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Development of Novel Polymorphic Microsatellite Markers in Catch Bowl Coral, *Isopora palifera* (Scleractinia; Acroporidae) Using Next-generation Sequencing

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Sung-Yin Yang, Wai-Ling Fong, Wenhua Savanna Chow, Chia-Min Hsu, Chia-Ling Carynn Chan, Shashank Keshavmurthy, and Chaolun Allen Chen (2018) Catch bowl coral, *Isopora palifera*, is a shallow-water scleractinian species distributed in the Indo-West Pacific region, and has been studied for its reproduction, symbiont diversity, and population genetics. In order to develop microsatellite markers to reveal the genetic connectivity of *I. palifera* in the Kenting reefs, southern Taiwan, we applied a stepwise approach including Illumina sequencing, primer screening, and validation. DNA sequences of each 6,363,035 read pairs were assembled with high coverage and sequencing depth, and 1,173,835 potential SSRs were identified. A set of 60,986 primers were designed and tested, and six novel microsatellite markers with three type motifs, including 3 di- and 3 tetra- repeats, were successfully isolated. The ranges in number of alleles per locus and observed and expected heterozygosities were 3-5, 0.444-0.538, and 0.375-0.565, respectively. Application of these loci to the genetic diversity of an *I. palifera* population that experienced bleaching events in the Kenting reef between 1998 and 2015 showed a signature admixture of three clusters without temporal variation. These loci are useful for studying population genetics in the genus *Isopora*. Our results suggest that next-generation sequencing technology is convenient and cost-effective and can be utilized to isolate microsatellites in other reef-building corals.

Key words: *Isopora palifera*, Coral, Microsatellites, Next-generation sequencing.

BACKGROUND

Microsatellites are simple sequence repeat (SSR) motifs scattered throughout the genome and have been isolated and utilized in population genetics, molecular ecology, conservation biology, and genetic mapping of marine organisms (Grover and Sharma 2016). Microsatellite marker isolation and validation was traditionally time-consuming and expensive until it began to be applied to assess genetic variation in target organisms (Vieira et al. 2016). For reef-building

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corals in tropical waters, obtaining pure genomic materials for SSR library construction is difficult due to contamination by symbiotic organisms (such as *Symbiodinium*), microbes (bacterial communities), and parasitic crustaceans, which can all complicate the screening process of primer design and validation (reviewed in Márquez et al. 2002). Sperm can overcome contamination because it is the source of *Symbiodinium*-free genomic DNA, can be collected on predicted spawning nights, and has been successfully utilized in isolating microsatellites from several species of reef-building corals (Miller and Howard 2004; Shearer and Coffroth 2004; Baums et al. 2005; Isomura and Hidaka 2008; Wang et al. 2009; Concepcion et al. 2010; Tang et al. 2010; Shinzato et al. 2014; Nakajima et al. 2015). Nevertheless, the development of microsatellite markers can be strenuous for species that do not release gametes into the water column (*i.e.*, brooders) or species that there is no spawning information on.

Rapid advances in next-generation sequencing (NGS) technology have shown several advantages to overcoming the problems in developing microsatellites for reef-building corals. First, NGS can generate large amounts of SSRs in a short period of time and at a relatively low cost (Grover and Sharma 2016). For example, NGS has been used to successfully generate 27 microsatellite loci to differentiate two sympatric types of *Galaxea fasicularis* (Nakajima et al. 2015). Second, reference coral genomes (Shinzato et al. 2011) can be utilised to eliminate potential *Symbiodinium* loci in coral SSR libraries generated from regular tissue samples. Third, sequencing the barcoded SSR amplicons using NGS and following bioinformatics procedures for genotyping could directly assess population genetics; this technique has recently been developed to estimate the connectivity of *Acropora hyacinthus* in the Micronesian Islands (Cros et al. 2016).

In this study, we applied NGS technology to develop microsatellites for catch bowl coral, *Isopora palifera*, a common brooding Acroporidae coral found in the Indo-Pacific region (Wallace 1999). *I. palifera* population genetics have been studied using allozymes (Benzi et al. 1995), reproductive periodicity (Kojis and Quinn 1984), and shuffling of symbiont communities (Chen et al. 2005; Hsu et al. 2012; Keshavmurthy et al. 2014). Microsatellites developed in the present study can be utilized to examine genetic connectivity in the genus *Isopora.*

MATERIALS AND METHODS

Sample collection and DNA extraction

Samples before 2015 were used from Chen et al. (2005) and Hsu et al. (2012), sampling permission for 2015 was granted by the Kenting National Park Headquarters, permit no.1040008112. *Isopora palifera* was collected from the reef flat at 3 m depth in the Tantzi Bay (TZB), Kenting National Park (KNP), Taiwan (21°57'00.37"N, 120°46'31.38"E). In order to obtain *Symbiodinium*-free coral tissues for DNA extraction, one coral sample was bleached with menthol following the protocol developed by Wang et al. (2012). The bleached coral tissues examined by the Diving-PAM (Heinz Walz GmbH, Germany) showed a significant reduction in photochemical efficiency, and further confirmed the absence of *Symbiodinium* observed under a microscope (SZ61, Olympus Corporation). *Isopora palifera* genomic DNA was extracted using the phenol-chloroform method (Hillis et al. 1996).

Illumina paired-end sequencing, data filtering, and genome assembly

DNA libraries for Illumina sequencing were generated using Illumina TruSeq DNA Sample Preparation Kit (Illumina, San Diego, CA) following the manufacturer's recommendations. 5 to 10 μg of input genomic DNA was fragmented on a BioRuptor sonicator (Diagenode Inc., Denville, NJ, USA) for 20 cycles to generate 200-400 bp fragments. DNA fragments were then end-repaired and treated with a poly-A tailing reaction, followed by an adapter ligation. The adaptor-ligated DNA constructs were size-selected using agarose gel electrophoresis and constructs of 300-550 bp were isolated, amplified, and quantified using the Quant-iT dsDNA HS assay (Invitrogen). The hybridization-base microsatellite enrichment steps were then performed following the protocol described by Jennings et al. (2011), with three probes designed to enrich the motifs of $(CA)_{15}$, $(CT)_{15}$, and $(GATA)_{15}$. The microsatellite-enriched libraries were pooled in equimolar concentrations and submitted for 100 bp paired-end read sequencing on the HiSeq 2000 Sequencing Platform (Illumina, San Diego, CA).

The Illumina reads from each sample were first analyzed by FastQC (Andrews 2010); reads with median base quality score lower than 25 were trimmed by Trimmomatic (Bolger et al. 2014) and reads shorter than 5 bp were discarded directly.

The reads were paired and assembled *de novo* by SSR_Pipeline (Miller et al. 2013) with parameters of min-olap 10, max_olap 70, mismatch ratio 0.25; 40 bp of flanking sequences were preserved for primers targeting sites. Both simple and compound microsatellites were identified based on the following criteria: monomeric repeats > 10, dimeric repeats > 7, trimeric and tetrameric repeats are defined as > 5 repeats. The assembled paired-end sequences are limited to 200 bp, so the pentameric and hexameric repeats were both limited to 5 repeats. *de novo* assembly may generate variable tandem repeats of alleles within identical flanking regions, so in order to avoid such artifacts highly homologous sequences were clustered using software bundle CH-HIT-EST (Li and Godzik 2006; Fu et al. 2012) to reduce sequence redundancy. The sequence identity threshold was set to 0.95 to remove potentially redundant sequences from assembled reads. The outcome sequences containing only non-redundant sequences were then used for primer design.

Microsatellite identification and primer design

Customized scripts were used to extract tandem-repeat frequencies, which divided by permutation combinations of different allele types from monomeric to hexameric repeats. Online service BatchPrimer3 v1.0 (You et al. 2008) was used to design primers located on both flanking regions of assembled SSR-rich sequences under searching strategy set to “SSR screening and primer design”, with PCR products set to 100-300 bp in length and primer return number set to 1.0 to generate candidate primers. The outcome of designed primer sets was subsequently utilized to screen and validate polymorphisms.

Primer polymorphism test

A total of 150 primer pairs were selected to test amplification efficiency based on Tm values, GC content, and nucleotide composition. Forward primers were appended with an M13 tail (5'-TGT AAA ACG ACG GCC AGT -3') at the 5' end for later fluorescence attachment during PCR (Schuelke 2000); reverse primers were appended with an addition (GTTT) at the 5' end to ensure a non-templated adenylation for easier allele scoring later (Brownstein et al. 1996). The microsatellite loci were amplified through PCR using three primers: a sequence-specific forward primer with an M13 tail, a sequence-specific reverse primer with a GTTT addition, and the universal fluorescent-labeled M13 primer. The PCR was divided into 2 parts: 1) annealing and incorporation of the forward primer with its M13 tail into the accumulating PCR products; 2) annealing of the universal M13 primer to incorporate fluorescent dye into the PCR product. PCR cycling condition was: 1 cycle at 98°C (30 s); 25 cycles at 98°C (10 s), the primer-specific annealing temperature (Table 1) (30 s), and 72°C (30 s); 10 cycles at 98°C (10 s), 53°C (annealing temperature for the fluorescent-labeled M13 (-21) primer) (30 s), 72°C (30 s); and a final elongation of 10 min at 72°C. Following PCR, samples were electrophoresed on 5% denaturing polyacrylamide gels using Gel-Scan 3000 (Corbett Robotics, Australia) to check for polymorphisms. Allele size was determined using the software Gene Profiler 4.05 (Scanalytics) according to

Table 1. Details for six polymorphic microsatellite loci developed for *Isopora palifera* as calculated from the genotypes of 45 individuals

Locus	Primer sequences (5'-3')	Repeat motif	Optimal T_a (°C)	Size range (bp)	N_a	H_e	H_o	GenBank Accession no.
IP5	Forward: M13(-21)-TGTAAAGCCTAGCATCACTTG Reverse: GTTT-GGCAACGTACACTGTGTCTTA	$(AC)_{20}$	50	196-206	3	0.086	0.089	KX372302
IP181	Forward: M13(-21)-TTCAACTGAACTTGAAC Reverse: GTTT-GAAATGATCATAACCAAC	$(TA)7TG(TA)_4$	59	93-103	3	0.407	0.333	KX350086
IP235	Forward: M13(-21)-CCCAAGTAATTGACTGACTG Reverse: GTTT-AAGCAAGCAACTAAC	$(ATTG)_{10}$	50	145-185	8	0.737	0.644	KX350087
IP271	Forward: M13(-21)-CATGCATACATCATAGAAACG Reverse: GTTT-GGCCATTACTGACCTGTATTA	$(CA)_{23}$	56	169-199	8	0.617	0.622	KX350088
IP291	Forward: M13(-21)-AACTCGGAGTAGCAATTGAA Reverse: GTTT-CTTTCTATTTGCCGCATC	$(TATC)_{10}$	65	169-181	4	0.492	0.378	KX350089
IP297	Forward: M13(-21)-AACTCAACAGACACAGGGTAA Reverse: GTTT-CTCAGTGGTGGAAATACCTA	$(TATC)_6$	53	130-150	5	0.624	0.600	KX350090

T_a: Annealing temperature, N_a: Number of alleles, H_e: Expected heterozygosity, H_o: Observed heterozygosity.

the size marker (GeneScan-350-TAMRA Size Standard, Applied Biosystems). Six Microsatellite loci with polymorphisms were selected (Table 1). The PCR products of the six loci were cloned and sequenced to confirm the presence of a repeat motif. BLASTn was performed on all sequences against the *Symbiodinium* sequences in NCBI to confirm that no primer amplified *Symbiodinium*.

Genotyping and population genetic parameters

Forty-five samples collected from the TZB on May 2015 were genotyped and the number of alleles and mean observed and expected heterozygosities were calculated using GenAlEx v.6.502 (Peakall and Smouse 2012). Arlequin v.3.5 (Excoffier and Lischer 2010) was used to test for any significant departure from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium. None of the loci showed HWE deviation and linkage disequilibrium after Bonferroni correction (Rice 1989). The pairwise fixation index (F_{ST}) also was calculated for population differentiation in four samplings (Table S1). The genotyping data generated during this study are included in the table S2.

Temporal variation in genetic structure between 2000 and 2015

In order compare the temporal genetic variation of *I. palifera* populations in the TZB, a total of 91 *I. palifera* DNA samples collected in the TZB in four different years (2000, 2006, 2009, and 2015) (Table 2) were genotyped using six microsatellite loci developed from above procedures to examine if the TZB population changed over time. PCR was performed, followed by electrophoresis on Gel-Scan 3000 using the same protocol described above. The population genetic structure of *I. palifera* samples collected in different years was estimated using a Bayesian clustering approach implemented in STRUCTURE v.2.3.4 (Pritchard et al. 2000). Admixture model and allele frequency correlation were used. The number of genetic clusters (K) from 1 to 10 were tested by running three replicate simulations per K with 1,000,000 Markov chain Monte Carlo repetitions and 100,000 burn-in iterations. The most likely value of K was determined using the method from Evanno et al. (2005), implemented in a web-based software STRUCTURE HARVESTER (Earl 2012). Ten replicate simulations were run using the most likely value of K determined, the results of the ten runs were merged using FullSearch method in CLUMMP v.1.1.2 (Jakobsson and Rosenberg 2007) and then visualized using STRUCTURE PLOT (Ramasamy et al. 2014).

RESULTS

Data assessment of Illumina sequencing and identification of microsatellites in the *Isopora palifera* genome

Sequence quality was high for the 9.9 million (9,963,149) pair-end reads retrieved from Illumina Hiseq 2000. The quality scores are above 28 for all positions in reads (Phred quality score), with 0 sequences flagged as poor quality; GC content was 36%. R1 (146 bp) and R2 (126 bp) reads were assembled *de novo* then microsatellite-containing sequences with flanking regions were generated using program SSR_Pipeline. Since the *de novo*-assembled SSRs may contain identical sequence compositions on both flanking regions and the core of tandem repeats between flanking sites, filtering redundants - which have identical (identity ≥ 100%) or nearly identical (identify ≥

Table 2. Genetic summary statistics of six microsatellite loci from the TZB in the years 2000, 2006, 2009, and 2015 for *Isopora palifera*

Year	N	N_a	N_e	I	H_o	H_e	uH_e	F_{IS}
2000	21	4.000	2.527	0.992	0.475	0.538	0.552	0.111
2006	12	3.000	1.685	0.652	0.492	0.375	0.392	-0.264
2009	13	4.167	2.529	1.036	0.538	0.565	0.587	0.084
2015	45	5.167	2.304	0.918	0.444	0.494	0.499	0.089

N = No. of samples, N_a = No. of Alleles, N_e = No. of Effective Alleles = 1 / ($\sum p_i$^2), I = Shannon's Information Index = -1 $\sum$ (p_i * Ln (p_i)), H_o = Observed Heterozygosity, H_e = Expected Heterozygosity, uH_e = Unbiased Expected Heterozygosity = (2N / (2N-1)) * H_e, F_{IS} = Inbreeding Coefficient = (H_e - H_o) / H_e = 1 - (H_o / H_e). **Where p_i is the frequency of the i^{th} allele for the population & $\sum p_i$^2 is the sum of the squared population allele frequencies. ***No significance FIS after Bonferroni correction was observed

95%) flanking regions with same tandem repeats - were conducted by the program CD-HIT-EST. This stringent filter left a pool of 1,173,835 unique sequences containing monomeric to tetrameric repeats with at least 40 bp flanking regions for further primer design steps. Within the non-redundant SSR-containing sequence pool, tetrameric repeat sequences make up the highest portion (781,019) of all assemblages, while dimeric and monomeric repeats make up the second (234,860) and third (135,108) highest portions. These high-quality SSR-containing sequences were analyzed by online service BatchPrimer3 for microsatellite primer design. The dimeric SSR-rich sequences retrieved 15,917 designed primer sets and tetrameric SSRs retrieved 45,001 primer sets. Several primer sets from these datasets were selected and screened for polymorphisms of *Isopora* samples.

Primer tests, polymorphisms, and genetic diversity

Of 150 primer sets tested, 90 pairs (60%) yielding specific PCR products were selected to test the polymorphisms using 12 *Isopora palifera* individuals. Six microsatellite markers (0.04%) producing reliable PCR products with allele sizes ranging from 93 to 206 bp were submitted to NCBI; GenBank accession numbers are listed in table 1.

All six microsatellite loci were polymorphic, ranging in variability from three alleles (IP5, IP181) to eight alleles (IP235, IP271) (Table 1). Genetic diversity indices did not change much, with the average number of effected alleles per locus (N_e) remaining around 2.5, except the one in 2006 (Table 2). The observed heterozygosity (H_o) ranged from 0.444-0.538, and the expected heterozygosity (H_e) ranged from 0.375-0.565. None of the inbreeding coefficients (F_{IS}) showed significant departure from HWE towards homozygote excess after Bonferroni correction (Table 2). The F_{ST} of the population in 2000 was significantly different from those of 2006 and 2015, and 2006 was significantly different from 2015. The highest F_{ST} value was from 2000 to 2006, and next was from 2000 to 2015. The lowest F_{ST} values were from 2000 to 2009 and 2009 to 2015 (Table S1).

Temporal variation of genetic structure in the Tantzei Bay

For the genetic structure of *I. palifera* in the TZB, the most likely number of genetic clusters (K) determined by STRUCTURE and STRUCTURE HARVESTER was three (Fig. 1). At the four sampling times (year 2000, 2006, 2009, and 2015), the population in the TZB showed a similar pattern of admixture between the three clusters (Fig. 2).

DISCUSSION

NGS isolation of microsatellites in *Isopora palifera*

This study is the first attempt to develop microsatellites markers for *Isopora palifera*. NGS has been known to successfully isolate microsatellites in different coral species; for example, it was used for two species of *Acropora* (Shinzato et al. 2014), *Galaxea fascicularis* (Nakajima et al. 2015), and two species of *Pocillopora* (Nakajima et al. 2017), which generated 14, 11, and 13 microsatellite markers for single and cross species, respectively. This study generated six microsatellite markers and more sequencing reads for microsatellite detection - 9.9 million read pairs - than the other three studies, which generated 2.5 to 7 million read pairs (Nakajima et al. 2017). While the previous three studies used whole genomic data, this study enriched the microsatellite pools of dimeric and tetrameric repeats before sequencing. The merics and repeats set to design primers (Shinzato et al. 2014) for two *Acropora* species was 2 mer > 15 repeats, 3 mer > 10, 4 mer > 7, 5 and 6 mer > 4; Nakajima et al. (2015) for *G. fascicularis* was 3 mer: 60 and 4 mer = 60; and Nakajima et al. (2017) for two *Pocillpora* species was 4 mer: 10 - 15 repeats, 5 mer: 8 - 12 repeats, and 2 mer > 7, 3 and 4 mer > 5 repeats for this study. Trimeric repeats were not included in the final primer selection due to the possibility of its non-neutral characteristic in the genome, and they should be applied with caution for population genetics or phylogeny purposes (Goldstein and Schlötterer 1999). In spite of the different selection methods, the number of alleles and heterozygosities in this study were in a range similar to those in Shinzato et al. (2014).

Temporal genetic structure of *Isopora palifera* in the Tantzi Bay

Genetic structure inferred from the 6-microsatellite loci showed an absence of any temporal variation in the *I. palifera* population

structure in the TZB between 2000 and 2015. The *Acropora millepora* population in the Keppel Island, Great Barrier Reef (GBR) was also reported to lack temporal variation in its genetic structure (van Oppen et al. 2015). Several bleaching events reoccurred in GBR and caused high mortality in 1998, 2002, and 2006, but the population genetics of the *A. millepora* population surveyed in 2002 and 2008 did not show a bottleneck effect, and the structure remained similar across the two years. It is hypothesized that the bleaching may have caused partial colony mortality, from which *A. millepora* recovered rapidly (van Oppen et al. 2015). Our result is consistent with this hypothesis that *I. palifera* might be able to survive catastrophic stresses, such as thermal-induced bleaching and typhoons, and continue to withstand or replenish the population in the TZB. *Isopora palifera* is a

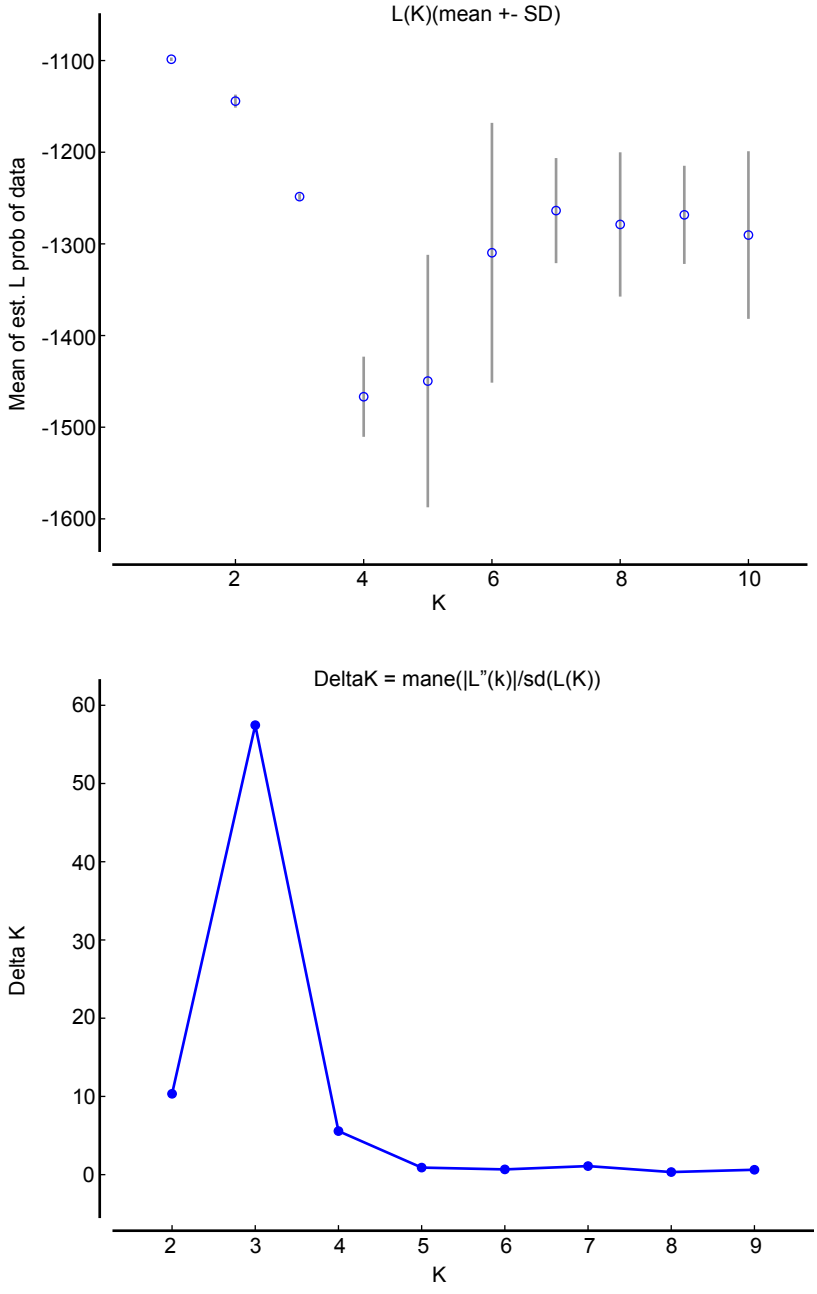

Fig. 1. Mean log-likelihood of K (top) and Delta K (bottom) values for STRUCTURE analysis of *Isopora palifera* samples in the TZB collected in the years 2000, 2006, 2009, and 2015. Values of K = 1-10 were tested by running 3 replicate simulations for each K. The most likely number of cluster (K) was three.

shallow-water species encrusted between the low intertidal zone and 3 m deep and is already exposed to a highly fluctuating environment; partial morality of encrusted *I. palifera* colonies was frequently observed during the bleaching survey. Several mass bleaching events have been observed in southern Taiwan throughout these 15 years, including the one each in 1998, 2007, and 2010 (Dai et al. 1999; Tung et al. 2007; Kuo et al. 2012; respectively). Large coverage of corals in KNP were bleached during these three events, including the population in the TZB.

Other life history traits, such as its association with the thermal-tolerant *Symbiodinium* species *S. trenchii* (D1a) and its ability to shuffle through time (Chen et al. 2005; Hsu et al. 2012; Keshavmurthy et al. 2014), might facilitate *I. palifera* to resist thermal-induced bleaching events. A survey of *Symbiodinium* diversity in *I. palifera* in 2000, one year after the 1998/1999 global bleaching event, suggested that *S. trenchii* is the dominant symbiont (Chen et al. 2005); but it may have been able to shuffle to *Symbiodinium* C3 dominated within 6 years when thermal stress was removed (Hsu et al. 2012). In addition, an *I. palifera* population in the reef exposed to long-term elevated seawater temperatures due to the presence of a nuclear power plant outlet (NPP-OL) are consistently predominant by *S. trenchii* (Hsu et al. 2012; Keshavmurthy et al. 2014). All of these findings support the idea that bleaching does not severely affect the genetic structure of *I. palifera* in the TZB.

On the other hand, typhoons might cause consistent physical damage, and result in the population decrease through time. Our result show that H_o was higher than the H_e in 2006, suggesting the population experienced a decline (Cornuet and Luikart 1996), probably caused by Typhoon Chanchu (Kuo et al. 2012). The genetics showed no sign of population decline in 2009 and 2015, implying that the typhoon may have caused low damage and the population showed consistent sexual reproduction. Colonies over 1 cm in mean radius may produce testes only, colonies will reach sexual maturity as hermaphrodites when greater than 5 cm (Kojis 1986). The growth rate of *I. palifera* could be roughly calculated as 2 cm/yr (Anderson et al. 2012), which is too small for valid reproduction and for our sampling in 2009. Therefore, the colonies sampled in 2009 should be those that escaped the typhoon Chanchu and regrew. The successful larval settlement rate was considered low in the Kenting region for *I. palifera*, yet evidence for recruitment was found in the TZB between 2011 and 2012 (Hsu et al. 2014). *Isopora palifera* is a brooding coral that gets fertilized internally then releases planulae (Kojis 1986). Once planulae are released, they tend to swim downward and crawl near the mother colony to find suitable substrate for settlement (Best and Resing 1987; Barid and Morse 2004). Although this limits larval dispersal and potentially increases the rate of self-recruitment sperm may successfully spread out more than ten metres (Aye and Miller 2006). The genetic data from previous studies suggest that the colonies mate randomly, even with individuals from nearby reefs (Benzie et al. 1995; Aye and Miller 2006). Since the population structure was similar, the inbreeding coefficient was low, and that F_{ST} was mostly high implies that the TZB population might receive constant gene flow from other nearby populations.

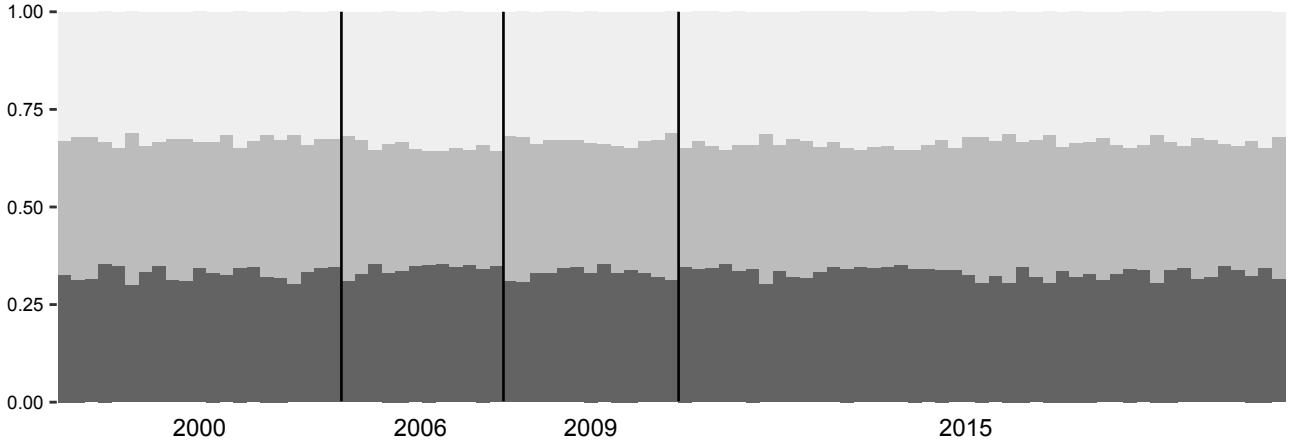

Fig. 2. *Isopora palifera* population structure in the TZB for four time-points (year 2000, 2006, 2009 and 2015) (K = 3). Horizontal bar plot on the bottom shows the results of the program STRUCTURE. Each individual is represented by a thin line in the bar chart, which is partitioned into different segments that indicate the individuals' cluster membership.

CONCLUSIONS

Six microsatellite markers isolated from this study were used to successfully construct the temporal population structure of *Isopora palifera* in Tantzei Bay, Kenting National Park, Taiwan. The *I. palifera* population exhibited panmixia throughout bleaching events and repopulated after typhoons, and this implies that it is highly resilient in the TZB. The level of influence the NPP-OL has in the KNP differs across the region; with the effects of typhoons and anthropogenic actives, knowing the genetic information of different populations would be useful information for management and conservation purposes. Further research using the microsatellite primers for *I. palifera* to investigate population structure diversity and genetic connectivity in the Kenting area is in progress.

List of abbreviations

SSR, Simple sequence repeat.
BGS, Next-generation sequencing.
TZB, Tantzi Bay.
KNP, Kenting National Park.
GBR, Great Barrier Reef.
NPP-OL, Nuclear power plant outlet.

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Competing interests: The authors declare that they have no conflict of interest.

Availability of data and materials: All sequences were uploaded to NCBI. The raw data are in supplementary section.

Consent for publication: Not applicable.

Sampling and field studies: All necessary permits for sampling and observational field studies were obtained by the authors from the competent authorities and are mentioned in the text.

Ethics approval consent to participate: Not applicable.

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Supplementary Materials

Table S1. The pairwise F_{ST} of *Isopora palifera* in different sampling years. (download)

Table S2. The supplement file is the raw data from six microsatellite markers scoring through all samples. (download)