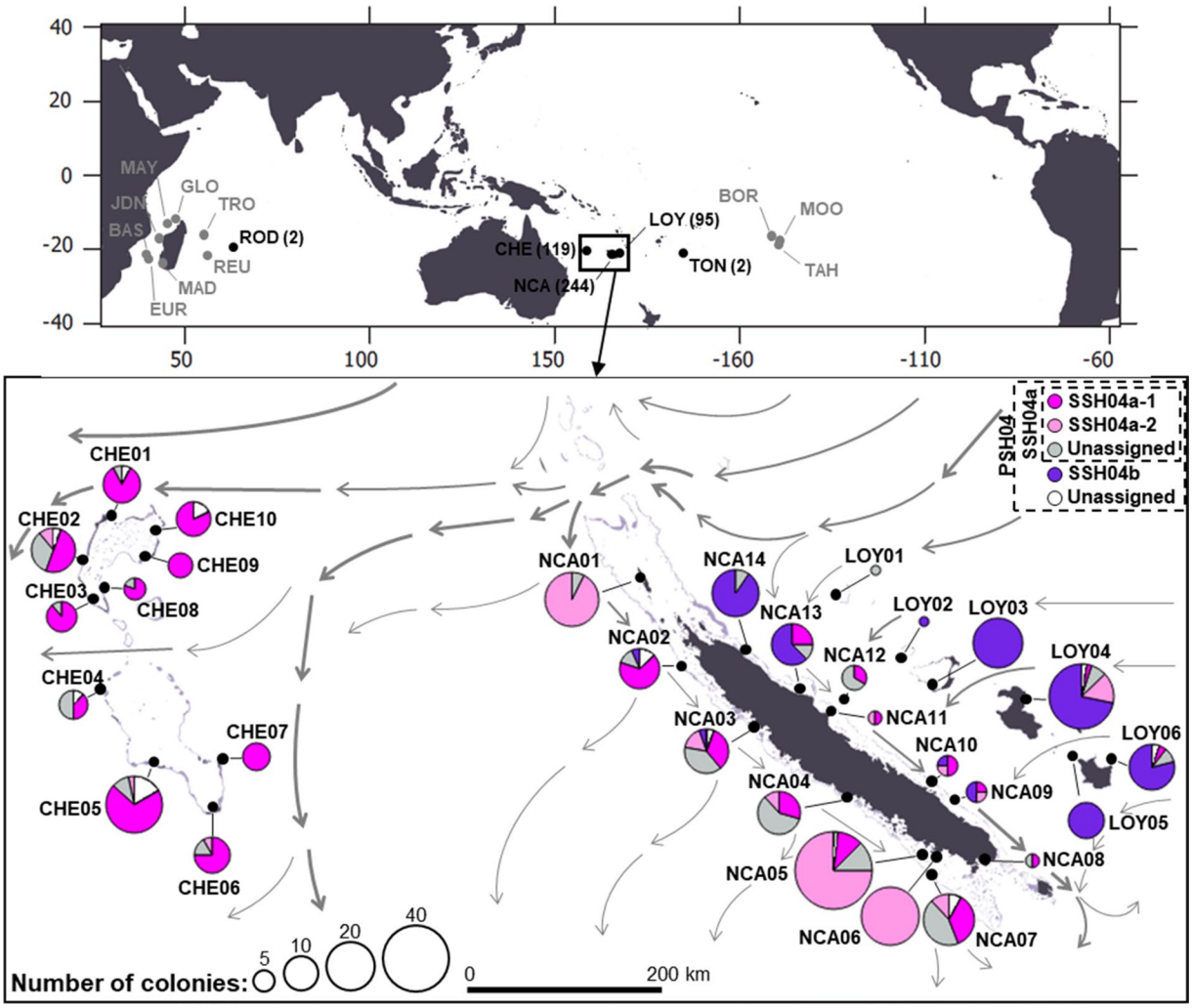

Fig. 1 Sampling localities of *Pocillopora* spp. colonies [from West to East and North to South: *GLO* Glorioso Islands, *MAY* Mayotte, *JDN* Juan de Nova Island, *BAS* Bassas da India, *EUR* Europa Island, *MAD* Madagascar, *TRO* Tromelin Island, *REU* Reunion Island, *ROD* Rodrigues Island, *CHE* Chesterfield Islands, *NCA* New Caledonia (Grande Terre), *LOY* Loyalty Islands, *TON* Tonga, *BOR* Bora-Bora, *MOO* Moorea, *TAH* Tahiti]. Localities for which colonies of *Pocillopora* PSH04 have been identified are in black (the number of colonies is indicated in parentheses). New Caledonia ecoregion is detailed below. Sites (detailed in Online Resource 4) are numerically identified from the locality code and the distribution of PSH04 clusters is represented [white and light grey represent the proportions of PSH04 colonies not assigned ($P < 0.75$) to a Secondary Species Hypothesis (SSH) or to an SSH04a cluster, respectively]. Arrows indicate the main currents (width proportional to speed; Vega et al. 2006)

(SMM) were calculated for all pairs of MLGs, and a threshold in the genetic distance distribution was determined, under which distinct MLGs were considered to belong to the same MLL.

Genetic partitioning exploration

All further analyses were performed on both the entire dataset (i.e., keeping all colonies) and a truncated one (i.e., keeping one representative per potential MLG per population, considering missing data as potentially identical alleles), as suggested in Alberto et al. (2005). Indeed, while repeated MLGs can bias estimators which are not designed for clonal populations, colonies with the same genotype participate equally to sexual reproduction and gene flow, and should not be ignored. Therefore, analysing both datasets should take into account the effect of clonality.

First, null alleles and other potential technical biases were assessed with Micro-Checker 2.2.3 (van Oosterhout et al. 2004), and genotypic linkage disequilibrium was tested with GenePop 4.7.0 (Raymond and Rousset 1995; Rousset 2008). Then, to infer the genetic partitioning within PSH04, we used and compared the results of assignment tests [Structure (Pritchard et al. 2000) and DAPC (Jombart et al. 2010)], Minimum Spanning Trees (MST; EDENetworks 2.18; Kivelä et al. 2015), allelic frequencies, and F_{ST}

(Weir and Cockerham 1984), as in Gélin et al. (2018a; see the Supplementary Method in Online Resource 3 for more details). We then defined the most likely number of genetically homogeneous groups (K) within the colonies as the highest K for which all previous methods were congruent for each dataset (i.e., the entire and the truncated). This level of differentiation should correspond to the SSH level.

In a hierarchical approach, and as a first level of structuration can hide a second one (see Gélin et al. 2018a), these analyses were repeated on each SSH separately. Once this second level of structuration has been tested, we defined the final number of genetic homogeneous groups (i.e., the clusters). Afterwards, we considered a population as all the colonies assigned to a given cluster with a probability of at least 0.75 (i.e., the minimum probability for which assignments in all datasets are strictly identical), sampled on the same site, at the same date (usually during a single dive and at the same depth). Colonies not assigned to a cluster with a probability ≥ 0.75 were considered admixed (i.e., assigned to more than one cluster due to hybridization, shared ancestry, or bad assignment due to missing data). NewHybrids 1.1 (Anderson and Thompson 2002) was then run with 10^6 iterations after a burn-in period of 10^5, to detect whether the admixed colonies could be considered as hybrids.

Genetic diversity and connectivity within each cluster

Allelic diversity

For each locus and each population, %NA, the percentage of missing data, Na, the number of alleles, and Np, the number of population-private alleles were estimated using FSTAT 2.9.3 (Goudet 2001). Then, for each population, mean indices ($\%NA_{pop}$, Na_{pop}, and Np_{pop}, respectively) were calculated overall loci, as the percentage of polymorphic loci (P). The clonal richness R (Dorken and Eckert 2001) was also estimated for each population, as previously.

Afterwards, only the populations with $N \geq 10$ were considered. The observed heterozygosity (Ho), the expected heterozygosity at Hardy–Weinberg equilibrium (He, gene diversity) and the inbreeding coefficient (F_{IS}; Wright 1931) were calculated with FSTAT 2.9.3 (Goudet 2001), for each locus and each population, and then for all loci within each population (noted Ho_{pop}, He_{pop}, and F_{ISpop}, respectively).

Genetic connectivity

Two genetic differentiation indices were estimated among populations: F_{ST} (Weir and Cockerham 1984) and D_{est} (Jost 2008). F_{ST} were estimated using GenePop 4.7.0 (Raymond and Rousset 1995; Rousset 2008), while D_{est} with the package 'diveRsity' (Keenan et al. 2013) of the software R 3.3.1 (R Core Team 2016). Directional gene flow between populations was assessed by constructing a relative migration network with divMigrate (Sundqvist et al. 2016), implemented in the R package 'diveRsity' (Keenan et al. 2013). Finally, for each cluster, a population-based network was built using EDENetworks 2.18 (Kivelä et al. 2015) and the F_{ST} distance (Weir and Cockerham 1984). Populations with $N < 10$ were included. All networks were built at the same threshold [defined at the lowest percolation threshold (Dpe) found among clusters; i.e., the lowest F_{ST} threshold, below which the network is fragmented; Rozenfeld et al. 2007] to allow their comparison.

Results

Sampling and *Pocillopora* species identification

Overall localities, more than 9000 colonies of *Pocillopora* were sampled, among which 462 colonies were assigned to PSH04 (Online Resource 2), all found in lagoons or upper reef slopes, between sea surface and 5 m depth (Online Resource 4). Among these 462 colonies, two were sampled in Rodrigues Island, in the southwestern Indian Ocean, and 460 in the tropical southwestern Pacific, within two ecoregions (sensu Spalding et al. 2007): New Caledonia ($N = 458$ colonies) and Tonga Islands ($N = 2$ colonies; Fig. 1). The colonies from New Caledonia ecoregion constitute the final dataset of this study. They were sampled on 30 sites within three localities: Chesterfield Islands (10 sites; CHE01-10; $N = 119$ colonies), Grande Terre (14 sites; NCA01-14; $N = 244$ colonies), and Loyalty Islands (6 sites; LOY01-06; $N = 95$ colonies; Fig. 1; Online Resource 4).

Global diversity and clonality

Among the 458 genotypes of the colonies retained, missing data represented 17.5%. Two loci (Poc40 and Pd3-009) showed very high levels of missing data (86.2% and 33.4%, respectively; Online Resource 1), and were not considered for further analyses, reducing the global proportion of missing data to 9.8%. An additional three loci (PV2, Pd3-005, and Pd3-EF65) showed relatively high levels of missing data ($15.3\% < \%NA < 19.7\%$) compared to the eight others ($3.2\% < \%NA < 8.6\%$; Online Resource 1). To deal with the high levels of missing data for these three loci without losing any crucial information, we decided to perform the analyses at 8 and 11 loci (%NA = 6.7% and 9.8%, respectively; Table 1; Online Resource 1). All 11 loci were polymorphic, with a number of alleles per locus varying from 5 for Pd3-004 to 22 for Pd3-005 (Online Resource 1).

At 11 loci, 454 and 420 different MLGs over 458 colonies were detected, considering missing data as different or

Table 1 Summary of the different *Pocillopora* PSH04 datasets analysed

N_{loci}	Dataset	N	%NA	NA treatment	N_{MLG}	R
11	Entire_11	458	9.8	Different	454	0.99
				Identical	420	0.92
	Truncated_11	423	8.7	–	420	0.99
8	Entire_8	458	6.7	Different	428	0.93
				Identical	393	0.86
	Truncated_8	395	5.8	–	393	0.99

N_{loci}, N, N_{MLG} numbers of loci, colonies and multi-locus genotypes (MLGs), respectively, *%NA* percentage of missing data, *NA treatment* treatment of missing data (different of potentially identical alleles) for the identification of MLGs, R clonal richness (Dorken and Eckert 2001)

potentially identical alleles, respectively. Thus, the clonal richness R of the dataset was comprised between 0.99 and 0.92, respectively (Table 1). Considering missing data as different alleles (GenClone method), over the 454 MLGs found, one MLG was repeated four times in NCA01, and a second twice in LOY03. For both MLGs, P_{SEX} (F_{IS}) was very low ($< 10^{-12}$), indicating that the colonies came from a single sexual reproduction event and belonged to the same genet. However, only two colonies actually shared the same MLG at 13 loci. Considering missing data as potentially identical alleles, at least 27 MLGs were shared by a minimum of two colonies, but only three were shared between two colonies sampled in different sites, distant from 200 to 280 km. When reducing the number of loci to 8428 and 393 different MLGs over 458 colonies were detected considering missing data as different or potentially identical alleles, respectively ($0.86 < R < 0.93$; Table 1). According to GenClone (missing data as different alleles), 16 MLGs were repeated in two-to-seven colonies from the same site, except for one MLG shared between colonies from NCA01 and NCA05, distant from more than 400 km. However, P_{SEX} (F_{IS}) was comprised between 10^{-1} and 10^{-17}, suggesting that some colonies sharing the same MLG are probably not ramets from the same genet. With the other method (i.e., missing data as potentially identical alleles), at least 37 MLGs were found more than once, sometimes among very distant colonies (> 750 km). Thus, keeping one representative per MLG and per population resulted in truncated datasets of 423 and 395 colonies at 11 and 8 loci, respectively (Table 1).

Both at 11 and 8 loci, no clear truncature was observed in the distribution of the pairwise genetic distances between MLGs under the IAM and the SMM (Online Resource 5), making it difficult to define the threshold distinguishing MLGs from the same MLL. We thus considered that MLGs belong to the same MLL when they differed from one allele (IAM) or two mutational steps (SMM), as in a previous study focusing on *P. acuta* (PSH05; Gélin et al. 2018b). Considering these thresholds, between 330 (IAM) and 389 (SMM) MLLs were detected at 11 loci, and between 161 (IAM) and 265 (SMM) at 8 loci.

Genetic partitioning exploration

Further analyses were performed on the four datasets summarised in Table 1 (i.e., an entire and a truncated one, both at 11 and 8 loci). No null allele nor other potential scoring error was detected with Micro-Checker. Moreover, no genotypic linkage disequilibrium was found. Thus, all loci (11 or 8 depending on the datasets) were kept.

All results were very similar for the four datasets (Online Resource 6), so we presented here the results for the most complete one (Entire_11), specifying when differences were observed. For $K = 2$ and $K = 3$, the five runs of Structure were similar and congruent with DAPC (Fig. 2; Online Resources 7-9). From $K = 4$, the results of the two methods became incongruent, with more differences between datasets (Online Resources 6-7). The MST retrieved the genetic groups at $K = 2$ (Online Resource 8c), but not at $K = 3$ (Fig. 2c). Moreover, at $K = 2$, the allelic frequencies of each group were very different (Online Resource 8d), especially for loci Pd3-008, Pd11, and Pd13 for which F_{ST} estimates exceed 0.250*** (Online Resource 8e). A pairwise F_{ST} of 0.203*** was calculated. At $K = 3$, the allelic frequencies were less different (Online Resource 9d), and pairwise F_{ST} varied from 0.117*** to 0.269***. Thus, considering the congruency among the five methods (Structure, DAPC, MST, allelic frequencies, and F_{ST}; Online Resources 6–9) within the four datasets, the optimal K was defined at 2. The two groups were designated SSH04a and SSH04b, and 440 colonies (96.1%) were assigned to a unique SSH ($P \geq 0.75$; $N_{SSH04a} = 327$; $N_{SSH04b} = 113$; Fig. 1; Online Resource 4). SSH04a mainly regrouped colonies from Chesterfield Islands and western Grande Terre, while SSH04b was composed of colonies from eastern Grande Terre and Loyalty Islands (Fig. 2). However, the two SSHs were found in sympatry in eight sites (Figs. 1, 2a). Moreover, 18 colonies (3.9%) were found admixed between the two SSHs: ten in Chesterfield Islands (CHE01-02-04-05-10),

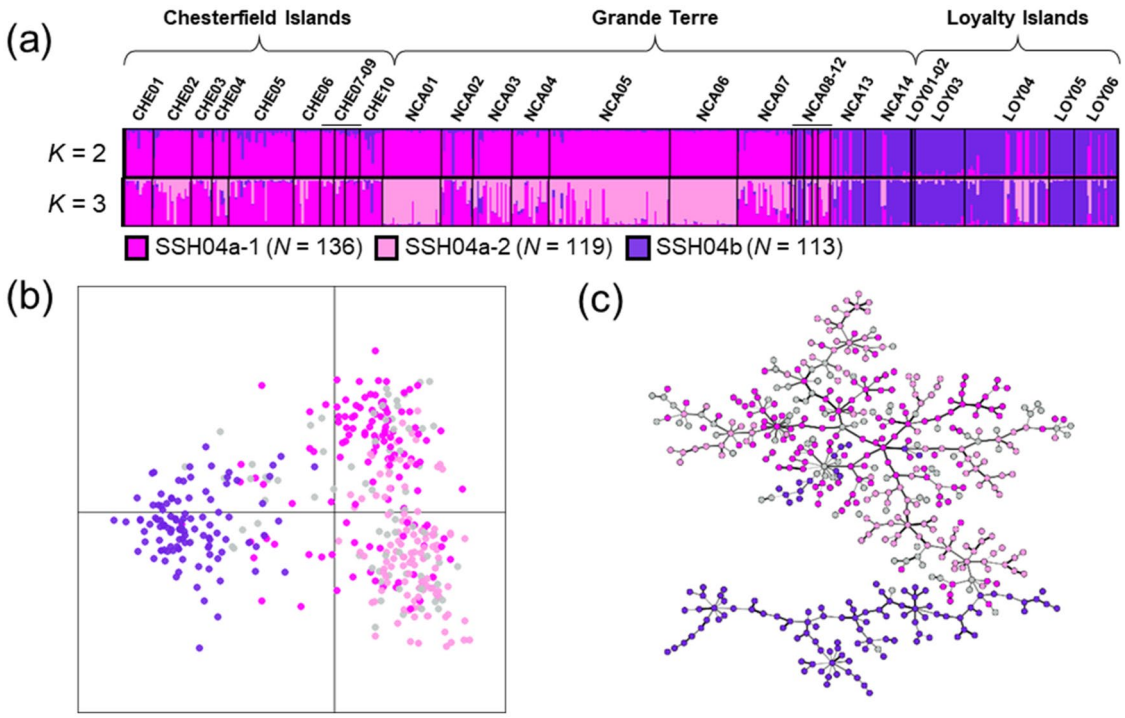

Fig. 2 *Pocillopora* PSH04 secondary species hypotheses (SSHs) and clusters (Entire_11 dataset). **a** STRUCTURE plots at $K=2$ and $K=3$, **b** DAPC assignments for $K=3$, and **c** minimum Spanning Tree. Colonies are coloured according to the clusters identified by STRUCTURE at $K=3$ (individual assignment probability ≥ 0.75). Colonies with an assignment probability inferior to 0.75 for each cluster were coloured in grey

six in Grande Terre (NCA02-03-05-07), and two in Loyalty Islands (LOY02-03), but only four presented no missing data in their genotypes. Thus, these admixed colonies probably correspond to bad assignments. Additionally, NEWHYBRIDS detected only six hybrids among the 458 colonies (1.3%; all F2), and almost all colonies (~96%) assigned to an SSH were detected as pure lineages.

Then, assignment tests were performed using STRUCTURE and DAPC for each SSH separately (Online Resource 10). For SSH04a, both methods were congruent only at $K=2$, and retrieved the two groups previously found for the overall dataset at $K=3$ (Online Resource 10). As previously, the MST was not congruent with STRUCTURE and DAPC (Online Resource 11), and a pairwise F_{ST} of 0.117*** was calculated between the two groups. As these two groups were already found within the overall dataset, and as STRUCTURE and DAPC gave very similar results, we considered that SSH04a was partitioned into two clusters, named SSH04a-1 and SSH04a-2. The two clusters were found in sympatry in 11 sites, SSH04a-1 being the main cluster in Chesterfield Islands, while SSH04a-2 being the most abundant in western Grande Terre ($N_{SSH04a\text{-}1}=136$; $N_{SSH04a\text{-}2}=119$; Fig. 1-2; Online Resource 4). However, both clusters had only three populations with $N\geq 10$ over 23 and 13 for SSH04a-1 and SSH04a-2, respectively (Table 2; Online Resource 4). A high proportion of the colonies (22.0%) were found admixed between both clusters, within all localities (CHE: 16.5%; NCA: 23.5%; LOY: 42.9%), possibly due to bad assignments due to missing data. Nevertheless, 41.7% of these admixed colonies were detected as F2 hybrids.

For SSH04b, STRUCTURE and DAPC were incongruent from $K=2$ (Online Resource 10). Thus, SSH04b was considered to be composed of a unique cluster, mostly found in eastern Grande Terre and Loyalty Islands. For this cluster, six populations over 11 had $N\geq 10$ (Table 2; Online Resource 4).

Genetic diversity and connectivity within each cluster

Allelic diversity

At 11 loci, among populations with $N\geq 10$, the percentage of polymorphic loci (P) varied from 73% for $NCA02_{SSH04a\text{-}1}$ to 100% for six populations. The percentage of missing data ($\%NA_{pop}$) ranged from 0.7% for $NCA01_{SSH04a\text{-}2}$ to 22.0% for $LOY05_{SSH04b}$, the mean number of alleles per locus (Na_{pop}; ± SE) varied from 2.6 ± 0.3 for $LOY05_{SSH04b}$ to 4.8 ± 0.8 for $CHE05_{SSH04a\text{-}1}$, and the mean number of population-private alleles (Np_{pop}; ± SE) varied from 0.0 for five

Table 2 *Pocillopora* PSH04 population summary statistics at 11 loci. Only populations with at least 10 colonies were considered. Sites are detailed in Online Resource 4

Population	N	N_{MLG}	R	$\%NA_{pop}$	P (%)	Na_{pop}	Np_{pop}	Entire_11			Truncated_11		
								Ho_{pop}	He_{pop}	$F_{IS\ pop}$	Ho_{pop}	He_{pop}	$F_{IS\ pop}$
SSH04a-1													
CHE01	11	10–11	0.90–1.00	10.3	91	3.1 ± 0.5	0.0 ± 0.0	0.30 ± 0.09	0.54 ± 0.07	0.40 ± 0.14**	0.30 ± 0.08	0.56 ± 0.07	0.40 ± 0.14*
CHE05	21	21	1.00	6.9	100	4.8 ± 0.8	0.2 ± 0.1	0.40 ± 0.09	0.48 ± 0.09	0.12 ± 0.07^{NS}	0.40 ± 0.09	0.48 ± 0.09	0.12 ± 0.07^{NS}
NCA02	10	9–10	0.89–1.00	7.3	73	3.1 ± 0.6	0.1 ± 0.1	0.49 ± 0.08	0.56 ± 0.07	0.11 ± 0.11^{NS}	0.45 ± 0.07	0.56 ± 0.07	0.15 ± 0.11^{NS}
Total	136	128–136	0.94–1.00	8.0	100	9.0 ± 1.3	–	–	–	–	–	–	–
SSH04a-2													
NCA01	25	22	0.88	0.7	100	4.3 ± 0.5	0.2 ± 0.1	0.36 ± 0.07	0.45 ± 0.07	0.22 ± 0.09^{NS}	0.35 ± 0.06	0.46 ± 0.07	0.25 ± 0.08**
NCA05	42	39–42	0.93–1.00	9.2	100	3.9 ± 0.5	0.0 ± 0.0	0.38 ± 0.08	0.45 ± 0.07	0.10 ± 0.12^{NS}	0.38 ± 0.08	0.45 ± 0.07	0.11 ± 0.12^{NS}
NCA06	31	24–31	0.77–1.00	11.3	91	3.5 ± 0.5	0.1 ± 0.1	0.41 ± 0.10	0.46 ± 0.07	0.11 ± 0.13^{NS}	0.42 ± 0.10	0.48 ± 0.07	0.12 ± 0.14^{NS}
Total	119	106–116	0.89–0.97	8.3	100	5.2 ± 0.8	–	–	–	–	–	–	–
SSH04b													
NCA13	10	10	1.00	2.7	100	3.1 ± 0.3	0.2 ± 0.1	0.45 ± 0.08	0.50 ± 0.06	0.08 ± 0.17^{NS}	0.45 ± 0.08	0.50 ± 0.06	0.08 ± 0.17^{NS}
NCA14	19	15–19	0.78–1.00	16.7	91	3.0 ± 0.4	0.0 ± 0.0	0.52 ± 0.08	0.48 ± 0.05	-0.09 ± 0.14^{NS}	0.53 ± 0.08	0.50 ± 0.05	-0.09 ± 0.14^{NS}
LOY03	23	22	0.95	10.7	100	3.4 ± 0.6	0.1 ± 0.1	0.61 ± 0.08	0.58 ± 0.04	-0.10 ± 0.16^{NS}	0.61 ± 0.08	0.58 ± 0.04	-0.10 ± 0.15^{NS}
LOY04	28	26–28	0.93–1.00	14.3	100	4.3 ± 0.4	0.3 ± 0.1	0.38 ± 0.07	0.45 ± 0.04	0.20 ± 0.11^{NS}	0.39 ± 0.07	0.48 ± 0.05	0.22 ± 0.10^{NS}
LOY05	12	10–12	0.88–1.00	22.0	82	2.6 ± 0.3	0.0 ± 0.0	0.40 ± 0.11	0.42 ± 0.08	0.09 ± 0.17^{NS}	0.40 ± 0.10	0.45 ± 0.08	0.11 ± 0.15^{NS}
LOY06	15	15	1.00	21.8	91	2.8 ± 0.5	0.0 ± 0.0	0.41 ± 0.07	0.47 ± 0.06	0.10 ± 0.13^{NS}	0.40 ± 0.07	0.45 ± 0.06	0.09 ± 0.12^{NS}
Total	113	104–112	0.92–0.99	14.5	100	6.2 ± 0.8	–	–	–	–	–	–	–
Total PSH04	458	420–454	0.92–0.99	9.8	100	11.1 ± 1.4	–	–	–	–	–	–	–

N number of colonies, N_{MLG} number of multi-locus genotypes (MLGs) for both treatments of missing data, R clonal richness (Dorken and Eckert 2001), $\%NA_{pop}$ percentage of missing data, P proportion of polymorphic loci, Na_{pop} *and* Np_{pop} mean numbers (±SE) of alleles and population-private alleles, Ho_{pop} *and* He_{pop} mean (±SE) observed and expected heterozygosities, F_{ISpop} mean (±SE) inbreeding coefficient [Wright (1931); ^{NS}non-significant ($P > 0.05$); **$0.001 < P < 0.01$]

populations to 0.3 ± 0.1 for $LOY04_{SSH04b.}$ Population clonal richnesses were high, varying from 0.88 to 1.00 when considering missing data as different alleles, or from 0.77 to 1.00 when considering missing data as potentially identical alleles (Table 2). Thus, the mean observed and expected heterozygosities (Ho_{pop} and He_{pop}, respectively; ± SE) and F_{IS} estimates were very similar between the entire and the truncated datasets. For the entire dataset (Entire_11), Ho_{pop} varied from 0.30 ± 0.09 for $CHE01_{SSH04a\text{-}1}$ to 0.61 ± 0.09 for $LOY03_{SSH04b}$ and He_{pop}, from 0.42 ± 0.08 for $LOY05_{SSH04b}$ to 0.58 ± 0.04 for $LOY03_{SSH04b}$. Almost all F_{IS} estimates (11/12) were not significantly different from zero (ranging from -0.10 ± 0.16^{NS} for $LOY03_{SSH04b}$ to 0.22 ± 0.09^{NS} for $NCA01_{SSH04a\text{-}2}$), except in $CHE01_{SSH04a\text{-}1}$ ($F_{IS} = 0.40 \pm 0.14$**; Table 2). All indices followed the same trends at eight loci (Online Resource 12).

Genetic connectivity

At 11 loci, for the entire dataset (Entire_11), F_{ST} estimates between intra-cluster pairs of populations ranged from 0.025^{NS} for NCA13/LOY06 (SSH04b) to 0.230*** for NCA14/LOY05 (SSH04b; mean ± SE = 0.118 ± 0.137), and D_{est} estimates ranged from 0.009^{NS} to 0.166*** for the same population pairs (mean ± SE = 0.065 ± 0.008; Online Resource 13a). Estimates were generally lower for the truncated dataset (Truncated_11: $0.028^{NS} < F_{ST} < 0.201$***; $0.009^{NS} < D_{est} < 0.163$***), but followed the same trends. For both datasets, genetic differentiation between populations from different clusters was higher, with F_{ST} ranging from 0.162*** to 0.407***, and D_{est} from 0.104*** to 0.448*** (Online Resource 13a). Again, at 8 loci, results were very similar (Online Resource 13b).

The relative migration network retrieved the three clusters, but no particular pattern of migration could be distinguished (Online Resource 14a), even when considering clusters separately (Online Resource 14b), possibly due to the restricted size and number of populations.

Finally, population-based networks were built for each cluster independently. For SSH04a-1 and SSH04b, the Dpe was defined at 0.11, while it was higher for SSH04a-2 (Dpe = 0.64). Thus, all networks were built at the F_{ST} threshold of 0.11 (Fig. 3). The populations from SSH04a-2 and SSH04b, almost all in eastern New Caledonia and Loyalty Islands, appeared weakly connected, while those from SSH04a-1, located in Chesterfield Islands and New Caledonia (East and West) were more connected (Fig. 3).

Discussion

Our results evidenced that *Pocillopora* PSH04 colonies from the tropical southwestern Pacific belong to two SSHs (SSH04a and SSH04b), found in sympatry among several sites. Moreover, SSH04a split into two less differentiated clusters (SSH04a-1 and SSH04a-2), also sometimes sympatric. This partitioning seems unrelated to *corallum* macromorphology (Online Resource 15), nor clonality. Indeed, a few repeated MLGs were detected among colonies, even when considering missing data as potentially identical alleles, and results were very similar whether keeping them or not. Finally, the studied populations appeared relatively differentiated (1) according to the relative abundance of the different SSHs and clusters (the connectivity units), and (2) among each connectivity unit, according to F_{ST} and D_{est} estimates.

A single or several species?

In this study, analysing the genetic partitioning within *Pocillopora* PSH04 using different assignment methods based on allelic frequencies or genetic distances estimated from microsatellite data, we found that colonies were divided into two divergent genetic groups (SSH04a and SSH04b), according to the congruence among the different methods used, and the different datasets analysed. These two groups were strongly differentiated ($F_{ST} = 0.203$***), especially for some loci, and seem geographically separated (one SSH on each side of Grande Terre; Fig. 1). However, they were found in sympatry in some sites. No distinction between the two SSHs based on *corallum* macromorphology seems obvious (Online Resource 15). In addition, SSH04a was divided into two sympatric clusters (SSH04a-1 and SSH04a-2), which are themselves less, but still highly differentiated ($F_{ST} = 0.117$***).

In a previous study (Gélin et al. 2017a), the species boundaries within the *Pocillopora* genus were redefined using species delimitation methods based on genetic information: 16 PSHs were defined within the genus, some of which splitting into several SSHs. For example, *Pocillopora* PSH05 (sensu Gélin et al. 2017a), corresponding to *P. acuta* (previously *P. damicornis β*), split into four SSHs (SSH05a, b, c, and d), SSH05a and SSH05b being in sympatry in the Pacific Ocean, and SSH05c and SSH05d in the Indian Ocean (Gélin et al. 2018b). However, no genetic partitioning was previously identified within *Pocillopora* PSH04, as the former analyses were conducted on colonies belonging to the entire genus ($N = 975$ colonies of which 102 were assigned to *Pocillopora* PSH04, more precisely to SSH04a), and each locality was represented by only a few colonies (Gélin et al. 2017a). In the present study, only colonies assigned to *Pocillopora* PSH04 were considered, representing a small proportion of the diversity of the genus, but a larger number of colonies per locality.

Discoveries of cryptic species are not rare within scleractinian corals. Indeed, many species were defined on the sole use of traditional taxonomic (often morphological)

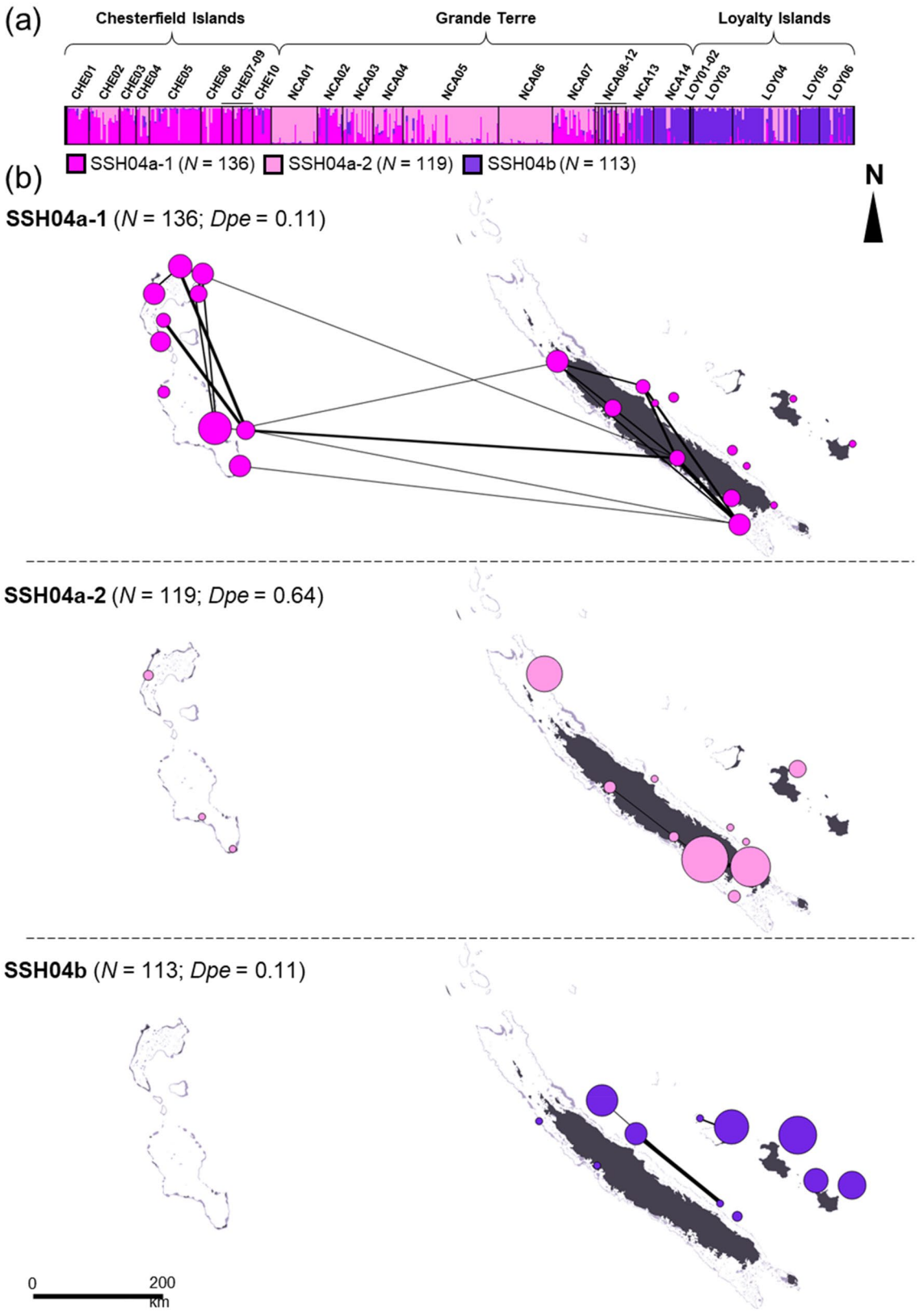

Fig. 3 Population-based networks for each cluster of *Pocillopora* PSH04 (Entire_11 dataset). **a** STRUCTURE plot at $K=3$; **b** population-based networks built with the F_{ST} distance (Weir and Cockerham 1984) thresholded at 0.11

characters (see Veron 2000), which are ambiguous due to phenotypic plasticity. Thus, many morphospecies previously thought to be distributed in the whole Indo-Pacific, were in fact complexes of different species, each restricted to the ocean basin [e.g., *P. damicornis* (Schmidt-Roach et al. 2014), *P. eydouxi/meandrina* (Gélin et al. 2018a), *P. verrucosa* (Oury, personal communication), *S. pistillata* (Keshavmurthy et al. 2013), and *L. pruinosa* (Arrigoni et al. 2020)]. Sympatric cryptic coral species are not unusual too. For example, four sympatric clusters were found within *Seriatopora hystrix* colonies from Lizard and Palm Islands (GBR; Warner et al. 2015), three in the Ryūkyū Archipelago (Japan; Nakajima et al. 2017). Misidentifying these cryptic species implies many consequences, such as incorrect biodiversity and connectivity assessments, themselves influencing the implementation of management and conservation plans.

In a previous study, Thomas et al. (2014) showed, using six microsatellite markers, that *P. damicornis s.s.* colonies of Ningaloo Reef and Muiron Islands (western Australia) belong to two distinct genetic clusters, one in the North of the sampling area, the other in the South, and in sympatry only at intermediate latitude sites. This pattern of genetic structuring is similar to the one observed by Gélin et al. (2017b), studying several populations of *Pocillopora* SSH05c in Reunion Island (southwestern Indian Ocean). A geographic barrier or asynchronous spawning of the most distant populations were mentioned to explain this pattern (Gélin et al. 2017b). A similar pattern was also observed within colonies of *Acropora tenuis* from Nansei Islands (Japan), suggesting the existence of two source populations at the geographic extremities of the sampling area, both mixing within the intermediate populations (Zayasu et al. 2016). In the present study, if the two SSHs, or the three clusters, constitute evolutionary units or distinct species, then reproductive barriers probably established among them. Ecological gradients, such as depth, were already evoked to explain the sympatry of *S. hystrix* clusters in the Ryūkyū Archipelago (Flot et al. 2008; Bongaerts et al. 2010; van Oppen et al. 2011; Nakajima et al. 2017). The same would apply to *Porites astreoides* (Lamarck, 1816) in Florida (southeastern USA; Serrano et al. 2016). However, depth does not appear to be the reproductive barrier here, as colonies sampled on the same site were usually at the same depth: all *P. damicornis s.s.* colonies were sampled in lagoons and upper reef slopes, between sea surface and 5 m depth. However, differential habitats on both sides of Grande Terre could explain the SSHs. Indeed, in the GBR, differences in habitat specificity were found among *S. hystrix* cryptic species, some being restricted to sheltered reefs, while others to exposed reefs (Warner et al. 2015). A similar trend could be hypothesised here, with SSH04a being mainly found in large and relatively sheltered lagoons (Chesterfield Islands and western Grande Terre), while SSH04b in more exposed habitats (eastern Grande Terre and Loyalty Islands; Fig. 1).

Clonality or not clonality?

Among the 458 colonies studied, only two MLGs were found repeated within sympatric colonies ($R = 0.99$) when considering all 11 loci and missing data as different alleles. The clonality increased when considering missing data as potentially identical alleles, but remained weak (27 MLGs repeated over 458 colonies; $R = 0.92$; 330–389 MLLs). At 8 loci, clonal richness was lower (R between 0.93 and 0.86, considering missing data as different or potentially identical alleles, respectively; 161–265 MLLs), but colonies sharing the same MLG at 8 loci had distinct MLGs at 11 loci. Moreover, high P_{SEX} (F_{IS}) values and colonies sharing identical MLGs distant from more than 750 km suggest an underestimation of the clonal richness at 8 loci. This relatively low rate of clonality can be explained by a non-optimal sampling to detect clonality (unlike random sampling for example). Nevertheless, with the same sampling (except in Reunion Island), Gélin et al. (2018b) found a high clonality rate in *Pocillopora* PSH05 [819 distinct MLGs (668–794 MLLs) among 1418 colonies over 13 loci, including the 11 ones from the present study; $R = 0.577$]. Thus, although the sampling was not appropriate for the study of clonality, if asexual propagation was common such as in *Pocillopora* PSH05, then a higher clonality rate should have been observed.

In other genetic studies focusing on *P. damicornis s.s.*, clonality rates were higher. Using six microsatellite loci, Thomas et al. (2014) obtained a global clonal richness of 0.52 ($N = 162$ colonies) but with a high spatial variability (R varying from 0.15 to 0.87 per site). Some MLGs were highly represented (up to 13 colonies sharing the same MLG) and others present in several sites (three MLGs present in two sites and one in three sites, distant from 6 to 240 km; Thomas et al. 2014). The same trends were found by Torda et al. (2013a, b) in the GBR region, using nine microsatellite loci. These differences in clonality rates could result from the lower number of loci used in the previous studies (and from their low polymorphism and/or possible homoplasy). It could also be the result of a spatial variation in clonal propagation within *P. damicornis s.s.* (e.g., due to different hurricane frequencies favouring fragmentation), or the result of differences in clonal propagation between cryptic species. As an illustration, *Porites lobata* colonies from the eastern tropical Pacific were disentangled in two species: *Porites lobata*, which reproduces mainly sexually, and *Porites evermanni*, which propagates through fragmentation by triggerfishes (Boulay et al. 2014). Different clonality rates were also found between western and eastern Caribbean *Acropora palmata* populations, possibly related to habitat characteristics (Baums et al. 2006). Whatever the cause of

these differences, clonal propagation in *P. damicornis s.s.* populations from New Caledonia appears relatively occasional and/or accidental.

Weak connectivity in New Caledonia

Considering the clusters as connectivity units, some populations, sometimes relatively distant (e.g., NCA13 and LOY06 within SSH04b, ~ 340 km apart), were genetically undifferentiated, while some populations belonging to the same locality, or even neighbours (i.e., a few tens kilometres apart), were highly differentiated. Such pattern could be a consequence of the restricted size and number of populations within each cluster (due to cryptic species), or suggests that population differentiation is not entirely related to geographic distance (isolation by distance hypothesis could not be tested). Currents could also be involved, as water exchanges between Grande Terre lagoons and the open ocean are weak and regional currents are dominated by a flow from East to West, resulting from the southeastern trade winds (Fig. 1; Vega et al. 2006). This suggests the existence of two reef systems hydrologically isolated on both sides of Grande Terre. Accordingly, populations of eastern Grande Terre (sinks) would be connected to Loyalty Islands populations (sources) by a unidirectional gene flow from East to West, following currents. Grande Terre would act as a geographical barrier for this flow, explaining why SSH04a-2 and SSH04b are almost restricted to the East of the ecoregion. However, it is conceivable that gene flow from eastern Grande Terre or Loyalty Islands will persist and reach Chesterfield Islands either directly through surface currents bypassing Grande Terre from the North, or indirectly through intermediate population(s) (stepping stone colonization). Unfortunately, we were unable to confirm this pattern of migration from the relative migration networks, again possibly due to the size and number of populations.

A high genetic differentiation was also observed between four *Pocillopora* PSH05 (*P. acuta*) populations from Grande Terre (corresponding to NCA01, NCA03, NCA05-06, and NCA11 herein; Gélin et al. 2018b). In addition, Postaire et al. (2017) showed a strong genetic differentiation between populations of the hydrozoan *Macrorhynchia phoenicea α* (sensu Postaire et al. 2016) in Chesterfield Islands, Grande Terre, and Loyalty Islands (many sites in common with this study), explained by the ecology of this species, often found attached at the base of *Pocillopora* colonies, although presenting a medusoid phase. Similar results were obtained from three new caledonian populations (close from NCA02, NCA05-06, and NCA10 herein) of two reef fishes: *Acanthurus triostegus* (larval stage duration: 60 days) and *Stegastes nigricans* (24 days; Planes et al. 1998). In contrast, the same three populations of another reef fish, *Epinephelus merra* (39 days), were found weakly differentiated in the same study (Planes et al. 1998). This difference was explained by (1) a limited sampling and (2) the high migratory capabilities of *E. merra* adults during spawning. Nevertheless, although several studies have revealed a weak connectivity in this ecoregion, the pattern seems not generalizable to all species. Indeed, using the mitochondrial COI, a high connectivity was found within populations of four squat lobster-like and a gastropod species from Isle of Pines and Norfolk seamounts (southern of Grande Terre; Samadi et al. 2006). It is thus necessary to multiply genetic connectivity investigations in this understudied Pacific region to achieve accurate multi-specific models.

Conclusion

Our results provide insights on the genetic diversity, structure, and connectivity of populations of *Pocillopora damicornis s.s.*, a recently delimited *Pocillopora* species that is thus largely understudied. In addition, this study focused on a marine province located in the South of the distribution range of this species: the tropical southwestern Pacific, largely understudied, as well. Thus, several evolutionary units, perhaps even several species, are distinguished under the name of *Pocillopora damicornis s.s*. Nevertheless, focusing on each evolutionary unit, their populations from the tropical southwestern Pacific appeared differentiated, probably due to restricted gene flow, limited by currents, habitat specificities, and/or geographical distances among populations. This low connectivity between populations, coupled with the existence of cryptic species, brings new insights to the connectivity pattern of New Caledonia ecoregion. Such results must be considered for setting up appropriate management plans for the Natural Park of the Coral Sea.

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Compliance with ethical standards

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

Ethics approval All applicable international, national, and/or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed, and all necessary approvals have been obtained (New Caledonia ecoregion: authorizations nos 2432-2012/ARR/DENV, 2660-2013/ARR/DENV, 60912-25-28-2012/JJC, 60455-15-25/JJC, and 6161-37/PR).

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