Science of the Total Environment 775 (2021) 145775

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Genetic structure of *Turbinaria peltata* in the northern South China Sea suggest insufficient genetic adaptability of relatively high-latitude scleractinian corals to environment stress

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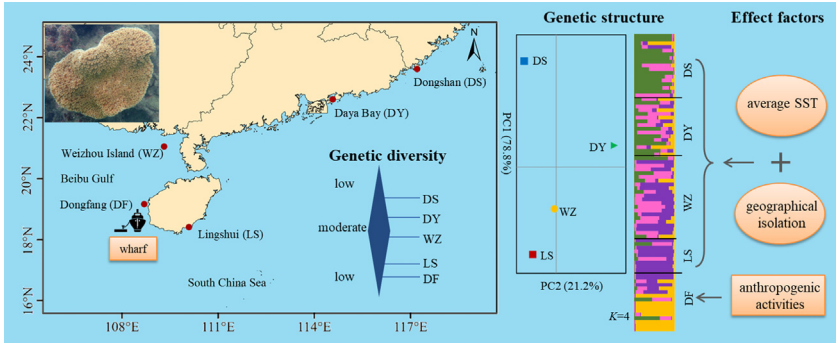
HIGHLIGHTS

- *Turbinaria peltata* has insufficient genetic adaptability under changing climate.
- Moderate genetic differentiation was observed among *T. peltata* populations in SCS.
- DF became a divergent population as a result of severe anthropogenic activity.
- Sea surface temperatures (SST) and geographic isolation have shaped the genetic structure of *T. peltata*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 November 2020
Received in revised form 12 January 2021
Accepted 6 February 2021
Available online 11 February 2021

Editor: Lotfi Aleya

Keywords:
Turbinaria peltata
Relatively high-latitude coral reef
Genetic diversity
Genetic differentiation
Coral refuge

ABSTRACT

With the rapid degradation of coral reefs due to global warming and anthropogenic impacts, relatively high-latitude areas, such as the northern South China Sea (SCS), are likely to become refuges for tropical coral species. Here we investigated the genetic features and adaptability of one dominant scleractinian coral species, *Turbinaria peltata*, in the northern SCS. A total of 81 samples from 5 sites were studied to explore potential mechanisms of adaptability to environmental stress as a result of climate change. Ten microsatellite markers developed in this study, one nuclear gene (internal transcribed spacer, ITS), and one mitochondrial gene (mitochondrial cytochrome oxidase subunit I gene, mtDNA COI) were used. Our results indicated that the genetic diversity of *T. peltata* in the northern SCS is low (A_r = 1.403–2.011, H_o = 0.105–0.248, H_e = 0.187–0.421) with the lowest in Dongfang population (DF) (A_r = 1.403, H_o = 0.22, H_e = 0.187). These results indicate that *T. peltata* has insufficient genetic adaptability and may unable to handle increasingly complex global changes. A significantly moderate genetic differentiation was observed among *T. peltata* populations (Φ_{ST} = 0.167), in addition to a high genetic differentiation between DF and other populations (F_{ST} = 0.272–0.536 > 0.25). The DF population near a fishing port was exposed to severe anthropogenic environmental stress, which may drive the extraordinarily high genetic differentiation between DF and other populations. Furthermore, the Mantel test results showed that the genetic differentiation of the other four populations was strongly correlated with the average sea surface temperature (SST) (R^2 = 0.82, Mantel test P < 0.05) and geographical distance (R^2 = 0.57, Mantel test P < 0.05).

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https://doi.org/10.1016/j.scitotenv.2021.145775

Our results suggest that the genetic structure of *T. peltata* in the relatively high-latitude of the SCS was significantly affected by average SST, geographical isolation, and anthropogenic activities. These findings provide a theoretical foundation for the protection of relatively high-latitude coral reefs.

1. Introduction

As one of the most diverse ecosystems, coral reefs provide habitat provisions for at least 30% of marine organisms (Badger, 1996). Corals are stenothermic and extremely sensitive to temperature changes (Carricart-Ganivet, 2004). Thus, rising sea surface temperatures (SST) threatens the survival of coral reefs worldwide, especially in low-latitude reefs (Price et al., 2019). Although the SST in relatively high-latitude areas (20–30°N) has also increased, this increment not only haven't exceeded the coral upper temperature limit (32 °C), but also has alleviated the low temperature pressure during the winter (Schleyer et al., 2008). Therefore, relatively high-latitude regions may serve as potential refuges for tropical coral species (Beger et al., 2014; Glynn, 1996; Riegl, 2003). Research is increasingly finding that native high-latitude reefs not only face environmental stress but also competition stress from northward-migrating tropical coral species (Chambers et al., 2012; Budd and Pandolfi, 2010; Haak et al., 2010). However, the mechanisms underlying the reaction of relatively high-latitude coral communities to environmental stress and their adaptability have yet to be elucidated.

The long-term viability of existing populations mainly depends on their genetic characteristics (Noreen et al., 2009). Characterizing genetic diversity and genetic differentiation using genetic molecular markers is key for defining protection priorities and predicting population-level responses to changing climate (Funk et al., 2012; Palsboll et al., 2007; Tulloch et al., 2015). Many population genetics studies have been conducted on the Great Barrier Reef in Australia and the Coral Triangle, in which many environmental factors have been evaluated, including ocean currents, breeding patterns, and geographical distances (Baums et al., 2012). These studies have demonstrated that the genetic characteristics of different coral species in disparate regions can vary enormously. For example, *Platygyra sinensis* retains high genetic diversity and high connectivity in Singapore, while *Pocillopora damicornis* was found to have strong patterns of differentiation and reduced genetic diversity in Western Australia (Tay et al., 2015; Thomas et al., 2017).

The South China Sea (SCS) is a critical region for coral reef connectivity, connecting the Indian and Pacific oceans, and its protection should be prioritized (Yu, 2012; Vujakovic, 2002). In recent years, the climatic refuges of tropical corals in the northern SCS have been confirmed by several studies (Qin et al., 2019; Tkachenko and Soong, 2017). However, the genetic characteristics of SCS coral reefs have barely been investigated, which has hampered the formulation of coral reef protection measures. Our previous study was the first to focus on large-scale genetic connectivity of a widespread coral species (*Porites lutea*) across the SCS and found high genetic diversity and connectivity (Huang et al., 2018). More genetic studies are urgently needed to improve our understanding of the genetic characteristics of dominant coral species in the SCS.

Turbinaria peltata is a dominant scleractinian coral species in relatively high-latitude of the SCS, and serves as a key ecological component in local coral communities. Shi et al. (2016) sequenced the complete mitochondrial genome of *T. peltata*, providing fundamental insights into the molecular study of this organism's development. The present study aimed to evaluate the genetic diversity, connectivity, and adaptability of *T. peltata* further. Firstly, we developed suitable microsatellite markers for *T. peltata* using meta-transcriptomic sequencing. Secondly, the genetic characteristics of *T. peltata* were calculated based on ten microsatellite markers, one nuclear genetic marker, and one mitochondrial marker. Thirdly, we analyzed the environmental factors affecting the resilience and adaptability of *T. peltata* to climate change. The results presented in this study will expand our understanding of the survival status and environmental stress adaptability of *T. peltata* in response to climate change.

2. Materials and methods

2.1. Sample collection and environmental data

Fragments of *T. peltata* were collected from five sampling sites in the northern SCS: Dongshan Island (DS, $n = 16$) in Fujian Province, Daya Bay (DY, $n = 18$) in Guangdong province, Weizhou Island (WZ, $n = 28$) in Guangxi province, Lingshui (LS, $n = 8$), and Dongfang (DF, $n = 10$) in Hainan province (Fig. 1). Due to seasonal low temperatures, the coral communities in DS and DY are represented by patch reefs (B. Chen et al., 2019). In WZ, the coral communities were diverse and healthy. The bowl coral *T. peltata* was one of the dominant coral species in DS, DY, and WZ, especially in DS. Although DS is located at the highest latitude for the natural distribution of scleractinian corals along the coast of mainland China, the *T. peltata* communities were large and healthy. With a low abundance of *T. peltata* along the coast of Hainan province, small numbers of *T. peltata* samples were collected from LS and DF with a similar sampling intensity.

Samples from a total of 81 *T. peltata* coral colonies were collected at depths of 2–15 m from the five aforementioned sampling sites (details in Suppl. Table 1). Each colony was separated by at least 5 m to minimize the chances of sampling the same ramet more than once. The colonies were sampled using pliers, taking into account the health of coral communities as a prerequisite. All fragments were transferred into collecting pipes containing 95% ethanol and stored at −20 °C before DNA extraction. As environmental factors may affect the population genetic characteristics, annual average SST (1982 to 2019) from all five sampling sites were obtained from the KNMI Climate Explorer (http://climexp.knmi.nl/registerform.cgi) (details in Suppl. Table 2). The geographical distance between sites was measured using ArcGIS 10.2.

2.2. Meta-transcriptome sequencing, microsatellite marker development and selection

Three healthy *T. peltata* from WZ were sampled and immediately frozen in liquid nitrogen. Then, samples were transported to Majorbio (Shanghai, China) for meta-transcriptome sequencing. Firstly, the total RNA was extracted and quantified for RNA-seq. Secondly, the *T. peltata* RNA transcriptome libraries were prepared using Illumina Truseq™ RNA sample prep Kit. The libraries were quantified and sequenced using the HiSeq X Ten platform (Illumina). Then the reads were trimmed and de novo assembled using Trinity (http://trinityrnaseq.sourceforge.net/) (Grabherr et al., 2011). All assembled transcripts were blast against the reported genomes of corals *Acropora digitifera*, *Stylophora pistillata*, and *Orbicella faveolata* (Prada et al., 2016; Voolstra et al., 2017; Yu et al., 2020). These transcripts could be confidently assigned to corals were used in the subsequent microsatellite marker detection.

The MISA software (http://pgrc.ipk-gatersleben.de/misa/) was used for SSR (Simple Sequence Repeats) locus detection and microsatellite marker development from the assembled transcripts. The online software Marker5 (http://frodo.wi.mit.edu/marker3/) was used for primer design (Mu et al., 2018). The microsatellite primers were selected by the repeat number of 2 nucleotide sequences ≥6, or the repeat number

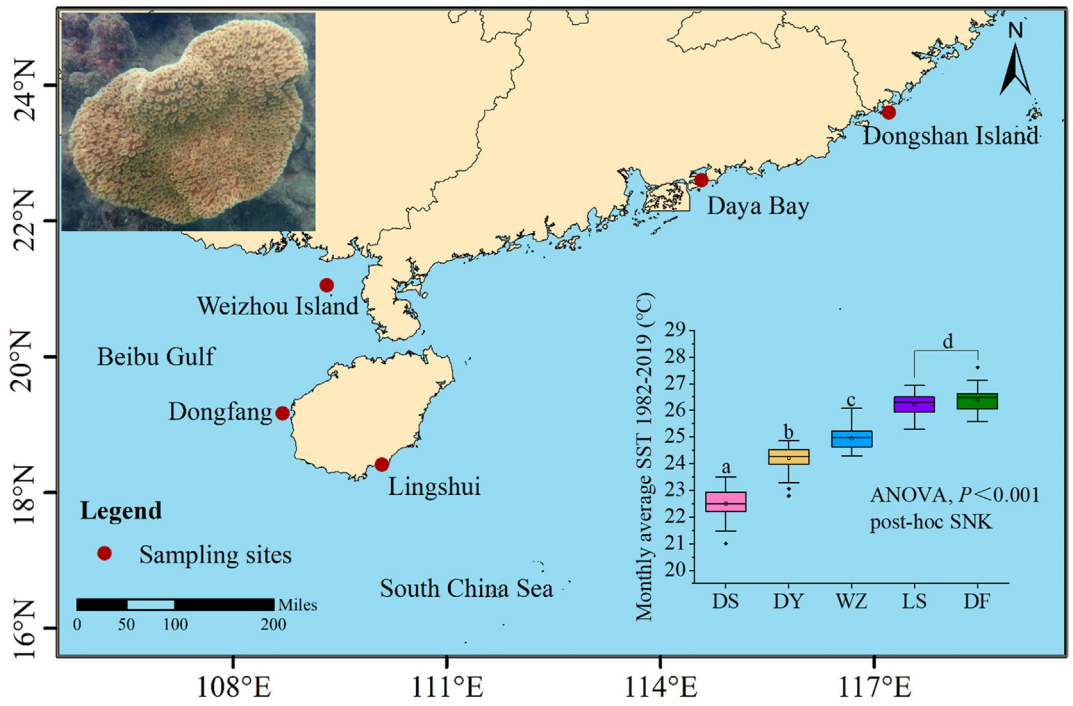

Fig. 1. Map of five sampling sites of *T. peltata* in the northern SCS. Box plots of annual mean, maximum, and minimum of SST from 1982 to 2019 in five sites. The bar within each box represents the median; the squares represent the mean value. Lowercase letters indicate post hoc comparisons (e.g., a, b, c, and d) with the significant factors among sampling sites. Inset is a colony of *T. peltata*.

of 3 or 4 nucleotide sequences ≥5, or the repeat number of 5 nucleotide sequences ≥4, or the repeat number of 6, 7, and 8 nucleotide sequences ≥3. During polymerase chain reaction (PCR) amplifications, the markers that produced a single bright band within the range of the target fragment were selected. Then, the selected markers were sequenced by TA cloning to determine whether they were correct simple repeat sequences. Ultimately, only confirmed microsatellite markers were subjected to PCR amplifications and capillary sequencing in batches. All sequencing experiments were completed by Sangon Biotech Company (Shanghai, China).

2.3. DNA extraction, PCR amplification, and sequencing

The genomic DNA of each sample was extracted using the TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Ten microsatellite loci, one nuclear genetic marker (the internal transcribed spacer, ITS) (Suppl. Table 3), and one mitochondrial marker (mitochondrial cytochrome oxidase subunit I gene, mtDNA COI) (Suppl. Table 3) were used to analyze the genetic diversity and genetic structure of *T. peltata*. The ten microsatellite loci were developed based on the above analysis, details see Table 1. For

Table 1
Description and summary data on the 10 polymorphic microsatellite loci for *T. peltata* developed in this study.

Locus	Marker sequence (5′-3′)	Repeat motif	Product size (bp)	T_a (°C)	H_o	H_e	N_a	N_e	A_r
T_1	F: AAGAAGGAGCCAGTGCAAC R: AGACCTGCAAGATCCGGTG	(AAG)^5	448	56	0.209	0.313	3.000	1.558	1.813
T_2	F: TGCTAATGAGGCCAAGGCG R: GTAATGCTGTCCCTGCACC	(AT)^6	395	57	0.038	0.037	1.800	1.041	1.103
T_3	F:TGTACAGGAATTTAGCGTGATTTG R: GGCGAGCGAATTATGTCCG	(CG)^6	406	57	0.046	0.381	2.800	1.856	2.580
T_5	F: TTTCCAGGTGCGACATAGG R: TAACGGCGCATACAACTGC	(AAC)^5	278	58	0.025	0.024	1.400	1.026	1.053
T_6	F: CGCGTGGATTTCTCTTGGC R: AGACTACAAGCATTACCGCTC	(CT)^7	299	58	0.151	0.234	2.400	1.462	1.616
T_8	F: CGGACTGAAGCACGAAAGTG R: AACGGTTAGGAGGGCCAAG	(CT)^6	421	59	0.015	0.037	1.400	1.041	1.108
T_9	F: AGCTAACCTGTGATCCGGC R: TGGTAACCTGCAATCATGGG	(AT)^8	398	59	0.359	0.601	4.400	2.840	3.092
T_{10}	F:TGTCTGATTCCGTCCACTCG R: ACGTCCGCTGCTGGATTAC	(AG)^6	383	61	0.506	0.553	3.200	2.306	2.246
T_{15}	F:TGTTAGGGATAATTTAGGTCGGTG R: CCTGTACTTGCTGGCCATTC	(AC) ^8	270	56	0.435	0.658	6.600	4.303	3.533
T_{17}	F: CATTGCTTCTGGAGCGTGG R: CGTGCTGGCAGAAAGATCC	(GGT) ^5	153	56	0.229	0.252	2.200	1.413	1.622

T_a, annealing temperature for PCR reactions; H_o, observed heterozygosity; H_e, effective heterozygosity; N_a, number of alleles; N_e, number of effective alleles; A_r, allelic richness.

nuclear genetic marker, we firstly conducted PCR using the "coral-universal" marker pair, A18S and A28S (Odorico and Miller, 1997). We obtained the full ITS rDNA intron region, including ITS1, ITS2, and the inserted 5.8S region with low success rate. So based on the obtained sequences, we developed two species-specific primers of ITS markers, which were described in Suppl. Table 3. The mtDNA COI gene was amplified using primers MCOIF and MCOIR (Fukami et al., 2004), details see Suppl. Table 3. Each marker was applied in 50-μL reaction volumes containing 2 μL of marker (20 μM), 0.5 μg of DNA template, 25 μL of *Premix Taq* (*TaKaRa Taq™* Version 2.0 plus dye), and ddH_2O to a total volume of 50-μL. PCR amplifications were conducted under the following conditions: an initial denaturation of 95 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 47–61 °C (see Table 1 and Suppl. Table 3) for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. All PCR products were sequenced by Sangon Biotech (Shanghai, China). The raw ITS sequences were submitted to GenBank under accession number MW407185-MW407244. The raw mtCOI sequences were deposited into GenBank (Accession Number: MW427697-MW427744 and MW456523-MW456553).

2.4. Data analysis

For microsatellite markers, GenepopV4 (Raymond and Rousset, 1995; Rousset, 2008) was used to analyze Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), heterozygote deficiency, and heterozygote excess for each locus (Table 1). All significance levels were adjusted with a strict Bonferroni correction for multiple tests ($P < 0.05$). GeneAlex (Peakall and Smouse, 2012) was used to determine the indices of genetic diversity, such as the number of alleles (N_a), effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), private alleles (PVA), indices of genetic differentiation, pairwise F_{ST} values ($F_{ST} < 0.05$ denotes low levels of genetic differentiation, $0.05 < F_{ST} < 0.25$ denotes medium levels, and $F_{ST} > 0.25$ denotes high levels). Analysis of molecular variance (AMOVA) was carried out in GeneAlex based on pairwise F_{ST} values. Principal coordinates analysis (PCoA) was also carried out in GeneAlex. GenClone2.0 was used to calculate the number of unique multilocus genotypes (MLG) for each population (Arnaud-Haond and Belkhir, 2007). FSTAT (Goudet, 1995) was used to acquire the allelic richness (A_r) and inbreeding coefficients (F_{is}) for each population. To inspect for the relationships between genetic differentiation (the pairwise F_{ST} values) and geographic distance and environmental factors (i.e., average SST and SST variance), Mantel test was run in IBD (Bohonak, 2002) under 1000 randomizations perform. The significance level P is 0.05. NeEstimator v2.1 (Do et al., 2014) was run for the estimation of contemporary effective population size (Nê) based on linkage disequilibrium method under $P_{Crit} = 0.02$.

CREATE1.37 was used to convert raw data to microsatellite file formats (Coombs et al., 2008). A one-way factorial analysis of variance (ANOVA) was used to test the annual average SST (1982–2019) within five regions in the northern SCS. STRUCTURE v2.3.4 (Pritchard et al., 2000) was used to analyze the genetic structure of *T. peltata* in the SCS with 1×10^5 step Markov chain runs followed by 1×10^6 iterations for each value of K (K = 1–4). Each value of K was replicated ten times. STRUCTURE HARVESTER (Earl and vonHoldt, 2012) was used to determine the best value of K. CLUMPP and CLUMPAK (Jakobsson and Rosenberg, 2007) were used to conduct repeat analyses. Finally, the optimal value of K was visualized using DISTRUCT (Rosenberg, 2004). The origin was used to portray the trend chart of SST from each population. SPSS was used to test the significance of SST differences among the populations.

The sequences of ITS and mtDNA COI were aligned using MEGA7.0 (Kumar et al., 2016) with further manual adjustment. DnaSP6.0 (Rozas et al., 2003) was used to determine the indices of genetic diversity, such as haplotype diversity (H_d), nucleotide diversity (π), and mean number of pairwise differences (K). Arlequin3.5.2.2 (Excoffier and Lischer, 2010) was used to analyze the pairwise F_{ST} values, conduct the Mantel test under 1000 bootstrap permutations, and analyze molecular variance (AMOVA) under 1000 bootstrap permutations. Although, the number of collected samples for each site is not very similar, genetic analysis software has taken it into consideration and the influence on sample size differences was minimized (Peakall and Smouse, 2012; Do et al., 2014).

3. Results

3.1. Microsatellite markers of T. peltata

Overall, three meta-transcriptome libraries of *T. peltata* were sequenced and 171,741,110 clean reads were acquired. De novo assembling yielded 112,220 unigenes. BLASTx top-hit species distribution of the 28,021 annotated unigenes exhibited highest homology to *Symbiodiniaceae microadriaticum* (46.36%), followed by *O. faveolata* (22.96%), *S. pistillata* (11.41%), and *A. digitifera* (6.67%). These transcripts could be confidently assigned to corals were used in the subsequent microsatellite marker detection.

A total of 449 pairs of SSR primers were developed from the meta-transcriptomic sequencing data in MISA. Then, 20 markers of *T. peltata* were sorted using PCR and TA-cloning from 44 randomly selected microsatellite markers. HWE and LD tests were conducted to remove markers that did not conform to HWE and maintain LD. Ultimately, a total of 10 species-specific microsatellite markers met the criteria and were used to conduct further genetic analysis. Details of the ten polymorphic microsatellite loci of *T. peltata* developed are provided in Table 1, including observed heterozygosity (H_o), ranging from 0.015 to 0.506, and expected heterozygosity (H_e), ranging from 0.037 to 0.658 (Table 1). At each site, the H_o values were close to the H_e values. Furthermore, the allelic richness (A_r) and number of alleles (N_a) were moderate, ranging from 1.053 to 3.533 and from 1.4 to 6.6, respectively. Thus, the ten microsatellite loci of *T. peltata* developed in this study were all moderately polymorphic. Significant heterozygote deficiency was found in nine of the ten loci ($P < 0.05$), except for T2. After Bonferroni correction, the ten polymorphic microsatellite loci of *T. peltata* met the Hardy-Weinberg equilibrium ($P < 0.05$).

3.2. Genetic diversity of T. peltata

For the microsatellite markers, the results obtained from GeneAlex and FSTAT demonstrated that the number of alleles (N_a) and the number of effective alleles (N_e) ranged from 1.600 to 4.600 and 1.413 to 2.842, respectively. H_o and H_e ranged from 0.105 to 0.248 and 0.187 to 0.421, respectively (Table 2). Allelic richness (A_r) ranged from 1.403 to 2.011 (Table 2). The inbreeding coefficient (F_{is}) ranged from 1.483 to 2.011 (Table 2). Private alleles (PVA) were detected in all loci, ranging from 1 in Lingshui to 23 in Weizhou (Table 2). The indices of alleles (N_a, N_e), heterozygosity (H_o, H_e), and allele richness (A_r) (Table 2) demonstrated that the genetic diversity of *T. peltata* in the northern SCS was low. Notably, DF population was lower than those for other populations. In addition, the clonal richness (R) values were high (R = 0.75–1.00) in

Table 2
Population genetic diversity statistics of *T. peltata* in the northern SCS based on 10 microsatellite markers.

Pop	N	N_g	R	PVA	N_a	N_e	H_o	H_e	A_r	F_{is}	Nê
DS	16	16	1.00	5	3.20	1.848	0.105	0.340	1.764	0.710	8.3
DY	18	18	1.00	5	3.20	1.898	0.209	0.381	1.832	0.487	23.7
WZ	28	26	0.93	23	4.60	2.842	0.248	0.421	2.011	0.440	42.8
LS	8	6	0.75	1	1.60	1.422	0.225	0.216	1.483	0.109	2.5
DF	10	3	0.33	3	2.00	1.413	0.220	0.187	1.403	−0.128	1.0
Total	80	69	0.863	32	2.92	1.885	0.201	0.309	1.977	–	–

N, number of samples; N_g, the number of unique multilocus genotypes; R, clonal richness; PVA, private alleles; N_a, number of alleles; N_e, number of effective alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; A_r, allelic richness; F_{is}, inbreeding coefficient; Nê, effective population size.

Table 3
Genetic diversity indices and neutrality test of *T. peltata* in the northern SCS based on ITS sequences and mtDNA COI sequences.

Gene	Pop	*N*	*H*	H_d	π	*K*	Tajima's *D*	Fu's *F*
ITS	DS	14	11	0.967	0.216	6.495	1.193	−2.834
	DY	13	13	1.000	0.246	7.372	−0.023	**−7.192****
	WZ	17	13	0.949	0.192	5.750	−0.091	**−4.403***
	LS	7	5	0.857	0.225	6.762	−0.717	0.881
	DF	9	4	0.583	0.148	4.444	0.968	2.286
	Total	60	39	0.962	0.229	6.859	0.214	−24.385***
mtDNA COI	DS	15	5	0.476	0.001	0.800	**−1.816****	**−2.103***
	DY	19	9	0.772	0.003	1.953	**−1.712***	**−3.597***
	WZ	27	9	0.513	0.002	1.464	**−2.354*****	**−3.769***
	LS	8	4	0.643	0.002	1.250	−1.448	−0.785
	DF	10	2	0.200	0.0003	0.200	0.000	0.000
	Total	79	24	0.551	0.031	0.903	**−2.639*****	**−∞*****

N, sample size; *H*, number of haplotypes; H_d, haplotype (gene) diversity; π, nucleotide diversity (average over loci); *K*, mean number of pairwise differences; (Tajima's *D* and Fu's *F*) neutrality test, Significant *P* values (sequential Bonferroni corrected *Significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$) marked as bold.

Table 5
Pairwise F_{ST} values between all 5 sampling sites across the northern SCS based on microsatellite markers, mtDNA COI, and ITS.

Gene	Location	DS	DY	WZ	LS	DF
Microsatellite loci	DS	0				
	DY	**0.141***	0			
	WZ	**0.173***	**0.069***	0		
	LS	**0.245***	**0.130***	0.012	0	
	DF	**0.306***	**0.272***	**0.310***	**0.536***	0
ITS	DS	0				
	DY	0.042	0			
	WZ	**0.138***	**0.096***	0		
	LS	**0.189***	**0.104***	**0.132***	0	
	DF	**0.228***	**0.163***	−0.028	**0.154***	0
mtDNA COI	DS	0				
	DY	0.031	0			
	WZ	−0.022	**0.038***	0		
	LS	0.014	0.027	−0.021	0	
	DF	−0.019	0.024	−0.029	0.021	0

* Significant F_{ST} values ($P < 0.05$) are denoted by asterisk and in bold.

all other populations, except for DF ($R = 0.33$). Effective population size (Nê) ranged from 1.0 to 42.8, with the lowest in DF (Table 2).

The haplotype diversity (H_d) values estimated by the mtDNA COI sequences were under 0.85 in each population. The maximum nucleotide diversity (π) values were only 0.003 (Table 3), and the pairwise differences (*K*) values were not beyond 1.953, showing a low genetic diversity level of *T. peltata*. Interestingly, the H_d values calculated by ITS sequences were between 0.583 and 1.000, the π values were between 0.148 and 0.246, and the *K* values were between 4.444 and 7.372 (Table 3). So the ITS results indicated a higher genetic diversity of *T. peltata*. For mtDNA COI sequences, the values of H_d, π, and *K* for the DF population were lower than those of other populations (Table 3). Most of Tajima's *D* and Fu's *F* values were negative. Only DF population experienced a positive selection based on ITS (Table 3).

3.3. Genetic structure of *T. peltata*

Based on the microsatellite markers, there was significant moderate genetic differentiation among the different *T. peltata* populations in the northern SCS ($\Phi_{ST} = 0.167$, $P < 0.05$; Table 4). The AMOVA results indicated that the genetic differentiation of *T. peltata* was mainly derived within populations (83.28%; Table 4), with little derived among populations (16.72%; Table 4). Pairwise F_{ST} statistical analysis showed that nine F_{ST} values from a total of ten pairs were at moderate or high levels, as well as being significant ($F_{ST} = 0.069$–0.536, $P < 0.05$; Table 5), in which between DF and other populations were at high levels ($F_{ST} > 0.25$; Table 5), while other F_{ST} values were at an intermediate level ($0.05 < F_{ST} < 0.25$; Table 5). In contrast, the value between WZ and LS was at a low level and not significant ($F_{ST} = 0.012$, $P > 0.05$; Table 5). The STRUCTURE results showed that $K = 2$ (the maximum value of delta *K*) was the most likely number of *T. peltata* genetic clusters (Fig. 2c). The red genetic cluster was almost entirely composed of samples from the DS and DF populations, whereas samples from the DY, WZ, and LS populations were assigned to the blue genetic cluster (Fig. 2c). Notably, when split into a total of four clusters at higher resolution,

Table 4
Analyses of molecular variance (AMOVA) based on microsatellite markers, mtDNA COI, and ITS of *T. peltata* in the northern SCS.

Gene	Source	d.f.	Sum of squares	Variance components	Percentage of variance (%)	Fixation indices (Φ_{ST})	*P* value
SSR	Among Pops	4	59.480	0.420	16.72%	**0.167***	0
	Within Pops	155	324.08	2.090	83.28%		
ITS	Among Pops	4	31.825	0.414	11.78%	**0.118***	0
	Within Pops	55	170.509	3.100	88.22%		
mtDNA COI	Among Pops	4	3.015	0.007	1.15%	0.011	0.153
	Within Pops	74	47.491	0.642	98.85%		

* Significant Φ_{ST} values ($P < 0.05$) are denoted by asterisk in bold.

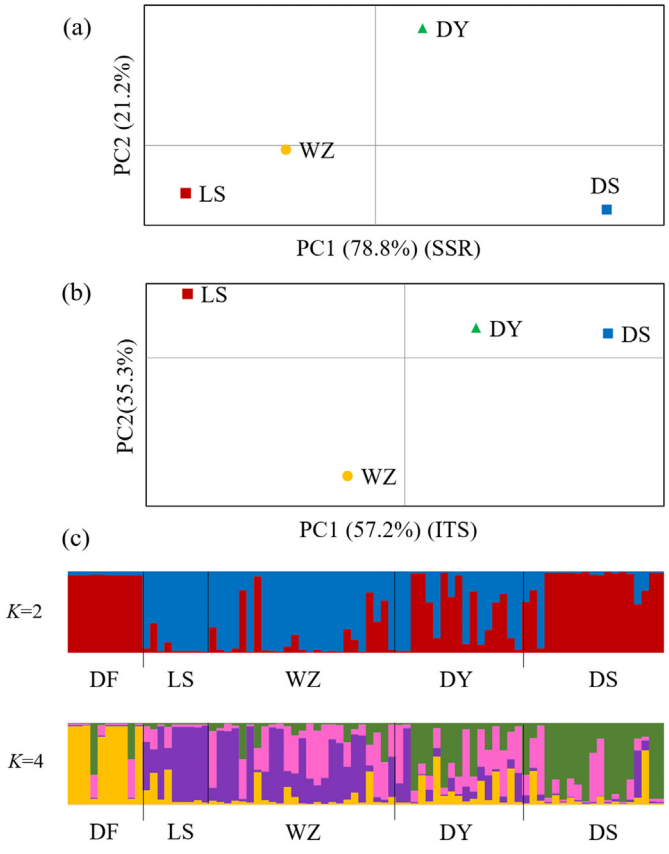

Fig. 2. Population genetic structure of *T. peltata* populations. (a, b) Principle component analysis (PCoA) among 4 *T. peltata* populations without DF population based on microsatellite markers (a) and ITS sequences (b). (c) Genetic structure of five *T. peltata* populations (included at the optimal $K = 2$ and the second-maximum delta $K = 4$).

the green cluster was composed of samples from the DS population, the yellow cluster of samples from the DF population, the purple cluster mainly of samples from the WZ and LS populations, and the pink cluster mainly of samples from the DY and WZ populations (Fig. 2c). The PCoA results showed that the *T. peltata* populations formed three genetic groups: DS and DY were in the same group, WZ and LS were in another group, and DF formed an independent group (Suppl. Fig. 1). When the DF population was excluded, the four remaining populations were distributed mostly along latitude (Fig. 2).

Based on the ITS sequences, the results also showed that there was moderate genetic differentiation among *T. peltata* in the northern SCS (F_{ST} = 0.096–0.228, $P < 0.05$; Table 5). The AMOVA results indicated that the genetic differentiation of *T. peltata* was mainly derived within populations (88.22%; Table 4), and little among populations (11.78%; Table 4). Among the pairwise F_{ST} values, only the values between DS and DY (F_{ST} = 0.042, $P > 0.05$; Table 5) and between WZ and DS ($F_{ST} = -0.028$, $P > 0.05$; Table 5) were at a low level and not significant. Other pairwise F_{ST} values were at a moderate level and were significant ($0.05 < F_{ST} = 0.096–0.228 < 0.25$; Table 5).

Based on the mtDNA COI sequences, the results showed that there was no significant genetic differentiation among *T. peltata* populations in the northern SCS. The AMOVA results indicated that genetic differentiation was mainly derived within populations (98.85%; Table 4), with little derived among populations (1.15%; Table 4), and differentiation were not significant (Φ_{ST} = 0.011, $P > 0.05$; Table 4). After Benjamini–Yekutieli correction, only one F_{ST} value from a total of ten pairs was at a low level, as well as being significant (between DY and WZ, F_{ST} = 0.38, $P < 0.05$; Table 5). Compared to the results of the microsatellite markers and ITS sequences, the resolution of the mtDNA COI sequences as a genetic marker for *T. peltata* was found to be very low. Therefore, they were not suitable for the genetic differentiation analysis of this species.

3.4. Relationships between environmental factors and the genetic differentiation of T. peltata

For microsatellite markers, the results of the Mantel test indicated that there were no significant positive correlations between genetic differentiation and geographic distance (R^2 = 0.02, Mantel test P = 0.60 > 0.05; Suppl. Fig. 2a), average SST (R^2 = 0.01, Mantel test P = 0.53 > 0.05; Suppl. Fig. 2a), and SST variance (R^2 = 0.02, Mantel test P = 0.59 > 0.05; Suppl. Fig. 2a). The relatively complicated environment within DF, where the fishing port is located, may result in genetic differentiation between DF and other populations. As such, we conducted another correlation analysis without the DF population. When the DF population was excluded, the correlation between the genetic differentiations and these three factors increased. In fact, a significantly positive correlation was observed between genetic differentiation and geographic distance (R^2 = 0.57, Mantel test P = 0.04 < 0.05; Fig. 3a) and average SST (R^2 = 0.82, Mantel test P = 0.0001 < 0.05; Fig. 3a). However, no significant or positive correlation was observed between the genetic differentiation and SST variance (R^2 = 0.03, mantel test P = 0.30 > 0.05; Fig. 3a).

For the ITS sequences, when the DF population was included, the Mantel test results indicated that there was a significant positive correlation between genetic differentiation and geographic distance (R^2 = 0.44, Mantel test P = 0.02 < 0.05; Suppl. Fig. 1b). Positive correlations were found between genetic differentiation and average SST (R^2 = 0.14, Mantel test P = 0.13 > 0.05; Suppl. Fig. 2b), and SST variance (R^2 = 0.04, Mantel test P = 0.26 > 0.05; Suppl. Fig. 2b), however, neither was significant. When the DF population was excluded, the correlation between genetic differentiation and geographic distance (R^2 = 0.51, Mantel test P = 0.04 < 0.05; Fig. 3b), average SST (R^2 = 0.45, Mantel test P = 0.08 > 0.05; Fig. 3b), and SST variance (R^2 = 0.36, Mantel test P = 0.07 > 0.05; Fig. 3b) was increased; however, the latter two indices were not significant. For the mtDNA COI sequences, the values of F_{ST} were used to perform a small correlation analysis.

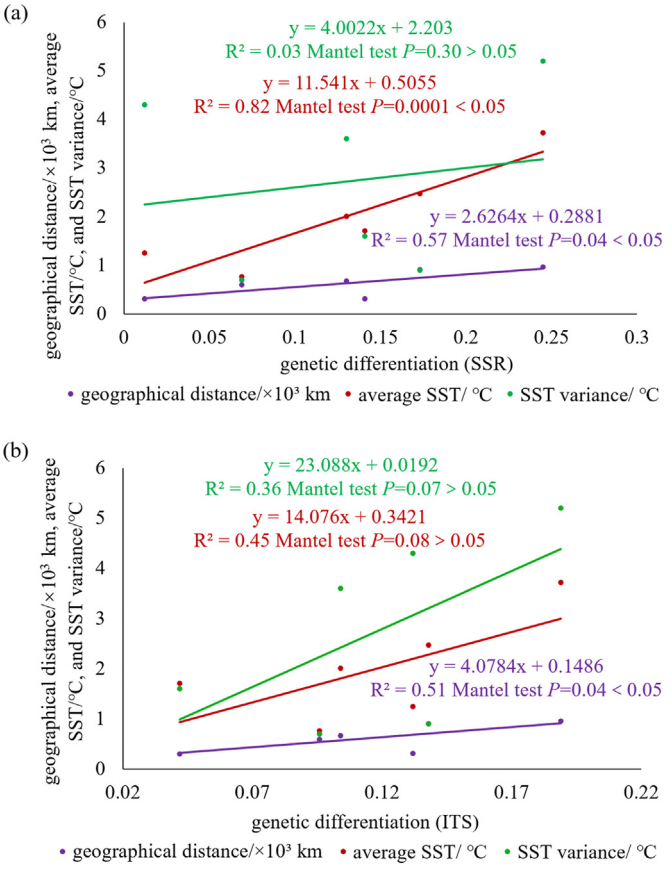

Fig. 3. Relationship between the genetic differentiation and geographic distance (purple), average SST (red), and SST variance (green) of *T. peltata* in the northern SCS without DF population based on microsatellite markers (a) and ITS sequences (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. The possibly poor genetic adaptability of T. peltata in the northern SCS

Based on the microsatellite markers and mtDNA COI sequences, the genetic diversity of *T. peltata* in the northern SCS was low, similar to other relatively high-latitude coral communities. In Eastern Australia, relatively high-latitude *Seriatopora hystrix* populations have significantly lower allelic richness than those in the southern Great Barrier Reef (Noreen et al., 2009). In a previous study, we found that a relatively high-latitude *P. lutea* population (Daya Bay) showed a lower genetic diversity than low-latitude populations in the SCS (Huang et al., 2018). However, the widespread species *S. hystrix* and *P. lutea* both have adequate population sizes and higher genetic diversity levels in the whole population, allowing these species to maintain viability and resist severe losses of genetic diversity (Huang et al., 2018; Noreen et al., 2009). Although *T. peltata* was dominant in the northern SCS, its geographical distribution was restricted, with no distribution in low-latitude areas, such as the Xisha Islands and Nansha Islands (X. Chen et al., 2019). With a low genetic diversity, limited distribution in relative high latitude and a relatively limited population size, *T. peltata* populations in the northern SCS may have difficulties in sustaining their long-term viability.

The survival of organisms depends on their capacity for adaptability and resilience under climate change. Genetic diversity and reproduction are two crucial factors related to adaptability (Bernhardt and Leslie, 2013; Somero, 2005). High genetic diversity could provide an adequate genotype to adapt to increasing environmental stress (Bernhardt and Leslie, 2013). However, sexual reproduction enhances the dispersal ability and increases the possibility of genetic variation (Harrison,

2011; Harrison and Wallace, 1990). The results indicated that sexual reproduction is dominant in most populations ($R = 0.75–1.00$), except for DF ($R = 0.33$). Sexual reproduction may improve the adaptability and resilience of *T. peltata* under climate change, although the genetic diversity in the northern SCS was low.

4.2. DF became a divergent population as a result of severe anthropogenic activity

In this study, DF was genetically distinct from other population, with the lowest genetic diversity and the highest genetic differentiation. Notably, the environment in sampling site DF was heavily modified by the construction of a fishing port and nearby wharf building (2007–2011). During their construction, riprap excavations and a large number of suspended particulate matter (SPM) had a destructive impact on the environment, resulting in a SPM concentration of 4000–6000 mg/L (unpublished data) in the wastewater, greatly exceed the safe range (10–50 mg/L). When the concentration of SPM is too high, corals can become fully covered and die. Even a moderate concentration of SPM can reduce seawater transparency, thereby hindering the photosynthesis of corals (Fabricius and De'Ath, 2004). Both during and after construction, the pollutants produced as a result (e.g. oil, organic matter, and heavy metals) had a large impact on the DF population. During the project, the oil-bearing water generated reached 1.96 t/d, the petroleum substances in the water reached 3.92 kg/d, and the lead content in each layer of seawater exceeded the standard by 100% (Lao et al., 2019). After the completion of the wharf, excessive docking and navigation activities continue to produce a large number of pollutants. This suggests that the DF population may have been influenced by human activities and most likely to experience a strong natural selection (Yang et al., 2015), where only adapted genotypes can survive in harsh environments (Yu, 2012). Indeed, the neutrality test result based on ITS showed a positive selection in DF (Table 3). Another plausible explanation was that with a small effective population size (DF: $N\hat{e} = 1.0$) after high mortality, genetic drift and bottleneck effects may drive DF to be a genetically distinct population. As a result, the genetic diversity of DF was low and gene communication was blocked, gradually forming a geographically and genetically divergent population. Isolated coral reefs depend on local larval sources to remain healthy. If these larval sources are affected, the replenishment levels can fall below the ecological recovery threshold, making these populations unsustainable (Gilmour et al., 2013; Hoegh-Guldberg et al., 2007; Smith et al., 2008). This suggests that corals in DF are susceptible to intense anthropogenic disturbances, resulting in low genetic diversity and significant genetic differentiation. Thus, this population may face the risk of local extinction in the near future if no protection action is taken.

4.3. SST and geographical isolation dominate the genetic differentiation of T. peltata

The results obtained using microsatellite markers and ITS sequences indicated significant moderate genetic differentiation among the *T. peltata* populations. As discussed in the previous section, DF became genetically distinct population due to severe anthropogenic impacts. In order to scientifically explore the specific cause of the genetic differentiation of *T. peltata* in the northern SCS, we excluded the DF population in subsequent analyses. As a result, PCoA showed that the genetic differentiation of DS, DY, WZ, and LS showed a latitudinal distribution trend (Fig. 2). The ANOVA results showed that the annual average SST increased significantly with downward latitude (Fig. 1). Thus, we hypothesized that the latitude-related genetic differentiation of *T. peltata* in the northern SCS may be due to geographic distance or significant SST differences among latitudinal gradient populations.

The Mantel test results showed significant positive correlations between genetic differentiation and geographical distance and the annual average SST (Fig. 3). Geographical distance, ocean currents and sexual reproduction strategy were three crucial factors determining larvae exchange between geographical populations (Van der Ven et al., 2016; Lukoschek et al., 2016). The surface currents in our sampling sites were complicated and lacking strong current. There were constant anticlockwise currents in Beibu Gulf (WZ, DF) and alongshore currents in DY. Currents in DS and LS were change direction with the monsoon (Huang et al., 1992). The distance between sampling sites were 150–1000 km. The sympatric *P. lutea* have low genetic differentiation (Huang et al., 2018). Thus, we speculated that the biological characteristic of *T. peltata* may dominate its genetic structure, such as sexual reproduction strategy and tolerance ability to environment stress. Unfortunately, sexual reproduction strategy of *T. peltata* was unknown, whether broadcast spawning or brooding.

Furthermore, the Mantel test results suggested that the average SST had a higher correlation index than geographical isolation on genetic differentiation. SST has important effects on the population structure and connectivity of marine organisms (Li et al., 2009; Parmesan, 2006), especially in stenothermic corals, which are extremely sensitive to fluctuations in temperature (Carricart-Ganivet, 2004). In the northern SCS, average SST was significantly lower along with the increasing latitude (Fig. 1). Low temperature in winter limited the distribution of scleractinian corals towards north and DS was the north most of scleractinian coral distribution along China mainland (Li et al., 2010). Hence, temperature may influence the genetic structure of *T. peltata* by two approaches. First, low temperature may be one of the natural selection factors to force genetic divergence. Temperature-related adaptation has been reported in other corals, such as *Pocillopora damicornis* (Ulstrup et al., 2006) and *Porites lobata* (Barshis et al., 2010). Similarly, in the eastern tropical Pacific, the genetic structure of the coral endosymbiont *Durusdinium glynnii* was affected by fluctuating environmental conditions (Pettay and LaJeunesse, 2013). Second, we observed that the spawning time of corals in DY lagged about one month behind WZ (unpublished data). The asynchronous spawning time is most likely caused by different SST in spring. This may block the genetic communication through larva and further enhance the differentiation across relatively high-latitude areas. However, empirical data were needed to validate those hypotheses, such as genetic selection evidence or divergent physiological responses of different latitude population to low temperature.

High genetic differentiation hinders the self-recovery and self-sustainability of coral populations in the Southern Hemisphere. Thomas et al. (2017) identified strong patterns of differentiation and reduced genetic diversity in relatively high-latitude populations using a genotyping-by-sequencing approach. Those studies found that restricted gene flow and local adaptation highlight the vulnerability of relatively high-latitude coral reefs to rapid environmental change (Thomas et al., 2017). Furthermore, higher genetic differentiation is not conducive to the recovery of coral communities after disturbances (Whitaker, 2006). This suggests that the high genetic differentiation among *T. peltata* populations in the northern SCS increases their vulnerability to environmental changes.

In summary, geographical isolation and factors caused by SST at different latitudes dominated the moderate genetic differentiation of *T. peltata* populations in the northern SCS, except for DF, which suffered severe anthropogenic activity. The low genetic diversity and moderate genetic differentiation indicated that *T. peltata* populations in the northern SCS may be sensitive to rapid changing climate.

4.4. Management implications under climate change

Compared the genetic diversity and structure of *T. peltata* to *P. lutea* in the SCS, we found that their adaptability to climate change varied among different coral species. The broadcasting species *P. lutea* has a high diversity, a low differentiation, and an excellent dispersal potential (Huang et al., 2018). These genetic characteristics indicate potential for adaptability to climate change, and this species could migrate

northward (Huang et al., 2018). However, *T. peltata* is unlikely to migrate northward, due to its limited dispersal and high differentiation under climate change. As one of the dominant coral species at a relatively high-latitude, *T. peltata* is also threatened by competition from northward migrating corals from low latitudes. With low genetic diversity and significant moderate genetic differentiation, populations of *T. peltata* at relatively high-latitude are more sensitive to environmental changes. Thus, the status of relatively high-latitude coral populations, such as *T. peltata*, is worrying. The genetically enhanced adaptability and resilience of relatively high-latitude coral communities requires immediate attention, in combination with the development of anthropogenic management approaches for their conservation and protection (Fuller et al., 2020).

Relatively high-latitude reef areas in China have a high population density, high levels of human activity, coastal engineering, and environmental pollution, in addition to other serious problems (Qin et al., 2019). Previous studies have shown that living coral cover in the northern SCS has been on the decline, with coral ecosystems being heavily destroyed as a result of increasing nutrient runoff, pollution, aquaculture, and sedimentation due to human activities (Chen et al., 2009; Zhang et al., 2014). Faced with multiple environmental stresses, genetic diversity of corals may gradually decrease, eventually reducing their resilience and adaptability. Although global warming will theoretically benefit relatively high-latitude coral communities, the Weizhou Island reef area in relatively high-latitude will remain unable to become the coral refuge in the SCS due to the influence of human activities (Yu et al., 2019). Ecological surveys and reports on the impacts of human activity on coral reefs in the SCS indicate that the function of coral refuges in the northern SCS has been restricted (Yu et al., 2019). To counteract this, firstly, effective ecological protection could be initiated by the government to manage and reduce the impact of human activities, such as reclamation and pollution discharge. Secondly, since only two coral species, namely *P. lutea* and *T. peltata*, have been studied across the SCS, more large-scale genetic studies are urgently needed to gain a full understanding of the coral reefs in the SCS. Thirdly, protection measures should be developed to protect coral larval corridors to enhance genetic exchange and maintain genetic diversity. Lastly, a genetic approach could be used to enhance the genetic adaptability of some key species in the future.

5. Conclusion

The results of this study demonstrate that *T. peltata* populations in the northern SCS are characterized by reduced genetic diversity and moderate genetic differentiation. High pairwise F_{ST} values and independent genetic clusters indicate that DF population has been genetically distinct from others as a result of high levels of human activity (wharf construction). Challenged by rapid climate change, relatively high-latitude coral communities with low genetic diversity have a poor adaptability and face a high risk of extinction. Consequently, it has become necessary to take protection measures, such as protecting larvae corridors and reducing human activities, according to the genetic characteristics of relatively high-latitude coral communities to help them cope with changes on a global level.

CRediT authorship contribution statement

Qian Wu: Conceptualization, Methodology, Data curation, Visualization, Validation, Writing - original draft. **Wen Huang**: Conceptualization, Resources, Methodology, Validation, Writing - review & editing, Project administration, Funding acquisition. **Biao Chen**: Methodology, Validation, Writing- review & editing. **Enguang Yang**: Software, Formal analysis, Visualization. **Linqing Meng**: Software, Formal analysis. **Yinmin Chen**: Software, Writing-review & editing. **Jingjing Li**: Investigation, Writing - review & editing. **Xueyong Huang**: Resources, Investigation, Writing - review & editing. **Jiayuan Liang**: Resources, Writing - review & editing. **Tzuen-Kiat Yap**: Writing - review & editing. **Kefu Yu**: Conceptualization, Resources, Project administration, Funding acquisition.

Declaration of competing interest

We declare we have no competing interests.

Acknowledgement

We thank Xin Wang (Guangxi Mangrove Research Center) for collecting specimens in Dongfang, and we thank Yanqiu Luo (Guangxi University, China) for constructive suggestions. We would like to thank Editage (www.editage.cn) for English language editing.

Funding

This study was supported by the National Natural Science Foundation of China (41866006, 42090041 and 42030502), the Guangxi Natural Science Foundation (2017GXNSFAA198196 and 2016GXNSFBA380028), and the Guangxi Scientific Projects (No. AA17204074, AD17129063 and AB17129022).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145775.

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