



Population genetics of the brooding coral *Seriatopora hystrix* reveals patterns of strong genetic differentiation in the Western Indian Ocean

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Abstract

Coral reefs provide essential goods and services but are degrading at an alarming rate due to local and global anthropogenic stressors. The main limitation that prevents the implementation of adequate conservation measures is that connectivity and genetic structure of populations are poorly known. Here, the genetic diversity and connectivity of the brooding scleractinian coral *Seriatopora hystrix* were assessed at two scales by genotyping ten microsatellite markers for 356 individual colonies. *S. hystrix* showed high differentiation, both at large scale between the Red Sea and the Western Indian Ocean (WIO), and at smaller scale along the coast of East Africa. As such high levels of differentiation might indicate the presence of more than one species, a haplotype analysis was conducted with the nuclear marker ITS2, confirming that the Red Sea populations are genetically distinct from the WIO ones. Based on microsatellite analyses three groups could be distinguished within the WIO: (1) northern Madagascar, (2) south-west Madagascar together with one site in northern Mozambique (Nacala) and (3) all other sites in northern Mozambique, Tanzania and Kenya. These patterns of restricted connectivity could be explained by the short pelagic larval duration of *S. hystrix*, and/or by oceanographic factors, such as eddies in the Mozambique Channel (causing larval retention in northern Madagascar but facilitating dispersal from northern Mozambique towards south-west Madagascar). This study provides an additional line of evidence supporting the conservation priority status of the Northern Mozambique Channel and should inform coral reef management decisions in the region.

Introduction

Coral reefs provide habitat for at least a quarter of all known marine species (Fisher et al. 2015) and sustain an estimated value of around US\$352,000 ha⁻¹ y⁻¹ of ecological goods

and services such as food, coastal protection and recreation (Costanza et al. 2014). Currently, coral reef degradation is occurring at an alarming rate due to a variety of local and global anthropogenic stressors (Hoegh-Guldberg et al. 2017). The main limitation that prevents the implementation of adequate conservation measures is that the connectivity and genetic structure of coral populations are poorly known. Resilience-based management, such as marine protected area (MPA) networks, aims to support coral reefs by maximising reef resistance and recovery to environmental changes, but in order to be effective, knowledge of the connectivity among reefs is essential (McLeod et al. 2019). Connectivity determines gene flow and subsequently the genetic diversity and genetic structure of populations: for instance, high connectivity can facilitate the restoration of heavily disturbed or degraded sites (Palumbi 2003; van Oppen and Gates 2006). As adult corals are sedentary, connectivity between reefs is established through dispersal of larvae. Therefore, traits such as the pelagic larval duration (PLD) and larval behaviour have been proposed to be crucial determinants of genetic population structure.

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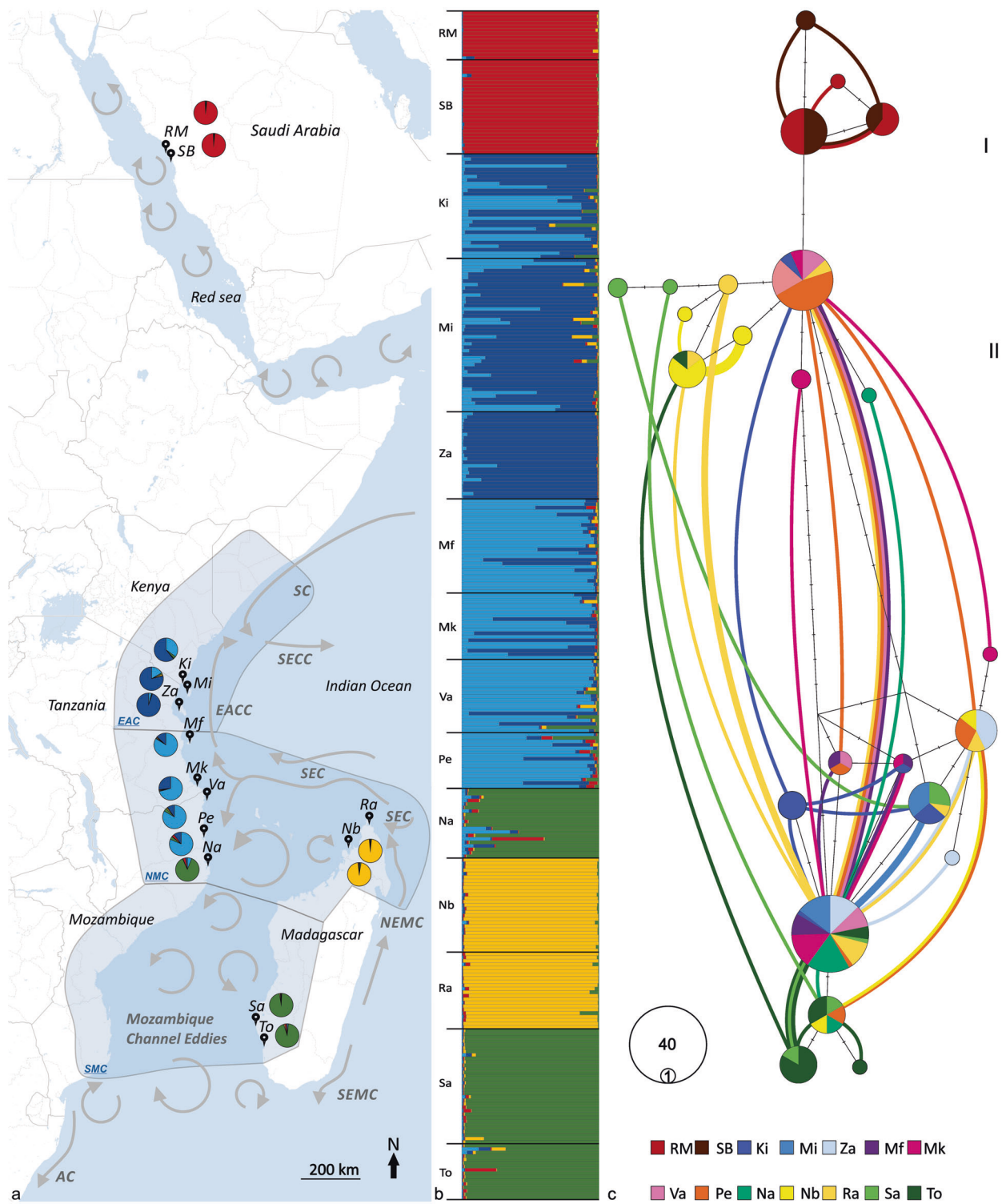
Additionally, dispersal is also constrained by environmental conditions, such as currents, oceanographic barriers and habitat suitability for larval settlement (Baird 2001; Cowen and Sponaugle 2009).

Larvae in scleractinian corals result mostly from sexual reproduction, which can broadly be divided in two strategies: broadcast spawning and brooding (Harrison 2011). Broadcast spawning corals release buoyant gamete bundles with eggs and sperm to the water column, where fertilisation and subsequent embryonal development take place. In brooding corals, only sperm is spawned, often during several months, with subsequent internal fertilisation and development of the larvae. Brooding corals are generally assumed to have a shorter PLD and consequently higher levels of genetic subdivision than found in broadcast spawning corals, but current evidence for this is limited. For example, the brooding coral *Seriatopora hystrix* was found to exhibit predominantly short-distance dispersal in Australia, where larvae mainly settled close to their natal colony (Underwood et al. 2007, 2009; van Oppen et al. 2008), and isolation by distance was found in the Red Sea on a small geographic scale (within 20 km, Maier et al. 2005). At the same time there is also evidence for occasional long-distance dispersal on reefs in Australia (Underwood et al. 2007; van Oppen et al. 2008).

Reproduction and connectivity form an integral component of studies on the long-term dynamics of coral species (e.g., McLeod et al. 2019; van Oppen and Gates 2006). However, unrecognised interspecific diversity, or cryptic diversity, challenges our understanding of connectivity patterns (Sheets et al. 2018). Morphospecies of scleractinian corals often do not correspond to “biological” species, particularly for corals with high morphological plasticity such as acroporids (Richards et al. 2016) and pocilloporids (e.g., Flot et al. 2010), including *Seriatopora* (Flot et al. 2008; Nakajima et al. 2017; Warner et al. 2015). Adding to the difficulty of resolving relationships, highly permeable reproductive boundaries have been described between some morphospecies of scleractinian corals (e.g., Vollmer and Palumbi 2002). This complicates current attempts to define spatial units of conspecific corals that exchange alleles. Such metapopulations could be used as units in conservation planning, but it is still unknown to what extent widespread coral morphospecies represent metapopulations that regularly exchange genetic material or form isolated subsets of populations (or even cryptic species). For all these reasons, species delimitation in corals requires comprehensive approaches that integrate molecular, morphological and distributional data (Schmidt-Roach et al. 2014; Arrigoni et al. 2020). In the case of cnidarians, the cytochrome *c* oxidase subunit I fragment typically used for DNA barcoding of animals is not variable enough to

distinguish most species (Shearer and Coffroth 2008). Even when more variable mitochondrial regions are available, as in the case of pocilloporid corals (Flot and Tillier 2007), mitochondrial markers, unlike nuclear markers, are haploid and can therefore only be analysed using distance-based and tree-based approaches (Flot 2015). The former assumes that intraspecific distances are smaller than interspecific ones, while the latter only detect species that are monophyletic (Fontaneto et al. 2015), two requirements that are not always met for coral species, because of their large effective population sizes and the possible occurrence of hybridisation. Nuclear markers, by contrast, can be analysed using tree-based, distance-based, as well as allele sharing-based methods (Fontaneto et al. 2015). Allele sharing-based methods such as haplowebs (Flot et al. 2010) can help delimiting species regardless of their monophyly (or lack thereof). Like other single-locus methods, haplowebs may be confounded by markers under selection or in case of recent introgression and shared ancestral sequences (Flot et al. 2010). However, simulation studies have shown that haplowebs tend to perform better than other approaches designed to infer which individuals in a data set are conspecific (Dellicour and Flot 2015, 2018), providing useful information for the implementation of adequate management of coral populations.

The coral reefs of the Western Indian Ocean (WIO) and the Red Sea offer a unique environment to study the connectivity of corals under variable environmental conditions along a north-south gradient covering ~6000 km. The WIO has fringing reefs along almost the entire African coast, and these reefs experience complex oceanographic conditions (Fig. 1): the South Equatorial Current (SEC) carries water westwards towards the southern coast of Tanzania and the northern coast of Mozambique, where it bifurcates southward into the eddies in the Mozambique Channel down to South Africa and northward into the East African Coast Current (EACC) along the coasts of Tanzania and Kenya (Hancke et al. 2014; Lutjeharms and Bornman 2010; Schott and McCreary 2001; Sofianos and Johns 2003). Connected through the shallow and narrow strait of Bab-el-Mandeb with the Gulf of Aden, the Red Sea is a peripheral region of the Indo-Pacific that is characterised by a high coral biodiversity. Currents in the Red Sea are formed by gyres and these circulation patterns are spatially and temporally variable (Sofianos and Johns 2003). Based on coral species diversity, three regions can be distinguished in the central WIO (Obura 2012): the reefs surrounding the Northern Mozambique Channel (NMC region) that form the high-diversity core region in the WIO, the East African Coast (EAC) region that is under the influence of the EACC, and the Southern Mozambique Channel region (SMC) under the influence of Mozambique Channel eddies (Fig. 1). To date,



studies on coral connectivity patterns within or between major biogeographical regions of the WIO and the Red Sea are not available, as research tends to be limited to smaller geographical scales. *S. hystrix* (Pocilloporidae; Dana 1846) is an ideal species for such study as it has a widespread distribution in the Indo-Pacific, occurring, e.g., in the Red

Sea, Madagascar, along the coast of East Africa in Kenya and Tanzania, as well as in the north and south of Mozambique (Veron 2000). It is common not only on shallow reefs and intertidal reef flats, but also at mesophotic depths (Prasestia et al. 2017; van Oppen et al. 2011; Veron 2000). *S. hystrix* is a brooding coral that releases planula

◀ **Fig. 1 Sample sites and spatial genetic structure of the stony coral *Seriatopora hystrix*.** **a** Map showing sample sites of *S. hystrix* in Saudi Arabia, Kenya, Tanzania, Mozambique and Madagascar. Major ocean currents during the Northeast Monsoon are indicated schematically (Collins et al. 2014; Hancke et al. 2014; Lutjeharms and Bornman 2010; Schott and McCreary 2001; Sofianos and Johns 2003). SEC South Equatorial Current, NEMC Northeast Madagascar Current, SEMC Southeast Madagascar Current, EACC East African Coast Current, SECC South Equatorial Counter Current, SC Somali Current, AC Agulhas Current. Biogeographic regions proposed by Obura (2012) are also indicated: EAC East African Coastal region, NMC Northern Mozambique Channel region, SMC Southern Mozambique Channel region; sample site codes: RM Ras Masturah, SB Shi'b Al Bayda, Ki Kisite, Mi Misali, Za Zanzibar, Mf Mafia, Mk Mikindani, Va Vamizi, Pe Pemba, Na Nacala, Nb Nosy Be, Ra Ramena, Sa Salary, To Toliara. The pie charts show the distribution of five genetic clusters ($K = 5$) at sample sites as revealed by the Bayesian clustering implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). **b** Bar plot showing estimated membership fractions in each of the five clusters, with each horizontal bar representing one individual. **c** Haplotype network (haploweb) based on 124 sequences of the ITS2 marker. Each pie chart represents one of the 24 haplotypes, its area proportional to the number of individuals in which it was found and with colours indicating the locations where it was sampled. Black lines show the mutational steps between haplotypes. Coloured curves connect haplotypes that co-occur within heterozygous individuals: the thickness of a curve connecting two haplotypes represents the number of individuals in which these haplotypes co-occur, with colours indicating the locations where these individuals were sampled. Within this haploweb two groups are distinguished in which all haplotypes are connected by heterozygotes: group I includes all individuals collected in the Red Sea and group II includes all individuals collected in the Western Indian Ocean.

larvae year-round in the tropics and has a short PLD ranging from 6–12 h to 1 week in aquarium experiments (Baird 2001; Isomura and Nishihira 2001; Prasetia et al. 2017). Due to its short PLD, the brooding coral *S. hystrix* is a good model species to investigate the influence of ocean currents on the dispersal of reef building corals. This study investigates the genetic diversity, population structure and connectivity patterns of the brooding coral *S. hystrix* at two different scales: first, by comparing the WIO and the Red Sea, and second, by comparing sites within the WIO. Based on the life history of *S. hystrix*, species richness distribution and ocean currents (discussed in Obura et al. 2019), the hypotheses we aimed to test were: (1) a genetic break separates the Red Sea and the WIO, (2) the NMC region is genetically distinct and characterised by a high genetic diversity, (3) the SMC region is genetically distinct and (4) a genetic break occurs between the NMC and EAC regions. The four hypotheses were tested using ten microsatellite markers and a complementary haploweb analysis of the nuclear ITS2 marker, a strategy also used by Adjeroud et al. (2014). Ultimately, we hope that this study provides important insights into the genetic structure of coral populations and the possible link between PLD and

oceanography, as well as reveal which spatial scales are relevant for conservation initiatives.

Material and methods

Study region and sampling

The WIO has fringing reefs along the coast, with patchier reefs in the north of Kenya that become more extensive and diverse southwards into Tanzania and then into the north of Mozambique (McClanahan et al. 2014; Obura 2012; Obura et al. 2017). There are smaller and patchier offshore reefs in the South of Mozambique (Motta et al. 2002; Ridgway et al. 2008), but they are separated from the northern reefs by an area devoid of coral reefs, because of freshwater discharge and sediments from the large-scale river outlets and mangrove forests of central Mozambique (Obura et al. 2017). Across the channel, the Madagascar coastline consists of many fringing and barrier reefs as well as banks separated by extensive mangrove forests (Obura et al. 2017). The Red Sea has mainly fringing reefs along most of both shores, with atolls and pinnacle reefs found in the southern and central Red Sea (Wilkinson 2002). A total of 356 individual *S. hystrix* colonies were sampled at two locations in Saudi Arabia and 12 locations in the WIO: one in Kenya, four in Tanzania, three in Mozambique and four in Madagascar (Fig. 1a and Appendix S1). It is important to note that in this study no *S. hystrix* colonies were found in Mozambique south of Nacala (for which the sampling campaign included Vilanculos, Pembane, Tofo, Xai-Xai, Inhaca and Ponta do Ouro). Colonies were randomly selected within a reef with a distance of at least 2 m between consecutive collections, in order to reduce the chances of sampling clones. Each sample was obtained by cutting a 2–3 cm fragment from a branch. The fragments were stored in a plastic container with seawater until fixation in absolute ethanol. Samples were stored in the dark at 7 °C until extraction.

DNA extraction and microsatellite genotyping

Fragments were cut manually into small pieces with a wire cutter and DNA was extracted following the animal tissue protocol Omega Bio-tek® E.Z.N.A.® Tissue DNA kit (Norcross, GA, USA) to a final volume of 100 µL. Samples were genotyped using ten microsatellite primer pairs designed for *S. hystrix* from the Red Sea and Australia (details of the DNA extraction, primers and PCR conditions given in Appendix S2). PCR products were analysed on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting electropherograms were scored manually using GeneMarker® (v. 2.6.0; Soft-Genetics, State College, PA, USA).

Genetic diversity analysis

The software GenAIEx 6.503 (Peakall and Smouse 2012) was used to determine the probability of identity (PI) and to identify identical multilocus genotypes that were likely to be the result of fragmentation of the coral colony. Departures from Hardy–Weinberg equilibrium were computed for each locus using FSTAT 2.9.3 (Goudet 1995) and presented as F_{IS} (calculated as smallF; Weir and Cockerham 1984) with significance levels based on 140,000 permutations. In all FSTAT analyses, the number of permutations was automatically determined based on either the number of loci multiplied by the number of populations in the experimental design (e.g., for F_{IS}), or calculated for all loci together as the number of possible pairwise comparisons (e.g., for pairwise F_{ST}). Micro-Checker 2.2.3 (van Oosterhout et al. 2004) was used to check for microsatellite null alleles and scoring errors. Linkage disequilibrium values were calculated using FSTAT with P values based on 450,000 permutations. Number of alleles per population, corrected for sample size and expressed as allelic richness (A_r), were also calculated using FSTAT, whereas observed, expected and unbiased heterozygosities (corrected for sample size) and private alleles were calculated using GenAIEx. Bottleneck 1.2.02 (Cornuet and Luikart 1996) was used to check for recent bottleneck events using a two-phased mutation model with 12% variance, 95% stepwise mutation model and 10,000 iterations, after which a one-tailed Wilcoxon test of heterozygote excess and a mode shift test were performed (Peery et al. 2012). Lastly, a one-sided comparison (9,999 permutations) of the allelic richness A_r was performed using FSTAT to find out whether this parameter was significantly different between the EAC, NMC and SMC regions of the WIO.

Population structure within the WIO and Red Sea

To investigate differentiation amongst populations, pairwise F_{ST} values were estimated using the method of Weir and Cockerham (1984), (θ) implemented in FSTAT and tested for significance with 91,000 permutations. Statistical significance for all pairwise tests was adjusted for multiple comparisons using a Bonferroni correction. Pairwise F_{ST} values were used in a Mantel test of isolation-by-distance with 9,999 permutations in GenAIEx. The pairwise geographical distance was measured as the shortest path possible by water between two sample sites in Google Earth Pro (www.google.com/earth). GenAIEx was also used to calculate pairwise D_{EST} (Jost 2008) as an additional measure of differentiation and significance was tested with 9,999 permutations. SPAGeDI (Hardy and Vekemans 2002) was used to test if the assumption of stepwise mutations was met, in which case R_{ST} would

reflect population differentiation better than F_{ST} . Additionally, the relative contribution of mutation versus migration to population differentiation over distance intervals was investigated using 20,000 permutations. A significant R_{ST} would emphasise the role of mutation relative to migration rate in the differentiation of distant populations.

The software STRUCTURE 2.3.4 (Pritchard et al. 2000), which is a model-based clustering method, was run without prior population information and under the admixture model to determine the most likely number of genetic clusters (K). STRUCTURE was run for $K = 1–14$, using 10 runs with a burn-in of 100,000 generations followed by 1,000,000 additional cycles. Additionally, two separate STRUCTURE analyses were run for the WIO ($K = 1–12$), and for the African mainland ($K = 1–8$), using the same settings. HARVESTER 0.6.94 (Earl and Vonholdt 2012) was used to visualise and analyse the STRUCTURE output by plotting the log probability $L(K)$ and ΔK (Evanno et al. 2005).

Patterns of differentiation were also explored using a Discriminant Analysis of Principal Components (DAPC) implemented in the *Adegenet* package (Jombart 2008) implemented in R (<https://www.r-project.org/>). The DAPC was run for the whole data set, as well as separately for the WIO, and for the African mainland. The most likely number of clusters (K) was determined based on the lowest value of the Bayesian information criterion (using the function *find.clusters*). The correct number of principal components to be retained in the analyses was then confirmed by cross-validation (using the function *Xval.dapc*).

Haploweb analysis using ITS2

To investigate the possible occurrence of cryptic species among our samples, a haplotype web (haploweb) of nuclear ITS2 sequences was constructed as described in Flot et al. (2010), using the coral-specific primers ITSc2-5 and R28S1 to amplify ITS2 for six to eight individuals from each sample site (details of the PCR conditions are provided in Appendix S3). The PCR products were sequenced in both forward and reverse directions using the same primers as for amplification. The resulting sequences were assembled and cleaned manually in Sequencher 4.14 (Gene Codes Corporation, Ann Arbor, MI, USA). The haplotypes of length-variant heterozygotes were determined computationally by combining the information from the forward and reverse chromatograms (Flot et al. 2006) using the online programme Champuru (Flot 2007). The haplotypes of the other heterozygotes were resolved using the programmes SeqPHASE (Flot 2010) and PHASE 2.1 (Stephens and Scheet 2005; Stephens et al. 2001), taking advantage of

the known phased previously obtained for length-variant heterozygotes. Finally, the haploweb itself was generated using the online programme HaplowebMaker (<https://eeg-ebe.github.io/HaplowebMaker>; Spöri and Flot 2020).

Results

Genotypic diversity

Of the 356 sampled colonies, 341 were found to harbour unique multilocus genotypes. PIs were low for all sites ($PI < 0.01$, except in Zanzibar $PI = 0.014$) and therefore adequate for population studies (Waits et al. 2001). Within the sample sites, nine pairs of identical multilocus genotypes were found: one pair at the sample site Shi'b Al Bayda, two pairs at Kisite, three pairs at Zanzibar, two pairs at Nosy Be, and one pair at Toliara. Additionally, seven identical multilocus genotypes were found in sample site Toliara. As these individuals were most likely the result of the fragmentation of a colony, only one individual from each multilocus genotype was kept in ensuing analyses.

Genetic diversity

The ten microsatellite markers amplified for all 14 sample sites except for locus Sh2-005 in sample site Ramena. Therefore, all analyses were performed both with and without locus Sh2-005. Four sample sites (Misali, Vamizi,

Pemba and Nosy Be) showed positive F_{IS} values and significant departures from Hardy–Weinberg equilibrium, indicating heterozygote deficits (Table 1). Further analysis with Micro-Checker found that two sample sites, Mikindani (locus Sh3-009) and Vamizi (locus Sh2-005), possibly had null alleles (Appendix S4). Null alleles were only suspected for two loci, each for a single site, therefore all loci were included in subsequent data analyses. Two loci (Sh3-009 and Sh4.28) were found to be polymorphic for all sample sites, whereas the other loci were monomorphic for at least one sample site (Appendix S5).

The allelic richness (A_r) ranged between 1.5 and 2.4, the observed heterozygosity (H_O) between 0.166 and 0.367 and the unbiased expected heterozygosity (uH_E) between 0.198 and 0.409 (Table 1). Private alleles were found more frequently in the southern sites, with a maximum of seven in Nacala. A separate analysis after grouping the populations from the Red Sea, Madagascar and of the other sample sites revealed 2, 12 and 18 private alleles, respectively. No significant linkage disequilibrium was detected. None of the populations showed evidence of a recent bottleneck and all sample sites showed normal L-shaped distribution of allele frequencies. The one-sided comparisons of A_r within the WIO showed significantly higher A_r when comparing sites within the EAC and NMC, with $A_r = 1.7$ and $A_r = 2.0$, respectively ($EAC < NMC$, $P < 0.05$), and higher A_r in the SMC ($A_r = 2.3$, $SMC > EAC$, $P < 0.05$; $SMC > NMC$, $P < 0.05$). However, since only two sample sites (Salary and Toliara) were analysed in SMC, these results are not

Table 1 Genetic diversity in *Seriatopora hystrix* from the Western Indian Ocean and the Red Sea.

Sample site	Site code	<i>n</i>	H_O	uH_E	A_r	F_{IS}	<i>PVA</i>
Ras Masturah	RM	14	0.202 ± 0.081	0.214 ± 0.084	1.742	0.058	0
Shi'b Al Bayda	SB	27	0.246 ± 0.077	0.248 ± 0.083	1.803	0.006	1
Kisite	Ki	30	0.217 ± 0.086	0.261 ± 0.088	1.721	0.170	0
Misali	Mi	44	0.232 ± 0.085	0.296 ± 0.096	1.856	0.217*	0
Zanzibar	Za	25	0.166 ± 0.076	0.198 ± 0.074	1.504	0.163	0
Mafia	Mf	27	0.309 ± 0.073	0.351 ± 0.079	1.950	0.122	0
Mikindani	Mk	19	0.252 ± 0.063	0.318 ± 0.091	1.931	0.210	0
Vamizi	Va	21	0.324 ± 0.070	0.406 ± 0.092	2.354	0.206*	1
Pemba	Pe	16	0.213 ± 0.084	0.295 ± 0.078	1.848	0.285*	0
Nacala	Na	20	0.255 ± 0.117	0.253 ± 0.116	1.906	−0.008	7
Nosy Be	Nb	27	0.183 ± 0.068	0.249 ± 0.085	1.837	0.266*	2
Ramena	Ra	22	0.250 ± 0.072	0.266 ± 0.069	1.840	0.061	2
Salary	Sa	33	0.361 ± 0.079	0.392 ± 0.079	2.224	0.081	1
Toliara	To	16	0.367 ± 0.101	0.409 ± 0.069	2.314	0.105	4

For geographical location of the sample sites see Fig. 1 and Appendix S1.

n sample size, A_r allelic richness calculated without locus Sh2-005, H_O observed heterozygosity (±SD), uH_E unbiased expected heterozygosity (±SD), F_{IS} departure from Hardy–Weinberg equilibrium, positive values indicate a deficit of heterozygotes, *PVA* number of private alleles.

* $P < 0.001$, significant departure from zero is based on 140,000 randomisations, *P* values have been corrected for multiple tests.

Table 2 Below the diagonal: pairwise F_{ST} values (Weir and Cockerham 1984) and levels of significance after 91,000 permutations based on nine microsatellite loci in *Seriatopora hystrix*.

Saudi Arabia		Kenya, Tanzania and Mozambique										Madagascar					
RM	SB	Ki	Mi	Za	Mf	Mk	Va	Pe	Na	Nb	Ra	Sa	To				
RM	0.015***	0.431***	0.454***	0.433***	0.446***	0.366***	0.330***	0.308***	0.333***	0.414***	0.417***	0.371***	0.351***				
SB	0.040*	0.441***	0.460***	0.437***	0.435***	0.355***	0.335***	0.306***	0.347***	0.452***	0.462***	0.375***	0.373***				
Ki	0.555**	0.531***	0.067***	0.052***	0.044***	0.043***	0.081***	0.120***	0.241***	0.370***	0.394***	0.238***	0.278***				
Mi	0.530***	0.531***	0.146***	0.083***	0.087***	0.068***	0.120***	0.117***	0.260***	0.322***	0.357***	0.225***	0.241***				
Za	0.614*	0.589***	0.157***	0.198***	0.096***	0.048***	0.082***	0.114***	0.258***	0.418***	0.429***	0.241***	0.306***				
Mf	0.477*	0.478***	0.091***	0.154***	0.204***	0.029***	0.089***	0.120***	0.286***	0.420***	0.407***	0.221***	0.251***				
Mk	0.465*	0.455***	0.100***	0.134***	0.052 ^{NS}	0.065*	0.040***	0.067***	0.224***	0.419***	0.412***	0.228***	0.273***				
Va	0.377 ^{NS}	0.391***	0.145***	0.188***	0.124***	0.065*	0.132***	0.092***	0.176***	0.361***	0.349***	0.182***	0.233***				
Pe	0.414 ^{NS}	0.417**	0.236*	0.210***	0.186*	0.125 ^{NS}	0.132 ^{NS}	0.114***	0.114***	0.390***	0.396***	0.176***	0.183***				
Na	0.512*	0.502***	0.435***	0.414***	0.507***	0.378**	0.269**	0.235 ^{NS}	0.235 ^{NS}	0.338***	0.312***	0.165***	0.134***				
Nb	0.492**	0.515***	0.487***	0.425***	0.562***	0.455***	0.391***	0.455**	0.478***	0.420***	0.075***	0.252***	0.223***				
Ra	0.544***	0.568***	0.554***	0.513***	0.610***	0.495***	0.432***	0.503**	0.508***	0.283***	0.075***	0.255***	0.194***				
Sa	0.386***	0.400***	0.314***	0.291***	0.343***	0.253***	0.199***	0.217**	0.241***	0.299***	0.369***	0.255***	0.076***				
To	0.384*	0.415***	0.368***	0.319***	0.428***	0.313***	0.238***	0.222*	0.220***	0.286***	0.327***	0.097***	0.076***				

Above the diagonal: pairwise D_{EST} values (Jost 2008) and levels of significance after 9,999 permutations based on eight microsatellite loci in *S. hystrix*. Sample site codes: RM Ras Masturah, SB Shi'b Al Bayda, Ki Kisite, Mi Misali, Za Zanzibar, Mf Mafia, Mk Mkindani, Va Vamizi, Pe Pemba, Na Nacala, Nb Nosy Be, Ra Ramena, Sa Salary, To Toliara. For geographical location of samples sites see Fig. 1 and Appendix S1.

^{NS} non-significant.

*Significant at the 5% nominal level; **significant at the 1% nominal level; ***significant at the 0.1% nominal level. *P* values have been corrected for multiple tests.

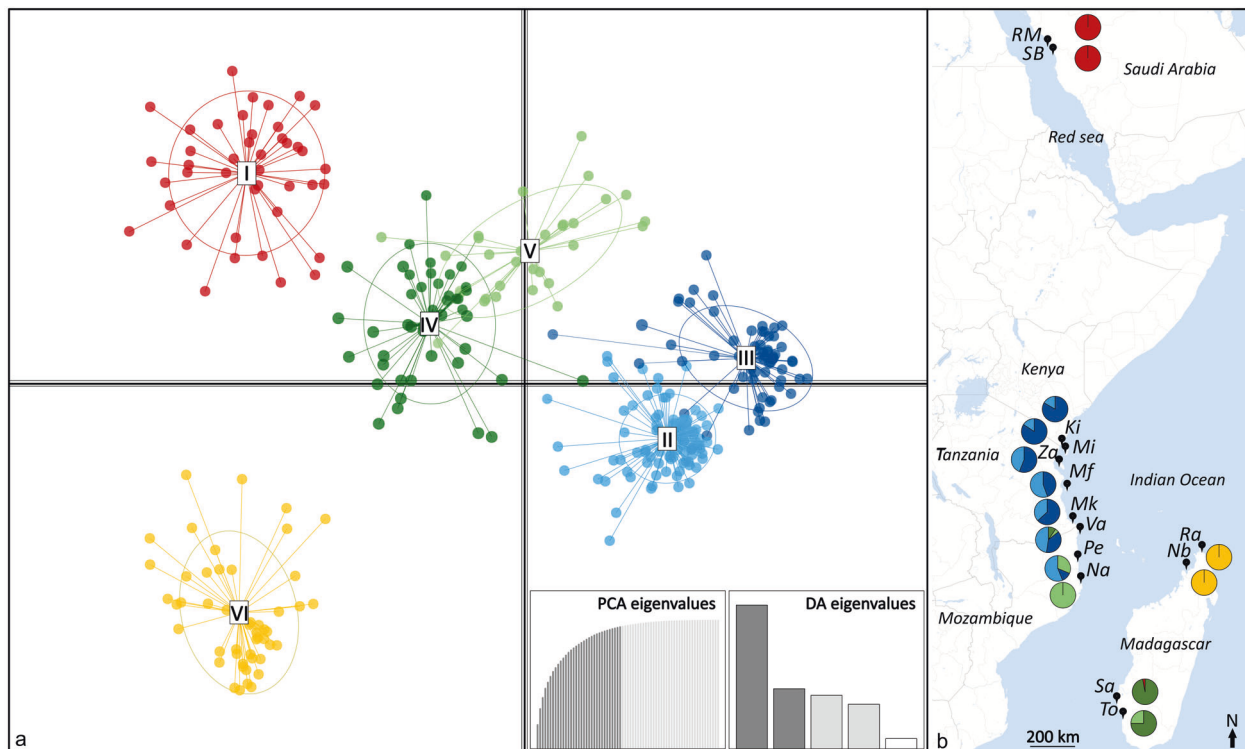


Fig. 2 Discriminant Analysis of Principal Components (DAPC) in *Seriatopora hystrix* based on ten microsatellite loci. (in Adegenet; Jombart (2008), 33 principal components retained) **a** DAPC plot: the dots represent individuals, while the different colours represent six clusters identified by Bayesian analyses: cluster I all individuals in the Red Sea; clusters II and III all individuals along the coast of Kenya and Tanzania and some individuals in the north of Mozambique; clusters IV and V individuals from north of Mozambique and south of

Madagascar; cluster VI all individuals from the north of Madagascar. **b** The pie charts show the distribution of the six genetic clusters identified by DAPC at the sample sites in the Red Sea and Western Indian Ocean. Sample site codes: RM Ras Masturah, SB Shi'b Al Bayda, Ki Kisite, Mi Misali, Za Zanzibar, Mf Mafia, Mk Mikindani, Va Vamizi, Pe Pemba, Na Nacala, Nb Nosy Be, Ra Ramena, Sa Salary, To Toliara.

included in the discussion. When considering the NMC without Madagascar, the NMC region still had a higher A_T ($A_T = 2.0$, $EAC < NMC$ mainland, $P < 0.05$).

Population structure

The sample sites were highly differentiated according to pairwise F_{ST} values, with significant differentiation found in 94% of the pairwise population comparisons (Table 2). The highest genetic differentiation was found for the Red Sea sites Ras Masturah (0.377–0.614) and Shi'b Al Bayda (0.391–0.589) versus all other sites. Two comparisons were not significant, Ras Masturah versus Pemba and Ras Masturah versus Vamizi, which can probably be explained by smaller sample sizes in these sites. Within the WIO, a strong differentiation between the East African mainland and sites in Madagascar was observed, especially for Nosy Be (0.283–0.562) and Ramena (0.327–0.610) versus all other sites, as well as for Salary (0.097–0.369) and Toliara (0.097–0.428) in south-west Madagascar. At the East African mainland, differentiation was lower in Kenya and northern

Tanzania, but higher in southern Tanzania and in Mozambique, with Nacala showing high differentiation compared to all other sites, except to adjacent reefs (Pemba and Vamizi) and sites in south-west Madagascar (Salary and Toliara). The Mantel test showed a significant correlation between population differentiation and geographical distance for all sites in the WIO ($R^2 = 0.32$, $P = 0.02$; Appendix S6) as well as for sample sites from the region comprising Kenya, Tanzania and northern Mozambique ($R^2 = 0.40$, $P = 0.0001$; Appendix S6).

D_{EST} was congruent with F_{ST} values (Table 2), indicating that F_{ST} did not underestimate the level and pattern of differentiation. Although similar gradients were observed for both measures, consistently lower values of D_{EST} were found for the East African mainland sites, suggesting a higher influence of migration over mutation rates. The contribution of stepwise mutations ($R_{ST} > F_{ST}$) was tested with a one-sided permutation test, but only with the five most polymorphic loci (Sh3-004, Sh2-005, Sh4-001, Sh3-009 and Sh4.28), as the permutation test was shown to be unreliable for loci with lower polymorphism (Hardy et al. 2003). Significant ($P < 0.05$) contributions of stepwise

mutations in allele size were detected for four loci. However, analyses of distance intervals showed that significant values were only found for distances beyond 738 km (loci Sh3-004, Sh4-001, Sh-009 and Sh4.28; Appendix S7). Additional testing of two groups, WIO (<4000 km) and Red Sea (>4000 km), revealed significant ($P < 0.05$) contributions of stepwise mutations for two loci (Sh3-004 and Sh4-001). The Bayesian clustering implemented in STRUCTURE showed both a plateau for the log probability and an optimum ΔK for $K = 5$, the most likely number of genetic clusters for all sample sites (Appendix S8 and Fig. 1a, b). Based on the proportion of each genetic cluster within each sample site, four groups of sample sites were identified: (1) Saudi Arabia (Ras Masturah, Shi'b Al Bayda); (2) sites in Kenya, Tanzania and northern Mozambique (Kisite, Misali, Zanzibar, Mafia, Mikindani, Vamizi, Pemba) forming a mixed group of two genetic clusters, although based on cluster distribution a shallow structure of northern sites (Kisite, Misali, Zanzibar) and southern sites (Mafia, Mikindani, Vamizi, Pemba) was observed; (3) one site in northern Mozambique (Nacala) and sites in south-west Madagascar (Salary, Toliara); and (4) northern Madagascar (Nosy Be, Ramena). These patterns were confirmed comparing different K values ($K = 1-14$, Appendix S8) as well as with separate STRUCTURE analyses for the WIO and for the African mainland (data not shown).

The DAPC revealed six clusters (Fig. 2, cluster I–VI) of *S. hystrix*, corresponding to the five clusters identified by STRUCTURE plus an additional cluster shared between northern Mozambique and south-west Madagascar. These clusters could be divided into four groups: (1) Cluster I in Saudi Arabia (Ras Masturah, Shi'b Al Bayda); (2) Clusters II and III in Kenya, Tanzania and some individuals from northern Mozambique (Kisite, Misali, Zanzibar, Mafia, Mikindani, Vamizi, Pemba); transitioning to group (3) formed by Cluster IV and Cluster V in northern Mozambique and south-west Madagascar (Vamizi, Pemba, Nacala, Salary, Toliara); and a separate group (4) formed by Cluster VI in northern Madagascar (Nosy Be, Ramena).

Haploweb analysis using ITS2

The ITS2 marker was successfully sequenced for 77 out of 85 samples of *S. hystrix*. There were 30 homozygous individuals and 47 heterozygous individuals, hence a total of 124 sequences corresponding to 24 haplotypes 566–572 bp long (Fig. 1c). In haplowebs, groups of individuals sharing a pool of alleles interconnected by allele sharing in heterozygotes are called fields for recombination (FFRs, following the terminology of Doyle 1995), each of which corresponds to one putative species (Flot et al. 2010). This analysis revealed two distinct FFRs: FFR I comprised all individuals collected in the Red Sea, whereas FFR II

comprised all individuals collected in the WIO. Within FFR I, four haplotypes were found in ten individuals from two locations in the Red Sea. Within FFR II, 20 haplotypes were found in 67 individuals from 12 locations in the WIO, of which the most prevalent haplotype was found in 40 individuals from 11 different locations. Because distinct FFRs suggest the occurrence of two different species, all analyses described above were also performed without the sample sites in the Red Sea, which provided similar results and interpretation (data not shown).

Discussion

This study investigated the large-scale genetic patterns of the brooding scleractinian coral *S. hystrix* within the Red Sea and WIO using microsatellite markers and the nuclear marker ITS2. Since *S. hystrix* is a brooding coral, higher genetic subdivision was expected than in other widespread species with a broadcast spawning reproduction strategy. This was the first coral population genetic study to include both the Red Sea and the WIO, and the first to include a substantial part of the East African coastline, as well as Madagascar.

Isolation of the Red Sea

The Saudi Arabian sample sites in the Red Sea are strongly differentiated from the sites in East Africa based on microsatellite data and the ITS2 marker. This confirms the a priori hypothesis of a strong genetic barrier between the Red Sea and the WIO, which may be explained either by historical and contemporary barriers or by interspecific boundaries. Sample sites in the Red Sea and the WIO belong to two different marine provinces (Spalding et al. 2007): the Red Sea/Gulf of Aden and the WIO. The Red Sea is a marine biodiversity hotspot with a high percentage of endemic species (5.5% of scleractinian corals species, 10% of crustaceans, 8.1% of echinoderms and 14.1% of fish; DiBattista et al. 2016). Strong isolation of Red Sea populations from the WIO has been previously reported for corals (Pinzón et al. 2013), fishes (discussed, e.g., in Froukh and Kochzius 2008; DiBattista et al. 2013, 2016), arthropods (e.g., Iacchei et al. 2016), echinoderms (Vogler et al. 2008), molluscs (e.g., Hui et al. 2016) and sponges (Wörheide et al. 2008), although some studies found no dispersal barriers for some fish species (DiBattista et al. 2013; Kochzius and Blohm 2005). The Red Sea was formed ~24 million years ago and, although currently connected to the Indian Ocean through the shallow Bab-el-Mandeb Strait, it experienced historic isolation from the Indian Ocean during lower sea levels in glacial cycles, causing periods of extreme desiccation and hypersalinity (Siddall et al. 2003).

Whether corals survived these conditions in refugia or recolonised the Red Sea from the Gulf of Aden remains under discussion (DiBattista et al. 2013). In addition to historical barriers, corals in the Red Sea experience dispersal barriers such as eddies in the central Red Sea region as well as turbidity in the southern Red Sea (Roberts et al. 1992). Strong isolation through historical and contemporary barriers could have resulted in a speciation and a subsequent interspecies boundary. In the ITS2 haploweb individuals from the Red Sea and WIO indeed belong to two distinct FFRs, suggesting that they correspond to two different species using the criterion of mutual allelic exclusivity (Flot et al. 2010). However, our sampling design did not include intermediate reefs and this preliminary result is based on a single marker (ITS2), for which the occurrence of paralogs could not be ruled out in this study. The putative species boundary between our WIO and Red Sea samples will need to be verified using morphological and novel multilocus molecular approaches based on RADseq and/or ultra-conserved elements and exons (Schmidt-Roach et al. 2014; Erickson et al. 2020; Arrigoni et al. 2020) before a definitive conclusion can be reached.

Strong differentiation of northern Madagascar

While the ITS2 analyses ruled out the occurrence of cryptic species in northern Madagascar (Nosy Be and Ramena), a strong differentiation from all other sites in the WIO was detected using microsatellite markers, indicating a possible contemporary barrier for larval dispersal. Oceanographic conditions in the NMC could play an important role in isolating these coral populations, specifically the cyclonic eddies that are generated and reside in front of the north-west coast of Madagascar (Collins et al. 2014). Different dispersal models support this isolation, showing that coral larvae released from north-west Madagascar are mostly self-recruiting, which is especially true for coral species with a short PLD such as *S. hystrix* (Crochelet et al. 2016; Gamoyo et al. 2019).

Contemporary genetic connectivity between northern Mozambique and south-west Madagascar

The individual-based cluster analyses, in which individuals from northern Mozambique are clustered with individuals from the south of Madagascar, confirm a degree of connectivity between northern Mozambique (NMC: Nacala) and south-west Madagascar (SMC: Salary and Toliara). Larval dispersal is likely under influence of anticyclonic eddies, generated in the Comoros Basin, after which they proceed southward into the Mozambique Channel. Potential connectivity between northern Mozambique and south-west

Madagascar was demonstrated in drifter experiments (Ramanantsoa et al. 2018). These results are also partly supported by a larval dispersal modelling study (Gamoyo et al. 2019), in which strong southward dispersal and subsequent transport in the Mozambique Channel eddies were found for larvae released from sites south of Pemba (Mozambique), although this study did not include sites south of Nacala. However, successful exchange of coral larvae is unlikely to be frequent, considering the high differentiation found here between northern Mozambique and south-west Madagascar. This is congruent with the results of a larvae dispersal model in the WIO by Crochelet et al. (2016), in which these sites were found to belong to two separate clusters with a lower potential exchange of larvae. Here, no *S. hystrix* colonies were found in Mozambique south of Nacala (for which the sampling campaign included Vilanculos, Pembane, Tofo, Xai-Xai, Inhaca and Ponta do Ouro), although *S. hystrix* was predicted to occur there (<https://www.coralsoftheworld.com>, accessed 13 Oct 2019). Indeed, recent expeditions recorded *S. hystrix* in Ilha Caldeira and Bazaruto (Vilanculos) in the Mozambique Channel. However, only *Seriatopora caliendrum* was recorded for sites further south along the coast (Schleyer and Celliers 2000, 2005). Further sampling and verification of records of *S. hystrix* south of Nacala will be required before definite conclusions on the regional substructure along the whole coastline of Mozambique can be drawn.

Genetic structure along the East African Coast (EAC)

Substantial subdivision was also found amongst coral reefs along the EAC: pairwise differentiation was high, although lower than amongst other sites in the WIO (except Nacala, as discussed above). However, when comparing the results of our different analyses, no definite genetic breaks were found, similarly to what was previously reported for a coral with a mixed reproduction strategy *Pocillopora damicornis* in Kenya (Souter et al. 2009), for the broadcast spawner *Platygyra daedalea* in Kenya (Souter and Grahn 2008), as well as for the broadcast spawner *Acropora tenuis* in Kenya and Tanzania (van der Ven et al. 2016). Although the overall level of genetic differentiation between sites in these studies was substantially lower than in the case of *S. hystrix* and no isolation-by-distance was found. This could be explained by differences in life histories of these corals, such as reproductive strategies associated with longer PLD and subsequent increased connectivity among surrounding reefs (Crochelet et al. 2016). Genetic connectivity without a clear genetic break was also found in molluscs (Ratsimbazafy and Kochzius 2018; Nehemia et al. 2019), arthropods (Rumisha et al. 2017) and fishes (Huyghe and Kochzius 2018). *S. hystrix* has a short PLD, resulting in high levels of self-recruitment with

the majority of larvae settling within 100 m of their mother colony in north-west Australia (Underwood et al. 2007), although longer distance dispersal can occur (van Oppen et al. 2008). Comparable levels of differentiation were found for *S. hystrix* on the Great Barrier Reef and in northern Western Australia (mean $F_{ST} = 0.20$ ~500 km, van Oppen et al. 2008), including isolation-by-distance (overall $F_{ST} = 0.198$ ~505 km, Underwood et al. 2009). Low differentiation was found in the Red Sea over a distance of 610 km, ($F_{ST} = 0.089$; Maier et al. 2005), but the lower mean F_{ST} value can be explained by the proximity of most of the sample sites, e.g., ~0.15 km and the use of only three microsatellite loci. Based on the prevalence of clusters in the Bayesian analyses, there is an indication for a gradient in the WIO, with one cluster more dominant in the north (EAC: Kisite, Misali and Zanzibar) and one or two clusters more dominant in the south (NMC: Mafia, Mikindani, Vamizi and Pemba; Fig. 1a, b), although Mikindani shows more resemblance to the northern sites in the DAPC analysis (Fig. 2b). This distinction between sites may be explained by different oceanographic conditions. The northern sites are under year-round influence of the EACC, while the southern sites are part of the NMC with influence of the Mozambique Channel eddies, the bifurcation of the SEC in front of the African continent and the transition of the SEC into the EACC (Lutjeharms and Bornman 2010; Schott and McCreary 2001). Since the southern sites are part of the NMC region, they present a similar genetic signature caused by an exchange of larvae between northern Mozambique and Mikindani, and further transport of larvae from Mikindani to Mafia, with high genetic connectivity between Mikindani and Mafia supported by lower pairwise F_{ST} values. This pattern was also suggested for *A. tenuis* (van der Ven et al. 2016) and is supported by the strong resemblance in coral species diversity between these sites (Obura 2012). Although no bottleneck was detected, we cannot exclude false negatives caused by small sample sizes and subsequent limited statistical power in this data set (Peery et al. 2012). The aforementioned gradient may therefore be the result of severe historic bleaching events that have impacted the northern and southern sample sites differently. Pocilloporids exhibit a low tolerance to elevated sea surface temperatures (Marshall and Baird 2000) and suffered substantial losses during the 1998 El Niño in the WIO (Wilkinson 2002), with up to 100 % mortality on some reefs in Kenya (McClanahan et al. 2001). However, reefs further south within the NMC seem to have evaded environmental conditions triggering bleaching events and still support bleaching-susceptible coral taxa (McClanahan et al. 2007a, 2014). Subsequent recolonisation of northern sites may have come from various source populations not sampled in this study, such as reefs further north and deeper refugia or mesophotic reefs, which can potentially contribute larvae to shallower reefs (Prasetia et al. 2017; van Oppen et al. 2011).

Genetic diversity in the Northern Mozambique Channel

Allelic richness was significantly higher at the sites in the NMC compared to sites further north, both when including mainland and Madagascar sites and for mainland sites alone. These results are consistent with the higher coral cover and diversity found in the NMC, which can be explained by current patterns (such as the inflow of the SEC transporting larvae) combined with the high connectivity caused by mixing in the NMC eddies, and lower environmental stress than north of the NMC (McClanahan et al. 2014; Obura 2012). These complex oceanographic patterns also influence coral reef communities, because of the differences in sensitivity to temperature anomalies between coral species: the EACC along the coast of Tanzania and Kenya shows more frequent temperature fluctuations, while the SEC has a stabilising influence on the temperature in the region (McClanahan et al. 2011, 2007b, 2014). This could explain the higher abundance of temperature-sensitive taxa, such as acroporids and pocilloporids in the NMC, although variable levels of bleaching did occur in this region, for instance in Mayotte (Eriksson et al. 2012; Marshall and Baird 2000; McClanahan et al. 2014; Obura 2012; Obura et al. 2018).

In conclusion, the brooding stony coral *S. hystrix* shows a limited dispersal capacity and a high differentiation both at a large scale between the Red Sea and the WIO (>6000 km), and at smaller scale for sites along the coast of Tanzania and Kenya (Kisite and Misali; <65 km).

Red Sea populations of *S. hystrix* could belong to a different species than the populations in the WIO, which should form the basis for further integrated taxonomy studies. Within the WIO, three groups can be distinguished inhabiting (1) the north of Madagascar, (2) the south-west of Madagascar together with one site in northern Mozambique (Nacala) and (3) all other sites along the coast of northern Mozambique, Tanzania and Kenya. These patterns of contemporary dispersal barriers can be explained by the short PLD of *S. hystrix* and by oceanographic conditions, such as eddies in the Mozambique Channel causing larval retention in northern Madagascar, while the prevailing northbound EACC likely facilitates higher connectivity along the northern EAC. The strong genetic population structure in this study is congruent with findings for the same species from other biogeographic regions such as Australia (van Oppen et al. 2008; Underwood et al. 2009) and Japan (Nakajima et al. 2017), as well as for other brooding corals with a similarly short PLD. When comparing brooding corals with broadcast spawning corals with longer PLD, the importance of the reproduction strategy on connectivity is shown, with substantially lower levels of differentiation found in the broadcast spawning coral in the same region, e.g., *A. tenuis* (van der Ven et al. 2016), or other biogeographic regions, e.g., *Acropora digitifera* in Japan (Nakajima et al.

2010). These insights into the population structure of corals with different life histories can help to understand which spatial scales conservation initiatives should consider. For instance, this study confirmed assumed connectivity within the EAC for *S. hystrix*. However, this study also showed that the NMC region is in fact split by contemporary dispersal barriers, probably caused by the short PLD of *S. hystrix*, with on one side northern Madagascar isolated by local cyclonic eddies and on the other side Mozambique and Tanzania influenced by southward moving anticyclonic eddies in the channel. The NMC is considered a priority region for conservation and management efforts, as this region is high in coral cover and species richness, probably caused by historical high connectivity and low or geographically variable levels of environmental stress (McClanahan et al. 2007a, 2014; Obura 2012). In a biophysical modelling study, Crochelet et al. (2016) appointed several sites in this region that are particularly essential for the connectivity of larvae, and Geysers Bank and Cosmoledo were suggested as priority sites for MPAs within the Mozambique Channel. Genetic connectivity studies, and the dispersal barriers described, provide guidance on how to prioritise conservation efforts for coral reefs in the future. However, substantial differences in spatial scales can be expected, even within functional and taxonomically similar assemblages, such as corals. Therefore, there may not be a “one-size-fits-all” solution for coral reef conservation, but rather a compromise, which should consider a variety of marine life with diverse life history characteristics.

Data availability

Microsatellite genotypes and ITS2 DNA sequence data available in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.gtht76hjz>.

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Compliance with ethical standards

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

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