Coral Reefs (2015) 34:301–311
DOI 10.1007/s00338-014-1206-6

REPORT

Genetic connectivity of the broadcast spawning reef coral *Platygyra sinensis* on impacted reefs, and the description of new microsatellite markers

Y. C. Tay · A. M. E. Noreen · Suharsono · L. M. Chou · P. A. Todd

Received: 13 October 2013 / Accepted: 1 September 2014 / Published online: 26 September 2014

Abstract As tropical coral reef habitats continue to be lost or degraded, understanding the genetic diversity and connectivity among populations is essential for making informed management decisions. This is particularly important in rapidly developing, land-scarce nations (such as Singapore) that require targeted conservation efforts. Sixty percentage of Singapore's coral cover has been lost over the past five decades, and with further coastal reclamation underway, it is imperative to understand the effects of development on coral connectivity. In this study, we used seven microsatellite markers, of which six are newly described here, to investigate the genetic diversity and connectivity of the massive hard coral *Platygyra sinensis* at nine sites in Singapore and three in the nearby Indonesian island of Bintan. Our results show that *P. sinensis* currently retains large effective population sizes, high genetic diversity, as well as high connectivity among sites within each locality, which suggest that these populations have good potential for continued survival provided that there are no island-wide disturbances. However, the Singapore Strait appears to be a mild barrier to gene flow, which may lead to an increased reliance on self-seeding at either location. We suggest some directions for their management based on these potential population boundaries, which can help pave the path for marine conservation planning in Singapore.

Keywords *Platygyra sinensis* · Genetic connectivity · Microsatellites · Singapore · Coastal impacts · Larval dispersal

Communicated by Handling Editor Ruth Gates

Electronic supplementary material The online version of this article (doi:10.1007/s00338-014-1206-6) contains supplementary material, which is available to authorized users.

Y. C. Tay (✉)
Evolutionary Biology Laboratory, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Block S1A, #05-16, Singapore 117543, Singapore
e-mail: yweechieh@nus.edu.sg

A. M. E. Noreen
Applied Plant Ecology Lab, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore

Suharsono
Research Center for Oceanography, Indonesian Institute of Sciences (LIPI), Jl. Pasir Putih I, Ancol Timur, Jakarta, Indonesia

L. M. Chou
Reef Ecology Laboratory, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore

P. A. Todd
Experimental Marine Ecology Laboratory, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore

Introduction

The genetic and demographic implications of connectivity play a vital role in conservation biology. Sessile marine invertebrates such as corals often have a planktonic larval stage that depends on their oceanic environment for dispersal and genetic exchange between populations (e.g.

Atchison et al. 2008; van Oppen et al. 2008; Nunes et al. 2011). Larvae of broadcast spawning corals are capable of dispersal up to several 100 km (Rodriguez-Lanetty and Hoegh-Guldberg 2002; Severance and Karl 2006; Nakajima et al. 2012), although localized recruitment is more often dominant (Baums et al. 2005; Severance and Karl 2006; Underwood et al. 2009). Dispersal processes are important drivers of population size and genetic diversity, which are some of the key factors in maintaining population stability and long-term persistence. The understanding of population boundaries, source–sink dynamics, and levels of genetic diversity can, and should, be applied in the prioritization of conservation efforts and design of marine networks (Palumbi 2003), prediction of population resilience to various stresses (reviewed in van Oppen and Gates 2006), and the management of invasive species spread (Darling et al. 2008; Yasuda et al. 2009).

Localized anthropogenic impacts on the coral reefs in Singapore due to coastal development and shipping activities, which intensified during the past five decades of rapid economic growth, have resulted in the loss of approximately 60 % of its coral reefs (Chou 2006). The extent of reclamation is evidenced by the unusually straight edges along the south-western coastline of mainland Singapore, and many reefs have been lost in the merging of adjacent offshore islands (Fig. 1). The remaining reefs, which host up to 255 species of scleractinian corals (Huang et al. 2009), are subject to high levels of chronic sedimentation (Dikou and van Woesik 2006). Furthermore, recent plans (NPTD 2013) indicate more reclamation projects that may impact the remaining reefs. There is therefore a need to understand the dynamics of local coral populations. Prior to the present study, nothing was known about the genetic structure or connectivity of coral populations within the Singapore Strait, or how these factors might influence the resilience of these heavily impacted populations.

Platygyra sinensis, one of the most common hermatypic coral species in Singapore (Huang et al. 2009), is a key ecological component of the local reefs. It is also one of the many species of local broadcast spawning corals that participate in the mass spawning event just after the wet northeast monsoon in March/April (Guest et al. 2005). Despite the prominence of *Platygyra* species on tropical reefs, the population genetics of this group is understudied as compared to other broadcasting species such as the acroporids; worldwide, only four studies have thus far analysed the genetic structure of *Platygyra* populations: in Hong Kong (Ng and Morton 2003), East Africa (Souter and Grahn 2008), the Great Barrier Reef, Australia (Miller and Ayre 2008), and southern Taiwan (Keshavmurthy et al. 2012). Mitochondrial and nuclear gene sequences utilized in the most recently published population genetic study on a *Platygyra* species might have had inadequate resolving power (Keshavmurthy et al. 2012); the slow rate of

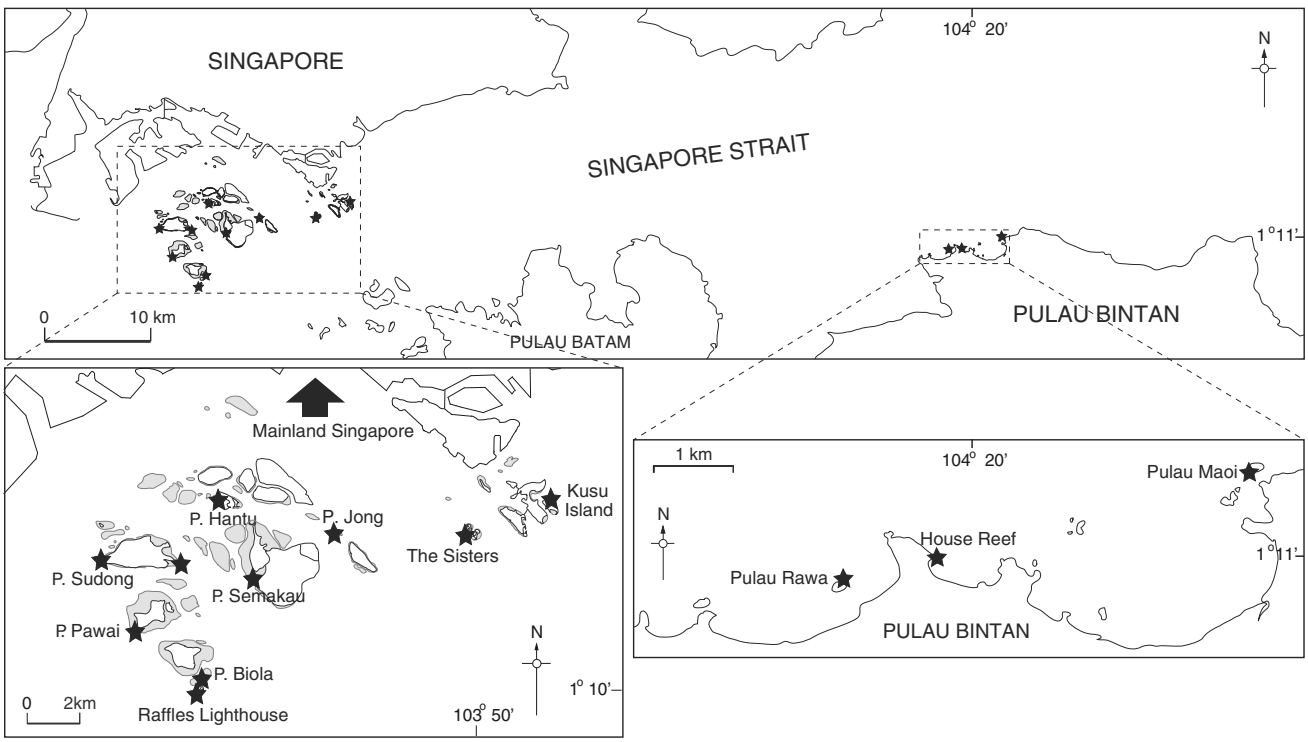

Fig. 1 Sampling sites within the Singapore Strait are marked by stars. *Bottom left panel* ten sampling sites within the Southern Islands of Singapore. *Bottom right panel* three sampling sites along the northern coast of P. Bintan

mitochondrial DNA evolution in corals (Shearer et al. 2002; van Oppen and Gates 2006; Huang et al. 2008) has mostly restricted coral population geneticists to using allozymes, amplified fragment length polymorphisms (AFLPs), and microsatellites (e.g. Combosch and Vollmer 2011; Brazeau et al. 2011; Keshavmurthy et al. 2012). Furthermore, there are few available microsatellite markers due to the small size of the coral genome (Márquez et al. 2000). Hence, we developed new polymorphic microsatellite markers specific to *P. sinensis* in this study.

Given the demographic dominance of *P. sinensis* within the Singapore Strait, their high fecundity, consistent participation during the annual mass spawning event in Singapore, positively buoyant gametes, and relatively long larval duration (almost 6 d; Babcock 1991; Tay et al. 2011), we expected a high level of larval exchange and genetic connectivity—although barriers to gene flow could have arisen due to anthropogenic impacts over the last few decades. Nevertheless, hydrodynamic simulations of broadcast coral larval transport within the Singapore Strait by Tay et al. (2012) also suggested the potential for relatively high connectivity among reefs within the Southern Islands of Singapore. Their model suggested a trend for east-to-west dispersal, likely due to the net westward current within the Singapore Strait during the annual mass spawning event, and indicated that reefs further upstream of the Singapore Strait (such as the Indonesian island Pulau Bintan; hereafter Pulau = P.) may be potential larval sources. However, some genetic structuring was expected between the populations in Singapore and P. Bintan because, for successful dispersal into or out of Singapore, larvae have to cross the >10 km width of the Strait, which is not only perpendicular to the main east-to-west direction of current, but also a major international shipping channel (Chua et al. 2000).

In the present study, we (1) document levels of genetic diversity and connectivity present in populations of *P. sinensis* in Singapore with comparisons to nearby Indonesian populations within the Singapore Strait, (2) investigate possible impacts on the genetic connectivity of *P. sinensis* within the Singapore Strait due to anthropogenic activities by comparing historical versus contemporary patterns of genetic structure, and (3) present six new microsatellite markers.

Materials and methods

Study site and sample collection, preservation and DNA extraction

A total of 428 *P. sinensis* samples were collected over a period of 2 years (2009–2010) from ten sites among the Southern Islands of Singapore, and three sites along the northern coast of P. Bintan, Indonesia (Fig. 1). Sample sites have been abbreviated in figures and tables as follows; Singaporean sites: Raffles Lighthouse (SGRL), P. Biola (SGBIO), P. Pawai (SGPW), P. Sudong (SGSUD), P. Hantu (SGHAN), P. Semakau (SGSEM), P. Jong (SGJO), The Sister's Islands (SGSIL), Kusu Island (SGKI); Indonesian sites: P. Rawa (BNPR), House Reef (BNHR), P. Maoi (BNPM). Samples from two sites at P. Sudong showed non-significant genetic differentiation and were pooled to increase the sample size for the connectivity analyses.

At each sampling site, a belt transect survey at least 50 m long was performed along the reef crest (~3 m depth) to search for *P. sinensis* colonies. A separate transect survey was conducted when the sample size from one transect was insufficient. The locations of each transect were determined with a Garmin 76CSx global positioning system (GPS), and the x and y coordinates of each colony with respect to the transect tape were recorded. To help identify any potential cross-contamination of coral DNA extracts with those of their algal symbionts, four samples were collected during the coral bleaching event in 2010 (Guest et al. 2012) and checked under a dissecting microscope to confirm the absence of zooxanthellae. Gonads were also isolated from one gravid sample under a dissecting microscope for additional symbiont-free coral DNA. All coral tissue samples were placed in ~950 µl CHAOS solution (4 M guanidine thiocyanate, 0.1 % N-lauroyl sarcosine sodium, 10 mM Tris buffer pH 8, 0.1 M 2-mercaptoethanol; Sargent et al. 1986) for at least 5 d at room temperature for tissue digestion before DNA extraction using the phenol–chloroform method with a phenol extraction buffer (100 mM Tris buffer pH 8, 10 mM EDTA, 0.1 % SDS; Fukami et al. 2004).

Marker selection and development

Five published microsatellite markers developed for the congeneric species *Platygyra daedalea* (Miller and Howard 2004) were tested on 38 DNA samples of *P. sinensis* from Singapore. Polymerase chain reaction (PCR) amplifications were modified from Miller and Ayre (2008) and Mangubhai et al. (2007). PCRs were carried out in 10 µl reactions containing 6 ng of DNA, 1× KAPA Buffer A, 0.8 U KAPA Taq (Genome Holdings), 0.2 mM of each dNTP, 0.5–0.65 µM of each unlabelled primer, and 0.5 mM fluorescence-labelled forward primers. Cycling protocol was as follows: 95 °C for 5 min, 30 cycles at 94 °C for 30 s, 57–63 °C (locus Pd29 at 57 °C, Pd31 at 57 °C, Pd48 at 60 °C, Pd61 at 63 °C, Pd62 at 57 °C) for 30 s, and 72 °C for 1 min, ending with an extra extension period at 72 °C for 10 min. Allelic variations were visualized on an ABI

Table 1 Details for six polymorphic microsatellite loci developed for *Platygyra sinensis* as calculated from genotypes of 38 individuals

Locus	Primer sequence 5′ → 3′	Repeat motif	Size (bp)	T_A (°C)	Fluorescence tag	N	N_A	H_O	H_E	Plex	Genbank accession no.
Plsi3.15	T: [a]CACTACACAATCAGGACTGC	AAC(10)	147–183	57	6-FAM	38	8	0.711	0.741	A	KM362853
	U: GTTTAAGGATTTGTTGCAGTTGTC										
Plsi4.02	T: [a]ACAATTCGGATATGTAGC	AAAC(11)	130–178	57	NED	38	12	0.842	0.869	B[c]	KM362854
	U: GTTTCTTTGGTTTGGTTTGTTCTC										
Plsi4.16	T: [a]GTATCACCCAGGCTACAAG	AAGT(8)	263–311	57	6-FAM	38	8	0.658	0.702	A	KM362855
	U: GTTTCACCAGGCAGAACTATAACG										
Plsi4.24	T: [a]TTATCTTGGTTCAGACAGACAG	ACAG(10)	111–159	62	VIC	38	11	0.579	0.856	C[d]	KM362856
	U: GTTTGACAACTCTAATGAAGGTCAG										
Plsi4.27	U: GTTTAACTGCCCAATCATTAACAC	AAAC(11)[b]	170–252	62	VIC	37	17	0.216	0.894	C[d]	KM362857
	T: [a]GTCTGCTTGTGTTTGAATTG										
Plsi4.48	U: GTTTGTGGTGGTGGAGAGATCTG	AAAC(13)[b]	147–193	57	NED	38	10	0.868	0.839	D[c]	KM362858
	T: [a]TGGTTTCTCTGCTGTTATCG										

The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag

T_A, annealing temperature for the PCR reactions; *Fluorescence tag*, the most commonly used fluorescence tag with the respective primer pair; N, number of individuals successfully genotyped, N_A, number of alleles observed; H_O *and* H_E, observed and expected heterozygosity, respectively; *Plex*, pairing of primer pairs in genotyping PCRs

[a] CAG tag (5′-CAGTCGGGCGTCATCA-3′) label

[b] Indicates imperfect microsatellites where allele sizes sometimes have increments of 2 bp instead of 4 bp as expected of tetranucleotide-repeat markers

[c] Genotyped together with loci not indicated in the tables, Plsi4.11 and Plsi4.14, respectively, which were discarded due to unreliable amplifications and large quantities of missing data

[d] Pd48 is added to plex C with a final concentration of 0.025 μM of each Pd48 primer

3100 capillary genetic analyser, and results were analysed using GeneMapper version 4.0 (Applied Biosystems). Of the five markers, only Pd48 gave clear, distinct peaks that were polymorphic and repeatable, despite repeats with an additional PIG-tail on the reverse primer (Brownstein et al. 1996). Hence, of these tested markers, only Pd48 was retained for use.

A new set of microsatellite markers specific to *P. sinensis* was therefore developed for the population genetic analyses in this study. Microsatellite enrichment and initial marker screening were performed following the protocol described in Lance et al. (2010). PCRs were carried out in 5 µl reactions containing 6 ng of DNA, 1× Qiagen PCR multiplex master mix (final concentration of 3 mM $MgCl_2$,), 0.13–0.17 µM of each fluorescence-labelled universal primer (Plsi3.15: 0.17 µM; Plsi4.02: 0.16 µM; Plsi4.16: 0.13 µM; Plsi4.24: 0.17 µM; Plsi4.27: 0.17 µM; Plsi4.48: 0.13 µM), and 0.05–0.125 µM final concentration of each unlabelled primer (Plsi3.15: 0.125 µM; Plsi 4.02: 0.066 µM; Plsi4.16: 0.05 µM; Plsi4.24: 0.06 µM; Plsi4.27: 0.11 µM; Plsi4.48: 0.09 µM). Cycling protocol was as follows: 95 °C for 15 min, 40 cycles at 94 °C for 30 s, 57–62 °C (specific annealing temperatures in Table 1) for 30 s, and 72 °C for 1 min, ending with an extra extension period at 60 °C for 30 min. Sixty primer pair sequences were screened (2 di-, 10 tri-, 48 tetra-nucleotide repeats; Electronic Supplementary Materials, ESM, Table S1), of which only six gave reliable, consistent peaks that were suitable for scoring. Each marker was also tested to ensure good amplification of the DNA samples extracted from symbiont-free samples. Conditions and characteristics of these markers were calculated using GenAlEx 6.4 (Peakall and Smouse 2006; Table 1).

Table 2 Population genetic indices for the 12 populations, and two locations

Population	*N*	$\bar{A}$	PVA	H_O	H_E	F_{IS_subset}
SGRL	40	9.95	1	0.610	0.819	0.097
SGBIO	27	8.76	2	0.551	0.786	0.213***
SGPW	36	8.63	1	0.594	0.773	0.090
SGSUD	37	10.16	0	0.616	0.805	0.135*
SGHAN	38	9.71	1	0.596	0.817	0.178***
SGSEM	28	9.49	1	0.581	0.801	0.159**
SGJO	27	9.04	0	0.769	0.789	0.088
SGSIL	26	8.59	0	0.619	0.767	0.052
SGKI	42	10.19	1	0.642	0.831	0.123**
SG	301	9.39	21	0.599	0.801	0.127
BNPR	36	8.94	1	0.616	0.794	0.119*
BNHR	38	9.63	2	0.620	0.800	0.121***
BNPM	38	8.81	0	0.623	0.786	0.115
BIN	112	9.12	5	0.619	0.793	0.118

N Number of successfully genotyped samples, $\bar{A}$ allelic richness, *PVA* number of private alleles, H_O observed, H_E expected heterozygosities, F_{IS}_subset the inbreeding coefficients excluding loci Plsi4.24 and Plsi2.27

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Genotyping

Multiplex PCRs (Table 1) were performed in 4 µl reactions containing 3 ng of DNA, keeping the PCR cycling conditions and final concentrations of all reagents the same as stated earlier. Ambiguous alleles, or private alleles detected using GenAlEx 6.4, were re-genotyped to ensure that the alleles were real. Genotyping for difficult samples was repeated up to three times, the last of which used new extracts of DNA samples. Samples that were not successfully genotyped after four attempts were assumed to be affected by null alleles. Plsi4.24 and 4.27 had a higher frequency of missing data (4.4 and 11.6 %, respectively). Scoring was checked manually across the entire data set to ensure consistency across runs.

Statistical analyses

Since many population genetic differentiation analyses rely on the assumptions of random drift mutations and marker neutrality, Lositan (Beaumont and Nichols 1996; Antao et al. 2008) was used to detect loci that may be under positive selection, using 100,000 simulations following the stepwise mutation model. Only Plsi4.27 was found to be under positive selection and was hence excluded from analyses that make assumptions about random drift mutation.

FSTAT v 2.9.3 (Goudet 1995) was used to conduct tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for each locus (ESM Table S2) and also to calculate allelic richness, HWE, and inbreeding coefficients (F_{IS}) for each population (Table 2). All significance levels were adjusted with the strict Bonferroni correction for multiple tests ($p < 0.05$). Private alleles were detected using GenAlEx. FreeNA (Chapuis and Estoup 2007) was used to calculate null allele frequencies under the assumption of HWE. Loci with high frequencies of null alleles (>0.3) were excluded from analyses that are sensitive to null alleles (Chakraborty et al. 1994; Carlsson 2008). BOTTLENECK (Piry et al. 1999) was used to determine whether a recent genetic bottleneck had occurred in all sampled sites, and within all sites in Singapore and P. Bintan. The Wilcoxon's test was used to test for significant deviations from population mutation-drift equilibrium, following the two-phase model (TPM) with 95 % single-step mutations and a variance among multiple steps of 12 as recommended for microsatellites.

Table 3 Pairwise F_{ST} values calculated in FSTAT

	SGRL	SGBIO	SGPW	SGSUD	SGHAN	SGSEM	SGJO	SGSIL	SGKI	BNPR	BNHR
SGRL											
SGBIO	0.000										
SGPW	0.002	0.000									
SGSUD	0.000	0.000	0.000								
SGHAN	**0.001**	**0.004**	**0.008**	**0.004**							
SGSEM	0.000	0.011	**0.018**	0.010	**0.009**						
SGJO	0.009	0.004	0.000	0.009	**0.015**	0.014					
SGSIL	0.000	0.000	0.000	0.000	**0.006**	0.012	**0.008**				
SGKI	0.004	0.000	0.007	0.009	**0.011**	**0.018**	0.005	0.007			
BNPR	0.003	**0.006**	0.000	0.002	**0.006***	0.010	0.000	**0.008**	0.004		
BNHR	0.004	0.004	0.000	0.000	0.002	**0.017**	0.001	0.006	0.004	0.000	
BNPM	0.000	0.012	0.008	0.003	**0.0111***	**0.006**	0.007	**0.012**	0.011	0.003	0.000

Negative values are converted to zero

In bold are $p < 0.05$; * significant after strict Bonferroni correction

Historical genetic differentiation among all sampled sites was assessed by calculating pairwise F_{ST} values in FSTAT v 2.9.3. As there was no significant genetic differentiation detected for sites within locations (Table 3), sites within locations were pooled for an among-location pairwise F_{ST} calculation. Retrospective power analyses were conducted in POWSIM v 4.1 (Ryman and Palm 2006) to check the adequacy of the remaining markers for resolving genetic structure among the populations, and between locations. Only individuals with complete genotype data were included in these analyses, and genetic drift to various levels of genetic divergence ($0 \leq F_{ST} \leq 1$) for populations of 1,000 and 10,000 individuals was each simulated 1,000 times (Fig. S1).

Contemporary differentiation patterns were assessed using the Bayesian clustering analysis implemented in STRUCTURE v 2.3.3 (Pritchard et al. 2000). Although STRUCTURE can also detect deep divergence, it is a better representation of contemporary divergence as it maximizes HWE while minimizing LD within genetic clusters. CONVERT (Glaubitz 2004) was used to format input data for use in STRUCTURE. STRUCTURE, a fully Bayesian clustering method, was used to estimate the most likely number of genetic clusters (K). Since admixture and allele frequency correlation are valid assumptions in the case of subtle population structure (e.g. $F_{ST} < 0.05$; Falush et al. 2003; Hauser et al. 2006), STRUCTURE analyses were run following these assumptions with no prior population information. Each run consisted of 1,000,000 iterations with a burn-in of 100,000, for each value of K from $K = 1$–16. For each K, the run was replicated ten times, and the most likely value of K was determined using STRUCTURE HARVESTER (Earl and VonHoldt 2011), which implements the method of Evanno et al. (2005). Due to stochasticity between each run in STRUCTURE, the ten independent iterations at the optimal value of K were matched using the Greedy algorithm in the cluster matching and permutation program CLUMPP (Jakobsson and Rosenberg 2007) and then visualized using DISTRUCT v 1.1 (Rosenberg 2004). Since this first set of runs suggested the possibility of subtle genetic structure between Singapore and Indonesia, the procedure was repeated with these two localities set as priors, for each value of K from $K = 1$–5. Spatial autocorrelation analyses were also conducted to determine whether fine-scale genetic clustering was present within populations. The autocorrelation coefficient, r, which measures correlation between coral genotypes and the geographic distance between individuals, was calculated for 10-m distance classes in GenAlEx v 6.4, with 999 permutations to test the null hypothesis of no spatial structure ($r = 0$), followed by 10,000 bootstrap replicates to determine the 95 % CI.

Due to the lack of genetic structuring within locations (Singapore and P. Bintan), population assignments and gene flow calculations were difficult and may have been more prone to errors (e.g. Wilson and Rannala 2003; Piry et al. 2004; Beerli 2009). Hence, even though estimations of migration were tested, they are not presented here.

Results

Genetic diversity

Only one set of two identical multilocus genotypes was detected, which is in accordance with low clonality of this genus as well as with the different morphologies of adjacent colonies observed in situ. Hence, all individuals were

included in the analyses. LD was not detected in any locus pair.

All loci were highly polymorphic, mostly with 13–16 alleles (Plsi4.27 had 33 alleles; ESM Table S2). Expected heterozygosities were high, ranging from 0.663 to 0.894. Additionally, allelic richness was high, ranging from 8.59 to 10.19, with a mean allelic richness of 9.33 ± SE 0.17. No significant heterozygote excesses were found, while significant heterozygote deficits were detected in five of the seven loci, and across most populations for Plsi4.24 and Plsi4.27. High inbreeding coefficients (>0.4) were found in Pd48, Plsi4.24, and Plsi4.27. The significant heterozygote deficits for Plsi4.24 and Plsi4.27 were likely due to the high frequencies of null alleles (up to 21.5 ± 0.011 and 25.0 ± 0.014 %, respectively; ESM Table S2). However, null allele frequencies exceeding 0.3 only occurred in Plsi4.24 and Plsi4.27 at P. Jong. Private alleles were detected in six loci; comparing among the 12 sites, low frequencies of seven private alleles were found in the sites in Singapore (0.012–0.045), and three from the sites in P. Bintan (0.014). F_{IS} values were calculated across all loci, and excluding loci with high null allele frequencies (Plsi4.24 and Plsi4.27). As expected, both the F_{IS} values and their significance levels were considerably reduced, although highly significant deviations from HWE persisted in a few sites in Singapore (Table 2). BOTTLENECK analyses found no signs of recent genetic bottlenecks in any of the 12 populations, as indicated by the non-significant probabilities of heterozygote excess ($p \geq 0.71$), and the L-shaped mode-shift graphs.

High levels of historical and contemporary genetic connectivity

Low and non-significant historical genetic structure within the nine Singapore sites (overall $F_{ST} = 0.005$) and the three Indonesian sites (overall $F_{ST} = 0$) was detected (pairwise F_{ST} values between sites in Table 3). Across the Singapore Strait, very subtle historical structure was detected between Singaporean and Indonesian populations (pairwise $F_{ST} = 0.003$, $p < 0.01$), which could be due to the two instances of significant genetic differentiation between P. Hantu in Singapore and two sites off of P. Bintan, although the absolute F_{ST} values were still low in these two comparisons ($F_{ST} = 0.006$ and 0.011, respectively; Table 3). Retrospective power analyses indicated sufficient power of the markers in resolving the low genetic structure among the individual sampling sites, with >99 % probability of detecting a true $F_{ST} = 0.006$ between individual sites, but a >91 % probability of detecting a true $F_{ST} = 0.003$ across the Singapore Strait (ESM Fig. S1).

With just three genetic clusters detected as the optimal number ($K = 3$), contemporary genetic structure analyses also indicated low genetic structure within the two locations (Fig. 2). All three clusters were detected in every population. However, as with historical differentiation, a subtle difference in genetic composition was found between the Indonesian and Singaporean populations. This structuring was more prominent when location was set as a priori in the STRUCTURE runs. Given the high frequency of genetic admixture at the individual level, however, it is also possible that there is no structure at all (where $K = 1$). Nevertheless, we found greater support for the subtle genetic divergence (when $K = 3$) due to the following reasons: (1) the posterior probability (LnPD) values were low at $K = 1$, and highest (least negative) at $K = 3$, and (2) the observed mixed population assignments in each individual were not symmetrical as they would be if there were truly no population structure (Pritchard et al. 2000).

Within sites (≤110 m transect), autocorrelation coefficients were not significantly different from random, including at the first distance class (0–10 m), indicating genetic homogeneity and a lack of fine-scale structuring (ESM Fig. S2).

Discussion

This paper presents the first population genetic analysis of *P. sinensis* based on highly polymorphic microsatellite loci, of which six were developed for the present study. Consistent with most other broadcast spawning coral species, the populations within the Singapore Strait propagate mainly by sexual reproduction that disperses gametes away

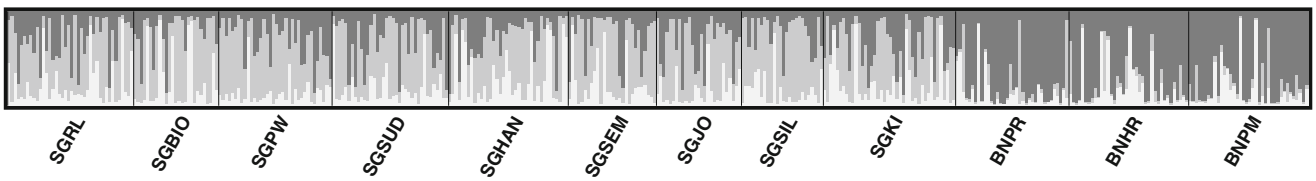

Fig. 2 Bayesian model-based cluster analysis as implemented by STRUCTURE when $K = 3$. Each *vertical bar* in the *bar plots* represents one sample, with the probability of membership of each individual to a genetic cluster represented by a *different shade*. Sampling localities are given on the *x* axis and separated by a *black line*; the nine sites on the *left* are from Singapore (SG-) and the three on the right from P. Bintan in Indonesia (BN-)

from the parental colonies and are genetically diverse. Analyses of contemporary as well as historical genetic structure indicate virtual panmixia within locations (<45 km), and subtle genetic differentiation between Singapore and a nearby Indonesian island, P. Bintan. This increase in genetic divergence suggests that the Singapore Strait is becoming a mild barrier to gene flow.

Population genetic structure

The overall low genetic structure among *P. sinensis* populations within the 70-km stretch of the Singapore Strait (F_{ST} values $\leq$0.018) is similar to the only other study of this species (i.e. Ng and Morton 2003: mean F_{ST} of 0.004 across all allozyme loci) conducted in Hong Kong, a site that is also heavily impacted by anthropogenic activities. Even though different markers were used, the similarity of these results suggests analogous population dynamics at both locations. The low genetic structure detected in our study, however, could also be due to the simultaneous formation of local coral reefs during the rapid flooding of the Sunda Shelf (Hanebuth et al. 2000; Bird et al. 2006) and maintained by high gene flow among populations. In support of the low overall F_{ST}, *P. sinensis* genotypes in this study were randomly distributed from the smallest (0–10 m) to the largest (0–110 m) scale examined, indicating that routine gamete dispersal is >100 m from parental colonies. Interestingly, a previous microsatellite study of *P. daedalea* on the GBR detected genotype clustering within 5–10 m of each other (Miller and Ayre 2008). Hence, even for closely related species, routine gamete dispersal distances may vary significantly depending on location. Routine transport of larvae >100 m from the parental colonies in Singapore and Indonesia is important from a management perspective, as it indicates that sites are more likely to be well-connected and that panmixia occurs over a larger scale than 'site'.

Significant heterozygote deficiencies, such as those found in the present study, are common among marine invertebrates, and especially in corals (e.g. Miller and Ayre 2008; Combosch and Vollmer 2011). Ng and Morton (2003) found only a few instances of heterozygote deficits in *P. sinensis* populations in Hong Kong, but this could be due to the lower allelic richness of allozymes. Heterozygote deficits in Singapore's *P. sinensis* populations are likely due to null alleles, as significant reductions in F_{IS} values were observed with the removal of the two loci with the highest null allele frequencies. Inbreeding effects are also possible as significant F_{IS} values persisted in some populations after removal of loci with high null allele frequencies—although this is unlikely due to the high connectivity within and among populations.

Land use and connectivity

Both historical and contemporary analyses suggest that *P. sinensis* in Singapore is an effectively panmictic population. This high genetic connectivity is reassuring, but surprising given the anthropogenic impacts over the past few decades. *P. sinensis* has a generation time of ~10 years (Carpenter et al. 2008), within the time frame of the rapid economic and coastal development in Singapore. Land reclamation activities in the 1980s, for instance, caused an annual average of ~0.8 % increase in Singapore's land area with a spike of ~3.5 % in the early 1980s (Tan et al. 2010). Given that environmental selection can bias the successful establishment of dispersed coral planulae (Hodgson 1990; Marshall et al. 2010), the massive reductions in coral reef populations (Chou 2006) and/or the fragmentation and degradation of the local reef environments (Dikou and van Woesik 2006) were expected to have caused changes in local larval dispersal processes (e.g. reduction in larval output or prevention of successful dispersal and recruitment of larvae), resulting in increased genetic divergence among sites over the past few decades. However, this result was not detected using available analyses and can likely be attributed to the high reproductive capacity and dispersal ability of broadcast spawning corals.

Furthermore, no significant difference in genetic diversity (assessed by paired *t* test of allelic richness, $p = 0.28$) was detected between populations in Singapore and the less-disturbed reefs on P. Bintan that were expected to have higher levels of genetic diversity. BOTTLENECK analyses also showed no signs of a genetic bottleneck, indicating that perhaps the anthropogenic activities and loss of coral populations thus far have not been sufficient to cause significant impacts to the local populations of *P. sinensis*.

At a larger spatial scale across the Singapore Strait, however, the observed patterns in historical and contemporary genetic structuring showed some evidence of increased genetic divergence: a distinct clustering of Singaporean versus Indonesian sites was detected in STRUCTURE analyses, whereas the F_{ST} value, despite being statistically significant, was extremely low at 0.003 (the probability of detecting a true F_{ST} of $\leq$0.003 with the current available loci is only $\leq$91.2 %; hence, the actual F_{ST} could potentially be lower; ESM Fig. S1), and the presence of low genetic structure between only one site in Singapore and two sites in P. Bintan suggests weaker historical genetic structure across the Strait. In addition, private alleles found in only either location (21 in Singapore, 5 in P. Bintan; Table 2) are signs of recent genetic divergence and relatively large population sizes. Here, we suggest that the Singapore Strait has been a mild barrier to gene flow due to the (1) strong currents within the Strait,

and/or (2) intensive shipping activities in one of the busiest and most important shipping waterways in the world (Chou 2006; Qu and Meng 2011): copper leached from ships can inhibit coral larval motility (Kwok and Ang 2013) and hence dispersal, while navigation-induced wave splash can interfere with the currents and cause larval displacement (Lechner et al. 2013).

Management implications

The high genetic connectivity of *P. sinensis* populations within the Singapore Strait has management implications at both local and cross-strait levels. For a reef system that potentially consists of multiple sources and sinks such as in Singapore waters, conservation efforts should focus on the prevention of multiple populations from being impacted simultaneously, such as by staggering land reclamation or dredging activities. Prioritization of conservation, if necessary, could target areas with higher genetic diversity such as P. Sudong or Kusu Island. The Sister's Islands and nearby reefs have recently been designated as the first Marine Park in Singapore. This will help to maintain the genetic connectivity of the local reefs, because this site has been predicted to be a good source site of coral larvae within the Southern Islands (Tay et al. 2012).

Resilience of *P. sinensis* populations in Singapore waters is likely high, due to its relatively widespread distribution and abundance (large effective population size), high genetic diversity, and indications of continued sexual reproduction. Its high connectivity within Singapore means these populations can likely recover quickly from extremely localized perturbations. This potential for recovery can also be inferred from the recent natural recruitment of corals on the artificial seawalls at P. Semakau within 15 years after their construction (Ng et al. 2012).

Given the high genetic relatedness across the Singapore Strait, the broadcast spawning coral populations in Singapore and northern P. Bintan may be managed as a metapopulation instead of individual reefs. While designating the entire Singapore Strait as an MPA is not realistic both politically and economically, transboundary management efforts between Singapore and Indonesia could include assistance in species reestablishment: species that are low in abundance or have become locally extinct in Singapore, for instance, could be re-established via transplantation of coral colonies from P. Bintan and vice versa, without much disruption to the genetic composition of these populations.

The adaptive abilities and long-term viability of Singapore's coral populations are nevertheless a concern given a wider context, i.e. the effects of climate change (e.g. two catastrophic thermal-bleaching events in 1998 and 2010; Guest et al. 2012), constant direct human pressures such as high sedimentation rates (Dikou and van Woesik 2006), and lack of good quality larval settlement sites. Even though their high genetic diversity can promote thermal-tolerance either directly or via internal symbionts (i.e. genetic resilience; Császár et al. 2010; LaJeunesse et al. 2010; Baums et al. 2013), as a whole, the reefs within the Singapore Strait may be vulnerable to large-scale disturbances. Recent evidence shows that it is the host, not the endosymbiont, that determines the coral's upper thermal-tolerance threshold (Wooldridge 2014), and as they share the same gene pool, they are probably susceptible to diseases or other impacts, as suggested by the mass bleaching of massive *Porites* coral populations in both Singapore (Guest et al. 2012) and P. Bintan (Y.C. Tay pers obs.) during the 2010 high sea surface temperature event. Furthermore, if the Singapore Strait becomes a stronger barrier to gene flow and Singapore's reefs become reliant on self-seeding, a large-scale disturbance could devastate the local population. Management efforts could therefore focus also on understanding the causes, and mitigating the effects, of the genetic barrier across the Singapore Strait.

Acknowledgments Permission to conduct research sampling in Indonesia was obtained through the Indonesian Institute of Sciences (LIPI) and the State Ministry of Research and Technology (RISTEK), under the research permit number 12/TKPIPA/FRP/SM/XII/2009. We thank S Lance and the Savannah Ecology Laboratory for the microsatellite library construction, members of the Marine Biology Laboratory for their assistance in the field, and the staff of the Banyan Tree Bintan Conservation Laboratory for their logistical support. We also thank VE Chhatre for his guidance on the use of several of the genetic softwares, D Huang and DP Araújo for their comments on early drafts of the manuscript, and CJ Starger for the insightful comments and suggestions to improve the manuscript. Special thanks to R Meier for his constructive advice, and allowing the free access to and use of his laboratory facilities for conducting the genetic work. This research was supported by a grant from the Technical Committee on the Coastal and Marine Environment of Singapore (Grant No. R-154-000-411-490). The first author would also like to acknowledge the World Future Foundation (WFF) for their support of this research by awarding her the WFF PhD Prize in Environmental and Sustainability Research.

References

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a F_{ST}-outlier method. BMC Bioinformatics 9:323

Atchison A, Sammarco PW, Brazeau DA (2008) Genetic connectivity in corals on the Flower Garden Banks and surrounding oil/gas platforms, Gulf of Mexico. J Exp Mar Biol Ecol 365:1–12

Babcock RC (1991) Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol Monogr 61:225–244

Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Mol Ecol 14:1377–1390

Baums IB, Devlin-Durante MK, Polato NR, Xu D, Giri S, Altman NS, Ruiz D, Parkinson JE, Boulay JN (2013) Genotypic variation

influences reproductive success and thermal stress tolerance in the reef building coral, *Acropora palmata*. Coral Reefs 32:703–717

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proc R Soc Lond B Biol Sci 263:1619–1626

Beerli P (2009) How to use Migrate or why are Markov chain Monte Carlo programs difficult to use? In: Bertorelle G, Bruford MW, Hauffe HC, Rizzoli A, Vernesi C (eds) Population genetics for animal conservation, conservation biology series, vol 17. Cambridge University Press, Cambridge, pp 42–79

Bird MI, Pang WC, Lambeck K (2006) The age and origin of the Straits of Singapore. Palaeogeogr Palaeoclimatol Palaeoecol 241:531–538

Brazeau DA, Sammarco PW, Atchison A (2011) Micro-scale genetic heterogeneity and structure in coral recruitment: fine-scale patchiness. Aquat Biol 12:55–67

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq Polymerase: primer modifications that facilitate genotyping. BioTechniques 20:1004–1010

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. J Hered 99:616–623

Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J, Delbeek JC, Devantier L, Edgar GJ, Edwards AJ, Fenner D, Guzmán HM, Hoeksema BW, Hodgson G, Johan O, Licuanan WY, Livingstone SR, Lovell ER, Moore JA, Obura DO, Ochavillo D, Polidoro BA, Precht WF, Quibilan MC, Reboton C, Richards ZT, Rogers AD, Sanciangco J, Sheppard A, Sheppard C, Smith J, Stuart S, Turak E, Veron JEN, Wallace C, Weil E, Wood E (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321(5888):560–563

Chakraborty R, Zhong Y, Jin L, Budowle B (1994) Nondetectability of restriction fragments and independence of DNA fragment sizes within and between loci in RFLP typing of DNA. Am J Hum Genet 55:391–401

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Chou LM (2006) Marine habitats in one of the world's busiest harbours. In: Wolanski E (ed) The environment in Asia Pacific harbours. Springer, Netherlands, pp 377–391

Chua T-E, Gorre IRL, Ross SA, Bernad SR, Gervacio B, Ebarvia MC (2000) The Malacca Straits. Mar Pollut Bull 41:160–178

Combosch DJ, Vollmer SV (2011) Population genetics of an ecosystem-defining reef coral *Pocillopora damicornis* in the Tropical Eastern Pacific. PLoS One 6:e21200

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. PLoS One 5:e9751

Darling JA, Bagley MJ, Roman J, Tepolt CK, Geller JB (2008) Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. Mol Ecol 17:4992–5007

Dikou A, van Woesik R (2006) Survival under chronic stress from sediment load: spatial patterns of hard coral communities in the southern islands of Singapore. Mar Pollut Bull 52:1340–1354

Earl DA, VonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N (2004) Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. Evolution 58:324–337

Glaubitz JC (2004) CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol Ecol Notes 4:309–310

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. J Hered 86:485–486

Guest JR, Baird AH, Goh BPL, Chou LM (2005) Seasonal reproduction in equatorial reef corals. Invertebr Reprod Dev 48:207–218

Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ, Campbell SJ, Yewdall K, Affendi YA, Chou LM (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PLoS One 7:e33353

Hanebuth T, Stattegger K, Grootes PM (2000) Rapid flooding of the Sunda Shelf: A late-glacial sea-level record. Science 288(5468):1033–1035

Hauser L, Seamons TR, Dauer M, Naish KA, Quinn TP (2006) An empirical verification of population assignment methods by marking and parentage data: hatchery and wild steelhead (*Oncorhynchus mykiss*) in Forks Creek, Washington, USA. Mol Ecol 15:3157–3173

Hodgson G (1990) Sediment and the settlement of larvae of the reef coral *Pocillopora damicornis*. Coral Reefs 9:41–43

Huang D, Meier R, Todd PA, Chou LM (2008) Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J Mol Evol 66(2):167–174

Huang D, Tun KPP, Chou LM, Todd PA (2009) An inventory of zooxanthellate scleractinian corals in Singapore, including 33 new records. Raffles Bull Zool 22:69–80

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Keshavmurthy S, Hsu C-M, Kuo C-Y, Meng P-J, Wang J-T, Chen CA (2012) Symbiont communities and host genetic structure of the brain coral *Platygyra verweyi*, at the outlet of a nuclear power plant and adjacent areas. Mol Ecol 21:4393–4407

Kwok CK, Ang PO Jr (2013) Inhibition of larval swimming activity of the coral (*Platygyra acuta*) by interactive thermal and chemical stresses. Mar Pollut Bull 74:264–273

LaJeunesse TC, Smith R, Walther M, Pinzón J, Pettay DT, McGinley M, Aschaffenburg M, Medina-Rosas P, Cupul-Magaña AL, Pérez AL, Reyes-Bonilla H, Warner ME (2010) Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. Proc R Soc Lond B Biol Sci 277:2925–2934

Lance SL, Light JE, Jones KL, Hagen C, Hafner JC (2010) Isolation and characterization of 17 polymorphic microsatellite loci in the kangaroo mouse, genus *Microdipodops* (Rodentia: Heteromyidae). Conserv Genet Resour 2:139–141

Lechner A, Keckeis H, Schludermann E, Loisl F, Humphries P, Glas M, Tritthart M, Habersack H (2013) Shoreline configurations affect dispersal patterns of fish larvae in a large river. ICES J Mar Sci. doi:10.1093/icesjms/fst139

Mangubhai S, Souter PB, Grahn M (2007) Phenotypic variation in the coral *Platygyra daedalea* in Kenya: morphometry and genetics. Mar Ecol Prog Ser 345:105–115

Márquez LM, Mackenzie JB, Takabayashi M, Smith CR, Chen CA (2000) Difficulties in obtaining microsatellites from acroporid corals. Proc 9th Int Coral Reef Symp 1:139–144

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S (2010) Phenotype-environment mismatches reduce connectivity in the sea. Ecol Lett 13:128–140

Miller KJ, Howard CG (2004) Isolation of microsatellites from two species of scleractinian coral. Mol Ecol Notes 4:11–13

Miller KJ, Ayre DJ (2008) Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. J Anim Ecol 77:713–724

Nakajima Y, Nishikawa A, Iguchi A, Sakai K (2012) Regional genetic differentiation among northern high-latitude island populations of a broadcast-spawning coral. Coral Reefs 31:1125–1133

National Population and Talent Division, NPTD (2013) The population white paper: a sustainable population for a dynamic Singapore. http://www.nptd.gov.sg/content/NPTD/news/_jcr_content/par_content/download_98/file.res/population-white-paper.pdf

Ng CSL, Chen D, Chou LM (2012) Hard coral assemblages on seawalls in Singapore. TMSI St John's Island 10th Anniversary Volume: Contributions to Marine Science pp 75–79

Ng WC, Morton B (2003) Genetic structure of the scleractinian coral *Platygyra sinensis* in Hong Kong. Mar Biol 143:963–968

Nunes FLD, Norris RD, Knowlton N (2011) Long distance dispersal and connectivity in amphi-Atlantic corals at regional and basin scales. PLoS One 6:e22298

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13:146–158

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155(2):945–959

Qu X, Meng Q (2011) The economic importance of the Straits of Malacca and Singapore: An extreme-scenario analysis. Transp Res Part E Logist Transp Rev 48:258–265

Rodriguez-Lanetty M, Hoegh-Guldberg O (2002) The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Plesiastrea versipora* in the Western Pacific. Mol Ecol 11:1177–1189

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of populations structure. Mol Ecol Notes 4:137–138

Ryman N, Palm S (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol Ecol Notes 6:600–602

Sargent TD, Jamrich M, Dawid IB (1986) Cell interactions and the control of gene activity during early development of *Xenopus laevis*. Dev Biol 114:238–246

Severance EG, Karl SA (2006) Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. Mar Biol 150:57–68

Shearer TL, van Oppen MJH, Romano SL, Wörheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol Ecol 11:2475–2487

Souter P, Grahn M (2008) Spatial genetic patterns in lagoonal, reef-slope and island populations of the coral *Platygyra daedalea* in Kenya and Tanzania. Coral Reefs 27:433–439

Tan HT, Chou LM, Yeo DCJ, Ng PKL (2010) Waste- and reclaimed land. In: Tan HT, Chou LM, Yeo DCJ, Ng PKL (eds) The natural heritage of Singapore, 3rd edn. Prentice Hall-Pearson Education South Asia Pte Ltd, Singapore, p 78

Tay YC, Guest JR, Chou LM, Todd PA (2011) Vertical distribution and settlement competencies in broadcast spawning coral larvae: implications for dispersal models. J Exp Mar Biol Ecol 409:324–330

Tay YC, Todd PA, Rosshaug PS, Chou LM (2012) Simulating the transport of broadcast coral larvae among the Southern Islands of Singapore. Aquat Biol 15:283–297

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2009) Ecologically relevant dispersal of corals on isolated reefs: implications for managing resilience. Ecol Appl 19:18–29

van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. Mol Ecol 15:3863–3883

van Oppen MJH, Lutz A, De'ath G, Peplow L, Kininmonth S (2008) Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. PLoS One 3:e3401

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Wooldridge SA (2014) Differential thermal bleaching susceptibilities amongst coral taxa: re-posing the role of the host. Coral Reefs 33:15–27

Yasuda N, Nagai S, Hamaguchi M, Okaji K, Gérard K, Nadaoka K (2009) Gene flow of *Acanthaster planci* (L.) in relation to ocean currents revealed by microsatellite analysis. Mol Ecol 18:1574–1590