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Dispersal, genetic variation, and symbiont interaction network of heat-tolerant endosymbiont *Durusdinium trenchii*: Insights into the adaptive potential of coral to climate change

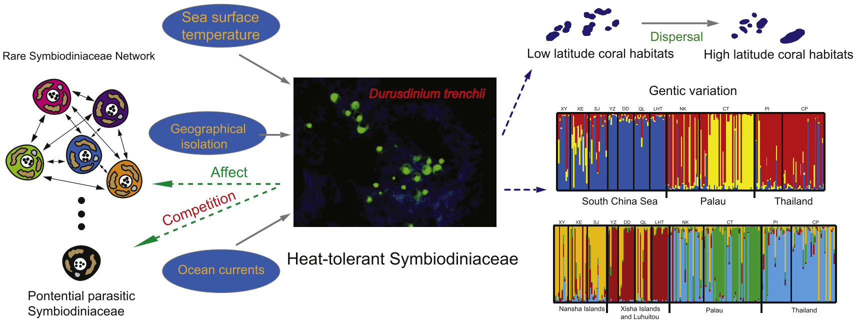
Biao Chen[a,b,c], Kefu Yu[a,b,c,*], Zhenjun Qin[a,b,c], Jiayuan Liang[a,b,c], Guanghua Wang[a,b,c], Xueyong Huang[a,b,c], Qian Wu[a,b,c], Leilei Jiang[a,b,c]

[a] *Guangxi Laboratory on the Study of Coral Reefs in the South China Sea, Guangxi University, Nanning, China*
[b] *Coral Reef Research Center of China, Guangxi University, Nanning, China*
[c] *School of Marine Sciences, Guangxi University, Nanning, China*

HIGHLIGHTS

- *Durusdinium trenchii* are widely distributed in the seawater from the South China Sea (SCS).
- *D. trenchii* has high genetic diversity and clone richness in the SCS.
- Geographic isolation and sea surface temperature has shaped the genetic variation of *D.trenchii* around the SCS.
- *D.trenchii* can affect the rare Symbiodiniaceae community.
- Heat-tolerance and high genetic diversity of *D.trenchii* enables it to help corals adapt to climate change.

GRAPHICAL ABSTRACT



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ABSTRACT

Global warming has degraded coral reef ecosystems worldwide. Some corals develop thermal tolerance by associating with heat-tolerant Symbiodiniaceae. Here, we studied the mechanisms surrounding the dispersal, genetic variation and symbionts interaction of heat-tolerant *Durusdinium trenchii* across 13° latitudes in the South China Sea (SCS), to explore the possible mechanisms underlying these changes. Our results showed that *Durusdinium trenchii* are widely distributed in the seawater from the SCS. Our analyses of microsatellite loci revealed that *D. trenchii* has a high genetic diversity in the SCS; STRUCTURE analysis indicated that *D. trenchii* can be divided into four populations within the SCS; There exist positive correlations between genetic variation and geographic isolation, average sea surface temperature (SST) and variations in SST. Network modelling inferences showed that *D. trenchii* is a key species in the Symbiodiniaceae communities in the tropical SCS and contributes the greatest number of co-exclusion relationships. These results indicated that *D. trenchii* can affect the rare Symbiodiniaceae community. The long lifespan and the monsoon-driven ocean currents have shaped the wide distribution of *D. trenchii*. But low SST limits the ability of *D. trenchii* to establish stable symbioses with coral in the subtropical habitats. Geographical isolation and SST have shaped significant genetic variation of *D.trenchii* around the SCS. Our data reveals the biogeography and genetic population characteristics of *D. trenchii* in the

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* Corresponding author at: Guangxi Laboratory on the Study of Coral Reefs in the South China Sea, Guangxi University, Nanning, China.
E-mail address: kefuyu@scsio.ac.cn (K. Yu).

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Indo-Pacific region, and suggests that heat-tolerance and high genetic diversity of *D. trenchii* aid the corals with their adaptation to climate change.

1. Introduction

Coral reef ecosystems harbour rich biodiversity and are known as the "tropical rainforest" of the ocean (Blackall et al., 2015; Reaka-Kudla, 1997). However, the impacts of global climate change and ocean warming have resulted in significant biodiversity losses in coral reef ecosystems (Brener-Raffalli et al., 2018; Carpenter et al., 2008; Hoeghguldberg et al., 2007; Hughes et al., 2017a; Hughes et al., 2017b). The third global massive bleaching event of 2016–2017 had a disastrous impact on coral reef ecosystems worldwide (Hughes et al., 2018a; Hughes et al., 2017a; Hughes et al., 2018b; Perry and Morgan, 2017; Stuart-Smith et al., 2018). Although the decline in coral cover is clearly related to sea surface temperature (SST) anomalies in the past two decades, coral loss shows significant spatial heterogeneity (Hughes et al., 2018b). Not all coral reefs exposed to extremely high SSTs have suffered a loss of live coral cover, such as the coral habitats in the northern portion of the Great Barrier Reef (Stuart-Smith et al., 2018). The spatial heterogeneity of the decline in live coral cover has been partly explained by local climatology (e.g., cyclones; Stuart-Smith et al., 2018) and spatial variations in thermal anomalies (e.g., the effect of upwelling; Maria et al., 2014), but the contribution of tolerant Symbiodiniaceae to the local adaptation of coral cannot be neglected. Symbiodiniaceae have been suggested to play an important role in the spatial heterogeneity of the decline of live coral cover and differences in bleaching resilience of coral hosts, as corals may survive bleaching events by associating with heat-tolerant symbionts (Baker et al., 2004; Stat et al., 2006; Hume et al., 2016; Stuart-Smith et al., 2018).

Symbiodiniaceae interact symbiotically with coral hosts by providing up to 95% of coral respiratory requirements and are thus critical for the maintenance and photosynthetic production of these ecosystems (Falkowski et al., 1984). Many studies have identified different physiological characteristics of various Symbiodiniaceae genotypes (LaJeunesse et al., 2018; Reimer et al., 2017; Rowan, 2004) and have suggested that stress-tolerant genotypes are involved in symbioses and adaption to climate change by associating with regional hosts (LaJeunesse et al., 2018; LaJeunesse et al., 2010). Ecological and biogeographic studies have tested the diversity and community composition of Symbiodiniaceae at the genus and sub-clade or type levels, which may be responsible for the tolerance of different Symbiodiniaceae-host combinations to climate change (Arif et al., 2014; Finney et al., 2010; van Oppen et al., 2005; Rowan and Powers, 1991; Sampayo et al., 2010; Tong et al., 2017; Ziegler et al., 2017). However, relatively few studies have focused on the diversity and distribution of Symbiodiniaceae at finer scales, such as at the population or individual clone levels (Thornhill et al., 2017).

Regional environmental factors (i.e., biogeographic barrier, sea surface current, sea surface temperature, and light intensity condition) can promote Symbiodiniaceae to produce individual local adaptive genotypes (Andras et al., 2011; Pettay and Lajeunesse, 2013; Howells et al., 2009), which are associated with the stress tolerance and bleaching sensitivity of corals (Pettay and Lajeunesse, 2013; Hume et al., 2016). Additionally, previous studies on open oceanic water have shown that free-living *Symbiodinium* (formerly clade A) and *Cladocopium* (formerly clade C) dominated in Symbiodiniaceae communities, which may provide symbiont reservoirs to repopulate coral reefs following stress-induced bleaching (Decelle et al., 2018). However, the conditions of distribution and dispersal of mutualistic heat-tolerant Symbiodiniaceae living in coral habitats and surrounding seawater across large-scale latitudes remain unclear. Mutualistic heat-tolerant Symbiodiniaceae has the potential to undergo sexual recombination in seawater (Thornhill et al., 2017) and establish direct symbiosis with coral larvae (Baird et al., 2009; Howells et al., 2009), indicating a more obvious effect on the resilience of coral-Symbiodiniaceae under stress conditions compared to free-living Symbiodiniaceae. In addition, the Symbiodiniaceae community is generally composed of one or two dominant symbiotic sub-clades (Tong et al., 2017; Ziegler et al., 2017) and contains a large number of rare Symbiodiniaceae members, which play important roles in the stability and environmental resilience of coral holobionts (Boulotte et al., 2016; Ziegler et al., 2018). However, non-mutualistic and opportunistic symbionts exist in the background Symbiodiniaceae community (Lee et al., 2016), which may increase carbon and nitrogen assimilation for themselves and reduce the proportion of photosynthates gained by the host (Baker et al., 2018). There are many potential co-occurrence and co-exclusion relationships among Symbiodiniaceae sub-clades, which construct the symbiont interaction network within the coral (Ziegler et al., 2018; Fabina et al., 2013). When Symbiodiniaceae community switching or shuffling events are induced by environmental stress, the interaction may be more obvious among the members of the background Symbiodiniaceae community (Boulotte et al., 2016). Therefore, studies of whether heat-tolerant dominant Symbiodiniaceae can affect rare symbionts community or inhibit some opportunistic members are urgently needed.

Durusdinium trenchii (formerly *Symbiodinium trenchii*) is a heat-tolerant Symbiodiniaceae species that has been widely examined (LaJeunesse et al., 2010; Lee et al., 2016). The abundance of *D. trenchii* increases following heat-based physiological trauma to the host; thus, assists the host in its adaptation to climate change (LaJeunesse et al., 2016; Pettay et al., 2015). Unexpectedly, The large-scale spread of *D. trenchii* has given rise to genetically similar clones across the greater Caribbean, which appears to have resulted in lower calcification by corals harbouring *D. trenchii* than those harbouring local symbionts, suggesting that coral-*D. trenchii* symbioses may ultimately endanger the stability and function of coral reef ecosystems (LaJeunesse et al., 2016; Pettay et al., 2015). However, heat-tolerant *D. trenchii* originated from the Indo-Pacific region and shows high genetic diversity and the potential for sexual reproduction in Thailand and Palau (Pettay et al., 2015; Thornhill et al., 2017), which may avoid the negative ecological impact caused by a single genotype. Few studies have examined the dispersal, genetic variation, symbionts interaction network, and environmental response rule of important heat-tolerant *D. trenchii* in different latitudinal coral habitats across the Indo-Pacific region. This information is critical for assessing the adaptability and resilience of coral reefs (Baums et al., 2014; Hedgecock et al., 2007; Wirshing et al., 2013).

The South China Sea (SCS) is located on the northern edge of the coral triangle (Spalding, 2001). Coral reefs are widely distributed from the Zengmu Reef (~4°N), near the equator, to the Leizhou Peninsula and Weizhou Island (~20–21°N) in the northern portion of the SCS (Yu, 2012; Wang and Li, 2009). Many coral communities are distributed along the northern edge of the SCS in sites such as Hongkong (~21–22°N), Dongshan Island (~23°N), and Fangchenggang (~21–22°N), and their distribution is controlled by seasonal low temperatures (Chen et al., 2007; Dong et al., 2008; Li et al., 2008; Ng and Ang, 2016). However, large-scale coral bleaching caused by global climate change has clearly affected tropical coral reef ecosystems in areas at intermediate and low latitudes within the SCS (Yu, 2012; Yu, 2012; Yu et al., 2006). *Galaxea fascicularis* survived a mass bleaching event and achieved ecological success in intermediate- and low-latitude coral habitats within the SCS under conditions of global climate changes (Wu et al., 2011; Zhou et al.,

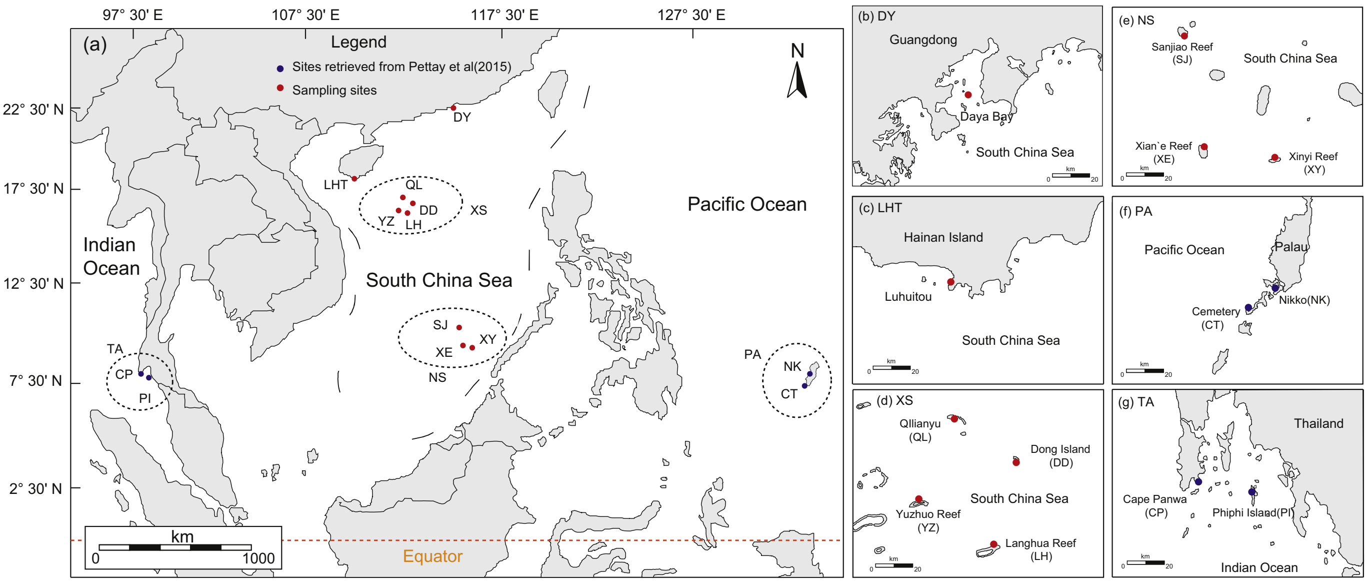

Fig. 1. Locations of samples collections around the SCS. (a) Overview of study locations (b) Daya Bay (DY) locate in the north of the South China Sea; (c) The southern Hainan Island, Luhuitou (LHT); (d) Xisha Islands including Qilianyu (QL), Yuzhuo reef (YZ), Dong Island (DD) and Langhua reef (LH) in the SCS; (e) Nansha Island including Sanjiao reef (SJ), Xinyi reef (XY) and Xian'e reef (XE) in the SCS; (f) The western Pacific Ocean, Palau. *D.trenchii* microsatellite loci date was retrieved in coral samples from Cemetery (CT) and Nikko (NK); (g) The north-eastern Indian Ocean, Andanma Sea, Thailand. *D.trenchii* microsatellite loci data was retrieved in coral from Cape Panwa (CP) and Phiphi Island (PI) (Pettay et al., 2015).

2017), likely because of its symbiotic relationship with *D. trenchii* (Zhou et al., 2017). Nevertheless, the tropical and subtropical coral habitats found within the SCS (Fig. 1; Spalding, 2001; Yu, 2012) span 19° latitude, indicating that these habitats are found within a gradient of SST within the SCS (Fig. 2) (Chen et al., 2019; Qin et al., 2019). The SST differences among coral habitats have shaped the symbiotic relationships between corals and Symbiodiniaceae subclades (Chen et al., 2019), resulting in different sensitivities to coral thermal bleaching (Qin et al., 2019). It has been shown that coral genotypes and genetic connectivity are partly determined by the SST (Huang et al., 2018). Therefore, Symbiodiniaceae genotypes may be affected and controlled by SST in the SCS. Overall, the specific environmental conditions and widely distributed *G. fascicularis* in the SCS aid in evaluating the dispersal, genetic variation, symbiont interaction network, and environmental response rule of *D. trenchii* in the Indo-Pacific region.

In this study, samples of *G. fascicularis* were collected from populations in several geographical provinces and regions spanning >1500 km in the SCS (Fig. 1). Next-generation sequencing (NGS) was performed to identify *D. trenchii* with high sensitivity to better understand the Symbiodiniaceae community composition. Eleven microsatellite loci (see Additional File 1: Table S1) were used to analyse the genetic diversity, genetic structure, and gene flow of the population. Moreover, network modelling inference was used to analyse Symbiodiniaceae interactions. We explored three fundamental hypotheses: (1) The genetic structure of heat-tolerant *D. trenchii* around the SCS has been affected by SST and geographic isolation; (2) Lifespan and ocean currents have shaped the wide distribution of *D. trenchii*; (3) Mutualistic *D. trenchii* has played an important role in the symbiont interaction network. To test these hypotheses, the dispersal, genetic variation, symbiont interaction network, and environmental response rule of *D. trenchii* in the SCS were examined. The analyses conducted here will increase our

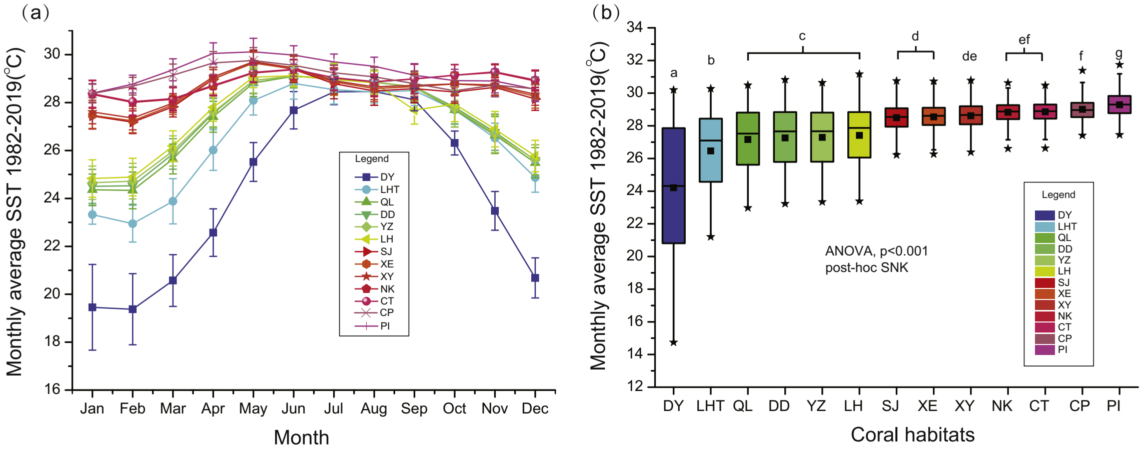

Fig. 2. Monthly averages of sea surface temperature (SST) from 1982 to 2019 for 13 sampling sites around the South China Sea (SCS). (a) Monthly averages and standard deviations of SST at all sampling sites around the SCS. (b) Box plots showing annual mean, maximum and minimum seasonal ranges by coral habitats. The bar within each box represents the median; the two bars above and below each box represent the upper and lower 25% quartiles, respectively; the whiskers represent the 1% and 99% values; the squares represent the mean value; the stars represent the minimum and maximum values. Lowercase letters indicate post hoc comparisons (e.g., a, b, c, d, de, ef, f and g) with the significant factors among sampling sites.

understanding of the biogeography and population genetics of heat-tolerant *D. trenchii* in the Indo-Pacific region and assess the adaptive potential of coral in the SCS to cope with climate change.

2. Materials and methods

2.1. Sample collection and environmental condition

A total of 108 fragments (~2–3 cm^2; see samples information in Additional File 2: Table S2) of *G. fascicularis* were collected by scuba diving from nine coral habitats in the SCS (Table 1). At each coral habitat, we collected morphologically distinct colonies of *G. fascicularis* along linear transects of at least 6 m apart at depths ranging from 2 to 15 m at each one of the four sites, which were separated by as much as 30 km. The samples were taken to the boat and cleaned with artificial sterile seawater (salinity: 35‰) to ensure that it was not contaminated with free-living Symbiodiniaceae in the seawater. All fragments were transferred into cryotubes (Jet Biofil, Guangzhou, China), 20% dimethyl sulphoxide (DMSO) buffer or 95% ethanol was added (Gaither et al., 2011), and samples were stored at −20 °C until DNA extraction. Because of logistical reasons, we were unable to collect coral samples at the same time, but we ensured that the samples were collected during the warm months (May to September 2017 and May to June 2018; Table 1). In addition, most of the Symbiodiniaceae communities are considered stable (Ziegler et al., 2017). Samples of seawater (5 L, $n = 3$ samples/site; see Additional File 2: Table S2) were collected during the voyage and filtered using 0.22-μm polycarbonate membranes (RephiLe, Shanghai, China). All filtered seawater samples were transferred to cryotubes, 95% ethanol or RNAlater® was added, and samples were stored at −20 °C until DNA extraction.

SST, upper-ocean surface current, and geographical distance among coral habitats were collected and statistically analysed as potential factors that affect the biogeographical distribution and genetic characteristics of coral-Symbiodiniaceae symbioses. Monthly average SST data of the Nansha Islands (NS, including Sanjiao Reef (SJ), Xian'e Reef (XE), and Xinyi Reef (XY)), Xisha Islands (XS, including Qilianyu (QL), Dong Island (DD), Yuzhuo Reef (YZ), and Langhua Reef (LH)), Luhuitou (LHT), and Daya Bay (DY) from January 1982 to July 2019 were acquired from KNMI Climate Explorer (http://climexp.knmi.nl/start.cgi) (Fig. 2a). In addition, the SST data of Thailand (TA, including Cape Panwa (CP) and Phi Phi Island (PI)) and Palau (PA, including Nikko (NK) and Cemetery (CT)), where ecological and genetic research of *D. trenchii* have been performed, were obtained (Fig. 2; LaJeunesse et al., 2010; Pettay et al., 2015). A one-way factorial analysis of variance (ANOVA) was used to test the monthly average SST (1982–2019) among different coral habitats (Fig. 2a and Additional File 3: Table S3). The Student-Newman-Keuls (SNK) test was used for post-hoc multiple comparisons of significant ANOVA results (Fig. 2b). The data on the upper-ocean surface current in the SCS were collected from the National Marine Science Data Center, National Science & Technology Resource Sharing Service Platform of China (http://mds.nmdis.org.cn/). The ocean current chart was plotted by ArcGIS 10.2. In addition, geographical distance among coral habitats was determined by ArcGIS 10.2.

2.2. DNA extraction and NGS of the internal transcribed spacer region

The Symbiodiniaceae sub-clades were identified by NGS of the internal transcribed spacer region 2 (ITS2). Nucleic acids were extracted using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). The obtained DNA was used as a template for PCR after filtering for quality and purity. ITSintfor2 (5′-GATTGCAGA ACTCCGTG-3′) (LaJeunesse and Trench, 2000) and ITS2-reverse (5′-GGGATCCATA TGCTTAAGTT CAGC GGGT-3′) (Coleman et al., 2010) were used as primers to conduct PCR amplification of the ITS2 region of the Symbiodiniaceae rDNA. The PCR was performed with 6 μL of a Tiangen Taq MasterMix II (Tiangen, Beijing, China), 2 μm primer, ~80 ng of DNA, and Tiangen-free water to a total volume of 20 μL. PCR amplification was carried out on an ABI GeneAmp® 9700 thermocycle controller with the following program: 3 min at 95 °C, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were purified using Qiagen Agarose Gel DNA Purification Kit (Qiagen). The purified PCR products were mixed in equal amounts followed by sequencing on an Illumina Miseq platform using the 2 × 300 bp mode at Majorbio (Shanghai, China). The raw IT2 sequencing dataset has been deposited in the NCBI Sequence Read Archive under accession number PRJNA542012.

2.3. Bioinformatics processing and network modelling inference

Quality control and sequence splicing of Illumina MiSeq Platform output data were conducted according to a previously described method (Arif et al., 2014; Chen et al., 2019; Ziegler et al., 2017). Briefly, Trimmomatic software was used to filter bases with a read tail mass value of <20 to ensure high quality reads for subsequent analysis. MOTHUR was applied to merge paired-end sequences and obtain full-length ITS2 rDNA sequences. CUTADAPT was used to trim the reverse and forward primers sequences. Sequences reads were quality trimmed and checked for chimeras using MOTHUR. The quality-filtered reads were aligned to the ITS2 database by BLASTN and the parameters were set following the pipeline detailed in Chen et al. (2019). The resulting counts of Symbiodiniaceae ITS2 sub-clades were merged for downstream statistical analysis. All *G. fascicularis* samples used in this research for multilocus genotyping were verified by this protocol to contain *D. trenchii* (the data of Symbiodiniaceae community composition are in Additional File 4: Table S4).

Symbiodiniaceae sub-clade interaction network analysis was applied using the co-occurrence network inference (CoNet) plugin for Cytoscape 3.7.2 (Faust et al., 2012) and networks were built for tropical and subtropical climate zones. Symbiodiniaceae ITS2 sub-clades as nodes were used to construct the symbiont interaction network because operational taxonomic units clustered by ITS2 read sequences at a 97% similarity cutoff will reduce the resolution of rare Symbiodiniaceae sub-clades (Arif et al., 2014; Ziegler et al., 2018).

Table 1
The samples of *G. fascicularis* from the SCS, including sampling regions, climate, the number of samples and sampling date information.

Regions	Climate	Sampling coral habitats	Coordinates	Monthly average SST (°C)	The number of samples	Sampling dates (yyyy.mm.dd)
Nansha Islands (NS)	Tropical	Xinyi Reef (XY)	N9°20′-9°21′,E115°54′-115°58′	25.6–31.8	12	2018.5.20–2018.5.25
	Tropical	Xian'e Reef (XE)	N9°20′-9°24′,E115°25′-115°26′		15	2018.5.27–2018.5.31
	Tropical	Sanjiao Reef (SJ)	N10°10′-10°13′,E115°16′-115°19′		16	2018.5.11–2018.5.18
Xisha Islands (XS)	Tropical	Langhua Reef (LH)	N16°0′-16°5′, E112°26′-112°35′	23.1–31.6	4	2017.5.31–2017.6.1
	Tropical	Yuzhuo Reef (YZ)	N16°18′-16°21′,E111°57′-112°5′		9	2017.6.10–2017.6.12
	Tropical	Dong Island (DD)	N16°39′ -16°40′,E112°43′-112°44′		14	2017.6.16–2017.6.18
	Tropical	Qilianyu (QL)	N16°54′ -16°58′,E112°11′-112°20′		15	2017.6.29–2017.6.30
Hainan Island	Tropical	Luhuitou (LHT)	N18°12′ -18°13′,E109°28′-109°29′	21.0–31.9	15	2017.8.20–2017.8.25
North of the South China Sea	Subtropical	Daya Bay (DY)	N22°34′ -22°39′,E114°33′-114°39′	19.5–30.2	8	2018.5.25–2018.5.27

These sub-clades with occurrence in at least two samples and at least two reads were selected.

Briefly, the construction of a network is divided into four steps: basic configuration, permutation, bootstrapping, and restoration of the network from random files. Two measures of correlation (Pearson and Spearman) and two measures of dissimilarity (Bray-Curtis and Kullback-Leibler) were used to estimate pairwise associations among Symbiodiniaceae sub-clades. Initial thresholds for all four measures were selected to retrieve 1000 positive and 1000 negative edges. For each measure and edge, 1000 normalised permutations and 1000 bootstrap scores were generated to mitigate the combinatorial bias. The measure-specific *p*-values were calculated and merged using Brown's method (Brown, 1975). After multiple tests using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995), edges with merged *p*-values below 0.05 were retained. Finally, the interaction networks were visualised with Cytoscape 3.7.2.

2.4. Microsatellite analysis

Eleven polymorphic microsatellite loci developed for *D.* (formerly clade D) were utilised (D1Sym9, D1Sym11, D1Sym14, D1Sym17, D1Sym34, D1Sym66, D1Sym92, D1Sym82, D1Sym87, D1Sym88, and D1Sym93; Pettay and Lajeunesse, 2010; Pettay et al., 2015; Wham et al., 2011). Each locus was amplified in 20 μL reaction volumes containing 2 μL primer, ~50 ng of DNA, 6 μL of Tiangen Taq MasterMix II, and Tiangen-free water. The reactions were performed under the following conditions: 94 °C for 2 min, followed by 32 cycles of 94 °C for 15 s, 55 °C (D1Sym 11, D1Sym14, D1Sym34, and D1Sym88), 56 °C (D1Sym 82), 57 °C (D1Sym 9, D1Sym17, D1Sym66, and D1Sym87) or 58 °C (D1Sym 92 and D1Sym93) for 15 s, and a final extension at 72 °C for 5 min (see Additional File 1: Table S1; LaJeunesse et al., 2014; Pettay and Lajeunesse, 2010; Wham et al., 2011). Products were analysed on an ABI 3730XL Genetic Analyzer at the Sangon Biotech company (Shanghai, China). Although many of these loci were used formerly for *D. boreum*, *D. eurythalpos*, and *D. glynni*, *D. trenchii* has experienced a whole or partial genome duplication making these loci diallelic (Pettay et al., 2015). When a single peak appears in microsatellite allele calling, we treated these loci as diploid, because *D. trenchii* exists in the haploid stage (microsatellite allele calls are in Additional File 5: Table S5; Pettay et al., 2015). Multilocus genotypes (MLGs) were constructed according to previously described methods (Pettay and Lajeunesse, 2013; Pettay et al., 2011; Pettay et al., 2015). To acquire a more comprehensive understanding of the *D. trenchii* genetic characteristics in the Indo-Pacific region, the MLG dataset of *D. trenchii* from Thailand (CP and PI) and Palau (NK and CT) was collected from the Dryad database (https://www.datadryad.org; Pettay et al., 2015).

Because populations of *D. trenchii* commonly reproduce asexually inside coral and tend to have a proportion of individuals with identical MLGs (Pettay et al., 2015), statistics based on allele frequencies can produce negative errors (Pettay and Lajeunesse, 2013; Pettay et al., 2015). Therefore, Genclone (Version 2.0) was used to test clonal richness (R) (Arnaud-Haond and Belkhir, 2010) and duplicate MLGs were removed, which caused all subsequent analysis and statistics to be based on a unique MLG in one sample. Because the number of samples for Langhua Reef (LH; Table 1) was insufficient for population genetic analysis and a *D. trenchii* population or individual was not found in Daya Bay (DY), they were removed from the sample library. The descriptive statistics included private alleles, information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), and the probability of identity (PI), which were conducted by GenAlex (Peakall and Smouse, 2010). STRUCTURE (Version 2.3.4) was used to analyse the genetic structure and Markov chain runs consisted of an initial “burn-in” of 1×10^5 steps followed by a final 1×10^6 iteration (Evanno et al., 2010). Five independent runs were performed for 1–11 Ks. STRUCTURE HARVESTER (Version 0.6.94) was used to determine the appropriate clustering of individuals (Earl and VonHoldt, 2012) (the results of Ln Pr(X\K) and deltaK are in Additional File 6: Fig.S1) and CLUMPP (Version 1.1.2; Jakobsson and Rosenberg, 2007) was used to fuse the results of the five independent runs for the chosen K. Lastly, graphic displays of structure plots were manipulated using DISTRUCT (Rosenberg, 2010).

Analyses of molecular variance (AMOVA) were conducted on geographic populations using GenAlex v.6.5 (see Additional File 7: Table S6 and Table 3; Peakall and Smouse, 2010). Correlations between geographical (i.e. geographical isolation) and genetic variance and between environmental (i.e. average SST and SST variance) and genetic variance were tested using Mantel tests (Mantel, 1967) performed with GenAlex v.6.5 (Peakall and Smouse, 2010). The microsatellite genotype data have been deposited in Additional File 5: Table S5.

3. Results

3.1. Biogeographic distribution of D. trenchii

Sequence-based analysis showed that *G. fascicularis* extensively participates in symbiosis with *D. trenchii* in 13 latitudes of the SCS, except for Daya Bay (Fig. 3). *Durusdinium trenchii* dominated the Symbiodiniaceae community of *G. fascicularis* in the tropical regions (Luhuitou, Xisha Islands, and Nansha Islands) in the SCS. In addition, the relative abundance of *D. trenchii* in each *G. fascicularis* colony was between 0.2% and 99.1% and the average relative abundance was 65.6 ± 37.5% (Luhuitou: 92.4 ± 11.2%, ranged from 53.7–98.4%; Xisha Islands: 70.3 ± 37.8%, varied from 0.2–99.1%; Nansha Islands: 51.5 ± 37.4%, ranged from 0.4–95.1%, Additional File 4: Table S4).

Moreover, *D. trenchii* was found to be widely distributed in the waters from Nansha Islands to the Luhuitou (relative abundance varied from 2.2% to 31.2%, Fig. 3f). Interestingly, the relative abundance of *D. trenchii* decreased gradually from the Nansha Islands to the Xisha Islands. The relative abundance of *D. trenchii* at the W5 seawater sampling site closest to the Nansha Islands was 31.2%, whereas only 2.2% of the relative abundance of *D. trenchii* was found at the W3 that is closest to the Xisha Islands (Fig. 3f). The seawater Symbiodiniaceae community accounted for 29.4% of the relative abundance of *D. trenchii* at the W2 sampling site, which is located between the Xisha Islands and Luhuitou (Fig. 3f). Therefore, the relative abundance of *D. trenchii* in water Symbiodiniaceae communities showed a decreasing-trend from the Nansha Islands and Luhuitou to Xisha Islands. Unexpectedly, *D. trenchii* was found in the reef seawater sample (W1) from Daya Bay, which was not detected in *G. fascicularis* (Fig. 3b and f).

There was notable latitudinal variation in the Symbiodiniaceae community composition of *G. fascicularis* in the tropical SCS. Apart from symbiosis with the dominant *D. trenchii*, *G. fascicularis* present symbiosis with C40, C27, and C3u in Nansha Island (Fig. 3e), whereas in the Xisha Islands, a symbiont community shift was observed as represented by an increased contribution of Symbiodiniaceae belonging to C21 and C21a (Fig. 3d). *Durusdinium trenchii* was dominant within the community (relative abundance is 92.4 ± 11.2%) of Luhuitou (Fig. 3c). The relative abundance of other symbionts (not belong to free-living Symbiodiniaceae), such as C3u, C27, and Cspc, in the water communities also showed a decreasing-trend from the Nansha Islands to the Xisha Islands. These symbionts were not among the top ten Symbiodiniaceae sub-clades at the W3 water sampling site (Fig. 3f). In the W2 water sampling site, the relative abundance of C3u and Cspc increased to 2.6% and 2.3%, respectively. In contrast, the A12 sub-clade dominated all tropical water samples, but this symbiont was not found in *G. fascicularis* or other widely distributed corals(Chen et al., 2019). Therefore, A12 may be a free-living Symbiodiniaceae sub-clade within the SCS.

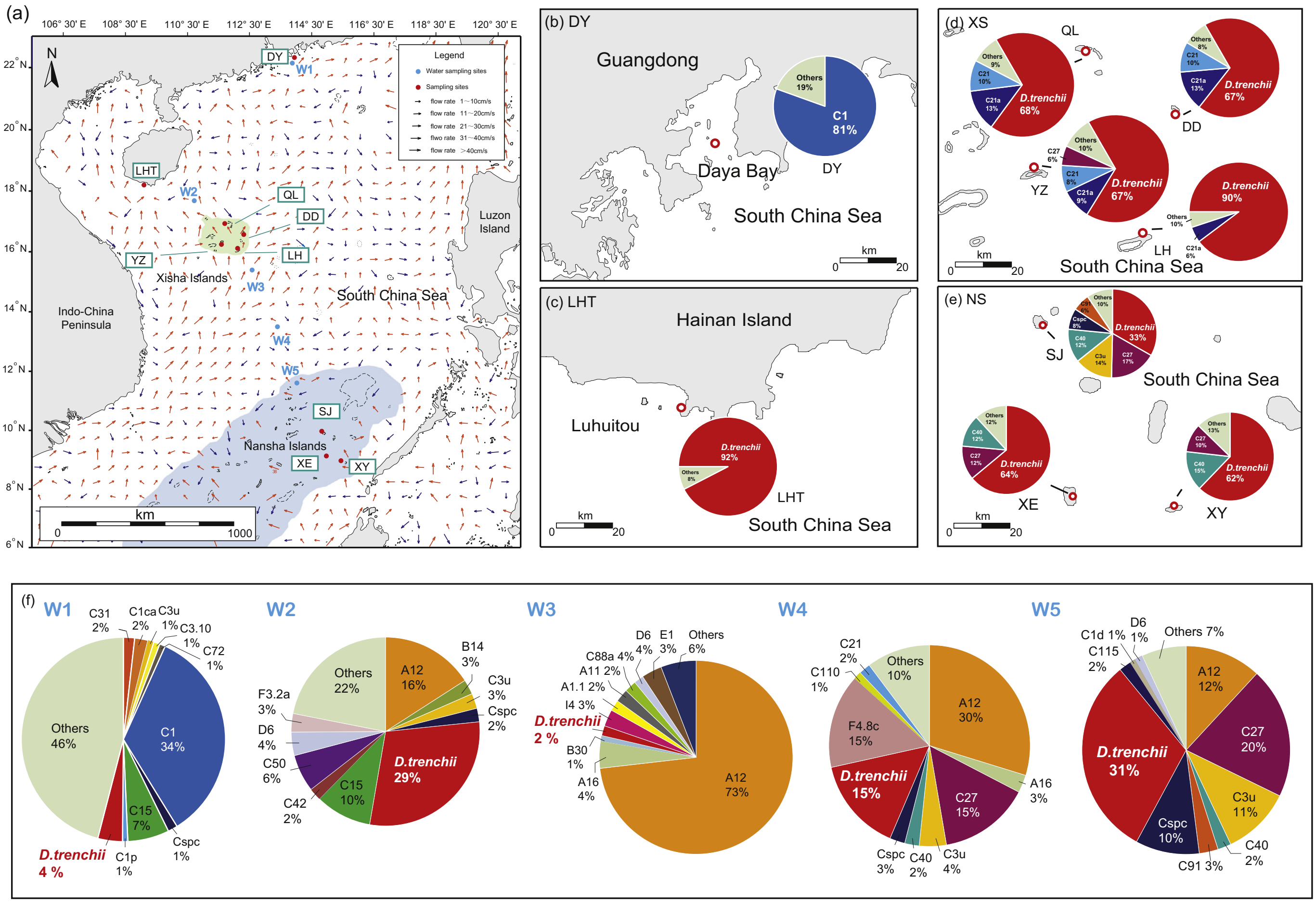

Fig. 3. Signatures of community composition of *G. fascicularis* Symbiodiniaceae ITS2 types in study sites. Overview is based on 108 *G. fascicularis* samples, fifteen water samples and summer ocean surface current. (a) *G. fascicularis* sampling sites ($n = 9$) and seawater sampling sites ($n = 5$) in the South China Sea. Red dots mean that coral samlping sites. Blue dots mean that seawater sampling sites. Orange and dark arrows mean that northward and southward surface ocean currents, respectively. Light blue and light green area represent Nansha Islands and Xisha Islands, respectively. Symbiodiniaceae community composition of *G. fascicularis* including (b) Daya Bay (DY), (c) Luhuitou (LHT), (d) Xisha Islands (XS) and (e) Nansha Islands (NS). (f) Symbiodiniaceae community composition of water samples in the South China Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Genetic diversity of D. trenchii

Durusdinium trenchii in the SCS showed high genotypic diversity. Genetic data analysis of 11 *D. trenchii* populations revealed 218 different genotypes among the 229 multilocus genotypes (MLGs) obtained. However, of the 96 *G. fascicularis* samples from the SCS, only 91 strains remained after excluding identical MLGs. The clonal richness (R) of *D. trenchii* ranged from 0.85 to 1.00 for each study site within the SCS, and duplicate genotypes were not detected in the population from the Nansha Islands (SJ, XE, and XY, Fig. 4 and Table 2). The total average clonal richness of all sampling sites was 0.95, which means that only ~5% of MLGs may be detected two or more times and that these repeated MLGs are distributed at the same sampling site (Fig. 4). Therefore, most coral samples containing *D. trenchii* only contained a single MLG, and MLGs from various samples, even those obtained from regions within a small geographic distance (<10 m), differed from each other.

The probability of identity ($P_{(ID)}$) of the sampling sites around the SCS (including Thailand and Palau) ranged from 5.5×10^{-7} to 1.1×10^{-10} (Table 2). For each sampling site in the SCS, the $P_{(ID)}$ ranged from 5.5×10^{-7} to 1.5×10^{-8}. Therefore, all $P_{(ID)}$ values were below 0.0001 (Waits et al., 2010), indicating that the probability of the same genotypes from different sampling sites having independent origins is extremely low (probability ranging from 1.8 million to 9.1 billion). Based on the above results, *D. trenchii* in the SCS has abundant unique MLGs and high clonal richness and thus exhibits rich genetic diversity.

3.3. Genetic structure of D. trenchii

The STRUCTURE results showed that K = 4 (lnP(D) = −5827.44), which was the most likely number of *D. trenchii* genetic clusters (Fig. 5b). The STRUCTURE HARVESTER test results also supported the subdivision of *D. trenchii* populations into four genetic populations around the SCS (K = 4 for the maximum value of deltaK). *Durusdinium trenchii* strains from the Xisha Islands and Luhuitou belonged to the same inferred population. The Nansha Islands (including XY, XE, and SJ), Xisha Islands-Luhuitou (including YZ, DD, QL, and LHT), Thailand (including CP and PI), and Palau (NK and CT) regions contained distinct and closely knit genetic units (Fig. 5b). In addition, orange and red clusters (Fig. 5b) were found at higher frequencies in samples from Nansha Islands, Xisha Islands, and Luhuitou, but with low and distinct frequencies in samples from Thailand and Palau. Notably, when K = 3 (lnP (D) = −5998.98), deltaK showed the second largest value, and examination of the posterior probabilities of each K value showed an increasing trend (Fig. S2). For K = 3, *D. trenchii* was divided into three inferred populations (Fig. 5a): SCS (including XY, XE, SJ, YZ, DD, QL, and LHT), Thailand (including CP and PI), and Palau (NK and CT). The yellow

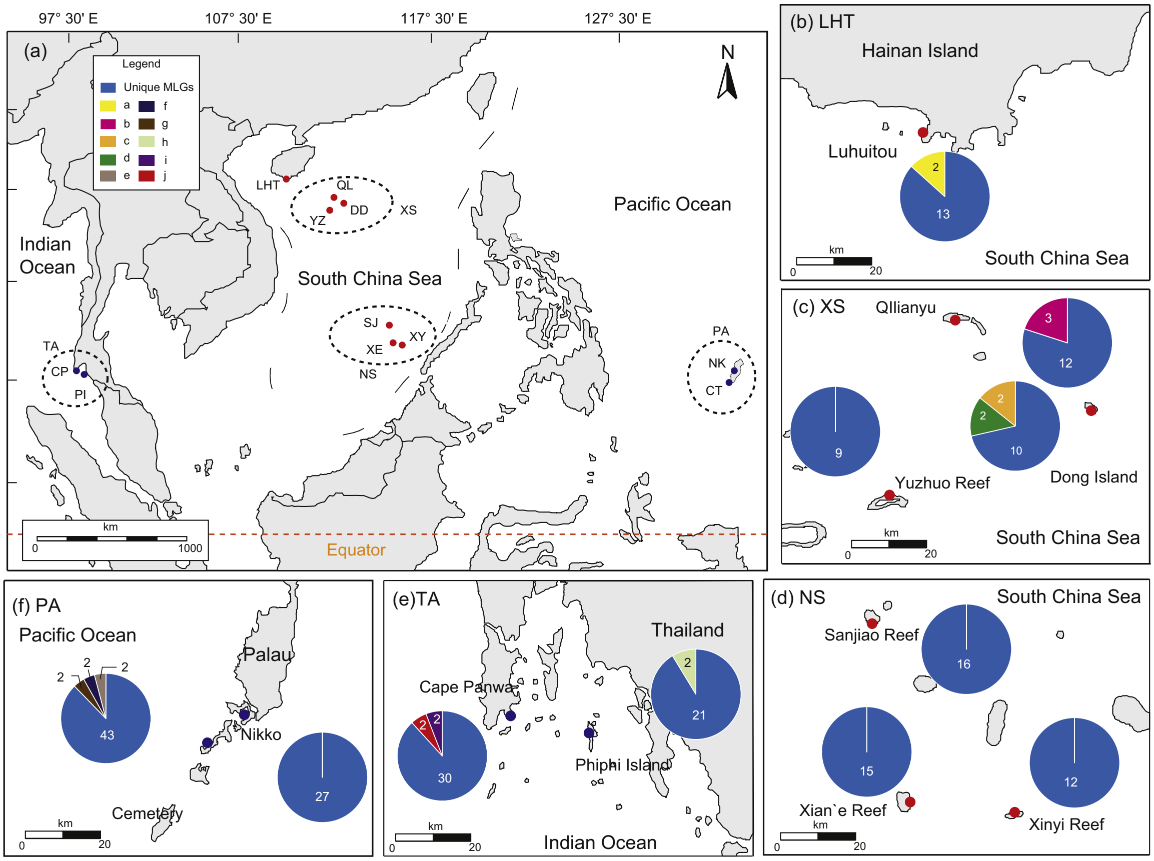

Fig. 4. The diversity of individual multilocus genotypes from populations of *D.trenchii*, collected from *G. fascicularis* in the South China Sea. The microsatellite data of population of *D.trenchii* from Thailand and Palau retrieved from Pettay et al. (2015). (a) Overview of sampling locations around the South China Sea. Most multilocus genetypes (unique MLGs are indicated by dark blue color.) characterized from *G. fascicularis* among (b) Luhuitou, (c) Xisha Islands and (d) Nansha Islands were unique; *D.trenchii* populations characterized from coral communities in (e) Thailand and (f) Palau, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and red genotypes contributed to populations in the Nansha Islands more than to those in the Xisha Islands and Luhuitou (Fig. 5a).

Φ_{PT} statistical analysis showed pairwise population structures among the 11 populations around the SCS. Differentiation among sampling sites was detected in this analysis, which was not observed in STRUCTURE analysis. For example, genetic variation was detected between XY and XE ($\Phi_{PT} = 0.092$) or XY and SJ ($\Phi_{PT} = 0.074$) within the SCS (Table 3). Geographical isolation and SST are important factors that may affect the genetic variation of *D. trenchii* and should be considered in correlation analysis. The Mantel test results indicated significant positive correlations between the genetic variation index Φ_{PT} and geographic distance ($R^2 = 0.1729$, Mantel test $p = 0.004 < 0.05$, Fig. 6), between the genetic variation index Φ_{PT} and the average SST ($R^2 = 0.3671$, Mantel test $p = 0.001 < 0.05$, Fig. 6), and between genetic variation index Φ_{PT} and the SST variance ($R^2 = 0452$, Mantel test $p = 0.021 < 0.05$, Fig. 6). Therefore, geographic isolation, the average SST, and the SST variance impact the genetic variation of *D. trenchii*.

3.4. Symbiodiniaceae co-occurrence and mutual exclusion relationships in G. fascicularis from the SCS

The results of Symbiodiniaceae community composition analysis showed that *D. trenchii* associated with corals were widely distributed in tropical coral habitats from the SCS and did not form symbioses

Table 2
Summary statistic by location, including number of coral samples, number of unique MLGs of *D.trenchii*, clonal richness(R), number of private alleles, infromation index (I), observed heterozygosity (H_o), expected heterozygosity (H_e) and probability of identity (PI).

Location	No.of samples	Unique MLGs	Clonal richness (R)	Private alleles	Information index (I)	Observed (Ho)	Expected (He)	Probability of identity (PI)
Xinyi Reef	12	12	1	3	1.10(0.17)	0.76(0.11)	0.58(0.07)	3.1E−08
Xian'e Reef	15	15	1	0	1.05(0.14)	0.62(0.08)	0.56(0.05)	8.9E−08
Sanjiao Reef	16	16	1	2	1.12(0.21)	0.71(0.10)	0.57(0.08)	1.5E−08
Yuzhuo Reef	9	9	1	0	0.91(0.16)	0.65(0.12)	0.54(0.06)	5.5E−07
Dong Island	14	12	0.85	0	1.06(0.20)	0.71(0.10)	0.58(0.08)	3.1E−08
Qilianyu	15	13	0.86	1	1.00(0.08)	0.74(0.09)	0.58(0.04)	2.0E−07
Luhuitou	15	14	0.93	0	1.05(0.17)	0.71(0.10)	0.57(0.08)	4.8E−08
Nikko	27	27	1	2	1.25(0.22)	0.73(0.07)	0.62(0.06)	1.3E−09
Cemetery	49	46	0.94	9	1.11(0.19)	0.79(0.10)	0.56(0.07)	6.4E−08
Phiphi Island	23	22	0.95	2	1.26(0.21)	0.72(0.07)	0.63(0.06)	1.3E−09
Cape Panwa	34	32	0.94	6	1.41(0.21)	0.76(0.06)	0.68(0.05)	1.1E−10

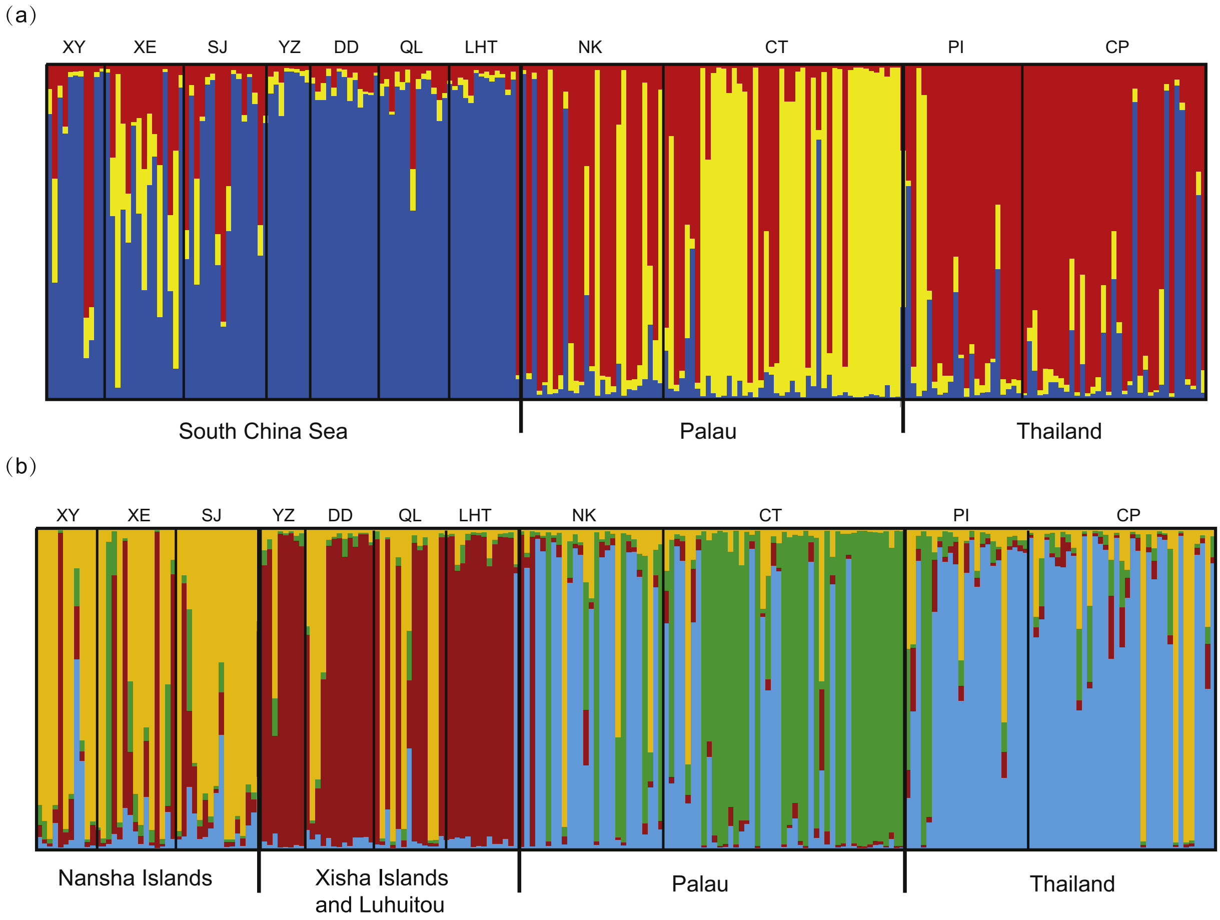

Fig. 5. The genetic structure of *D.trenchii* (based on 11 microsatellite locus, $n = 218$ distinct multlocus genotypes, MLGs) obtained from study locations around the South China Sea. (a) At second-maximum deltaK ($K = 3$), major differentiation occurred among the South China Sea, Palau and Thailand; (b) At the optimal $K = 4$, there are distinct differentiation appeared among the cluster of Nansha Islands, Xisha Islands-Luhuitou, Palau and Thailand.

with *G. fascicularis* in the subtropical Daya Bay (Fig. 3). Therefore, the co-occurrence and mutual exclusion relationships of the Symbiodiniaceae community composition in different climate zones were examined by network modelling. The tropical Symbiodiniaceae network led to the identification of 71 nodes and 215 interactions (Fig. 7a, see Additional file 8: Table S7 and Additional file 9: Table S8). The number of Symbiodiniaceae sub-clades associated with such interaction accounted for 33.0% of the total number of Symbiodiniaceae sub-clades identified

Table 3
A pairwise matrix comparing the genetic relationship (Φ_{PT}) between all 11 sampling locations of *D.trenchii* around the South China Sea.

		Nansha Islands			Xisha Islands			Hainan Island	Palau Islands		Thailand	
	Φ_{PT}	Xinyi Reef	Xian'e Reef	Sanjiao Reef	Yuzhuo Reef	Dong Island	Qilian Islands	Luhuitou	Nikko Bay	Cemetery	Phiphi Island	Cape Panwa
Nansha Islands	Xinyi Reef	–										
	Xian'e Reef	**0.092**	–									
	Sanjiao Reef	**0.074**	0.059	–								
Xisha Islands	Yuzhuo Reef	**0.162**	**0.189**	**0.233**	–							
	Dong Island	**0.176**	**0.194**	**0.216**	0.068	–						
	Qilian Islands	**0.158**	**0.181**	**0.207**	**0.208**	**0.170**	–					
Hainan Island	Luhuitou	**0.154**	**0.182**	**0.215**	0.113	**0.097**	**0.197**	–				
Palau Islands	Nikko Bay	**0.131**	**0.116**	**0.145**	**0.263**	**0.220**	**0.173**	**0.238**	–			
	Cemetery	**0.222**	**0.195**	**0.244**	**0.350**	**0.336**	**0.273**	**0.335**	**0.095**	–		
Thailand	Phiphi Island	**0.152**	**0.141**	**0.192**	**0.273**	**0.230**	**0.192**	**0.271**	0.030	**0.159**	–	
	Cape Panwa	**0.129**	**0.121**	**0.158**	**0.228**	**0.204**	**0.166**	**0.221**	**0.043**	**0.154**	0.033	–

†Significant Φ_{PT} values (sequential Bonferroni corrected $p < 0.05$) marked as bold.

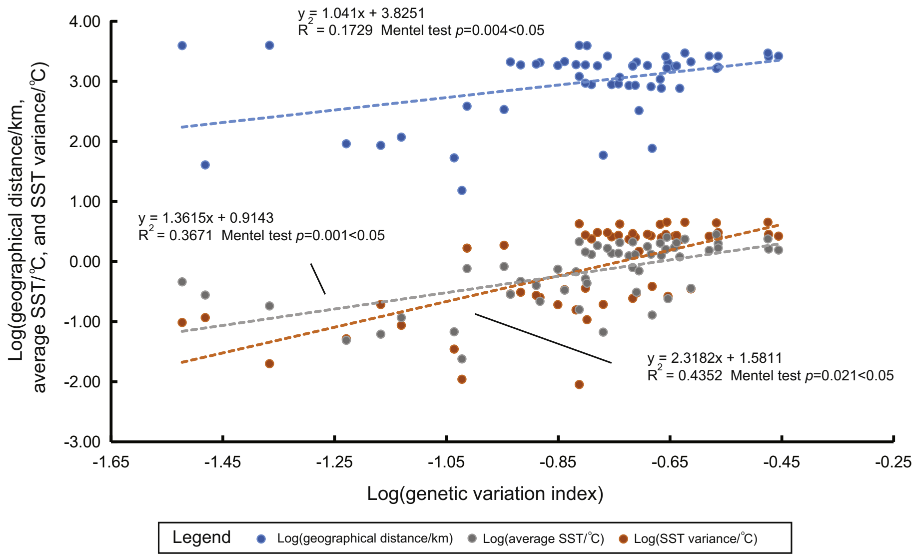

Fig. 6. Relationship between genetic variation and geographic distance (marked by blue), between genetic variation and average SST (marked by gray) and between genetic variation and SST variation (marked by orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the tropical region in the SCS ($n = 215$, see Additional file 8: Table S7), with co-occurrence as the main interaction form ($n = 181$, 84.2%, see Additional file 9: Table S8). In contrast, 58 nodes and 112 edges were identified in the subtropical Symbiodiniaceae network (Fig. 7b). The number of nodes accounted for 47.2% of the total number of Symbiodiniaceae sub-clades identified in the subtropical region in the SCS ($n = 123$, see Additional file 8: Table S7). The main interactions among Symbiodiniaceae sub-clade in subtropical SCS were co-exclusive ($n = 70$, 62.5%; see Additional file 9: Table S8), which is clearly distinct from the those in the tropical SCS. In addition, these two networks followed a scale-free degree distribution typical of biological systems, indicating that most Symbiodiniaceae sub-clades possessed few interactions, whereas a few sub-clades possessed many interactions (Fig. 7c and d).

Notably, *D. trenchii*, as a "hub" with the highest degree of interaction, has been identified in the tropical Symbiodiniaceae network (number of degrees = 34, Fig. 7a and c; Additional file 8: Table S7). Further, *D. trenchii* showed the largest number of co-exclusive relationships with other Symbiodiniaceae sub-clades ($n = 32$, Fig. 7c), accounting for 47.1% of the total number of co-exclusive relationships in the tropical Symbiodiniaceae sub-clade network from the SCS (Additional file 9 Table S8). Therefore, *D. trenchii* may play an important role in regulating interaction patterns and affecting the relative abundance of sub-clade in the Symbiodiniaceae community. Nevertheless, C1 was dominant in the Symbiodiniaceae community composition from the Daya Bay, but was not involved in the Symbiodiniaceae network. The interaction network of Symbiodiniaceae in the subtropic was completely constructed by rare Symbiodiniaceae sub-clades (Fig. 7d).

4. Discussion

4.1. Monsoon-driven sea surface currents provide a potential transport mechanism for the dispersal of long-lived D. trenchii

Physiological evidence suggests that Symbiodiniaceae are short-lived (~7 days in the environment) (Nitschke, 2015) and show poor swimming ability (3–10 m in 24 h) (Fitt and Trench, 1983), which may limit their dispersal among coral habitats without the help of a host. However, *D. trenchii* was identified in seawater samples from different latitudes in the SCS, even at W3 and W4 which were far from the coral habitats, indicating that *D. trenchii* has a longer lifespan or other hosts, such as foraminifera (Decelle et al., 2018). A heat-tolerant species of Symbiodiniaceae, *D. trenchii* from the Indo-Pacific Ocean, has spread widely among coral habitats of the greater Caribbean, suggesting that heat-tolerant *D. trenchii* has a longer lifespan and higher viability (Pettay et al., 2015). *Durusdinium trenchii* has invaded corals from the greater Caribbean through global human maritime transportation activities (Hallegraeff, 1998; Pettay et al., 2015), but this transport process requires longer than the estimated lifespan of other Symbiodiniaceae species, as shown in a previous study (~7 days, Fitt and Trench, 1983).

The relative abundance of *D. trenchii* in seawater decreased as the latitude increased between Nansha Islands and Xisha Islands, suggesting that *D. trenchii* in the SCS have migrated in this direction. During Pleistocene, persistent high temperatures and variable light conditions facilitated the development of numerous genetic and ecological differences in the adaptive radiation of *Durusdinium*, including *D. trenchii*, in a vast warm water zone across southeast Asia, Indonesia, and Northern Australia (LaJeunesse et al., 2010; Pettay et al., 2015). The Nansha Islands are in the Northern edge of the warm water zone in a relatively stable high temperature environment (Fig. 2), which is a potential suitable original habitat and dispersal source for *D. trenchii*. However, the relative abundance of *D. trenchii* in seawater decreased with increasing latitude, possibly because of its weak competitiveness in the free-living stage. The observations of Symbiodiniaceae in the open ocean suggested that *Symbiodinium* (formerly clade A) and *Cladocopium* (formerly clade C) have stronger competitiveness and various survival strategies, such as symbioses with plankton (Decelle et al., 2018). *Durusdinium* was mainly identified in the *piconano* size fraction (0.8–5 μm) and showed lower abundance in the open ocean (relative abundance < 1%), indicating that *D. trenchii* has weaker competitiveness and fewer survival strategies than *Symbiodinium* and *Cladocopium* in the open ocean (Decelle et al., 2018). Our results for the seawater Symbiodiniaceae community analysis support this view (Fig. 3f).

The long-distance dispersal of Symbiodiniaceae may rely on the sea current, as these individuals cannot cross hundreds of kilometres by swimming alone (Andras et al., 2011; Fitt and Trench, 1983; Thornhill et al., 2017; Wirshing et al., 2013). Therefore, the spread of *D. trenchii* with a potentially long lifespan may also depend on

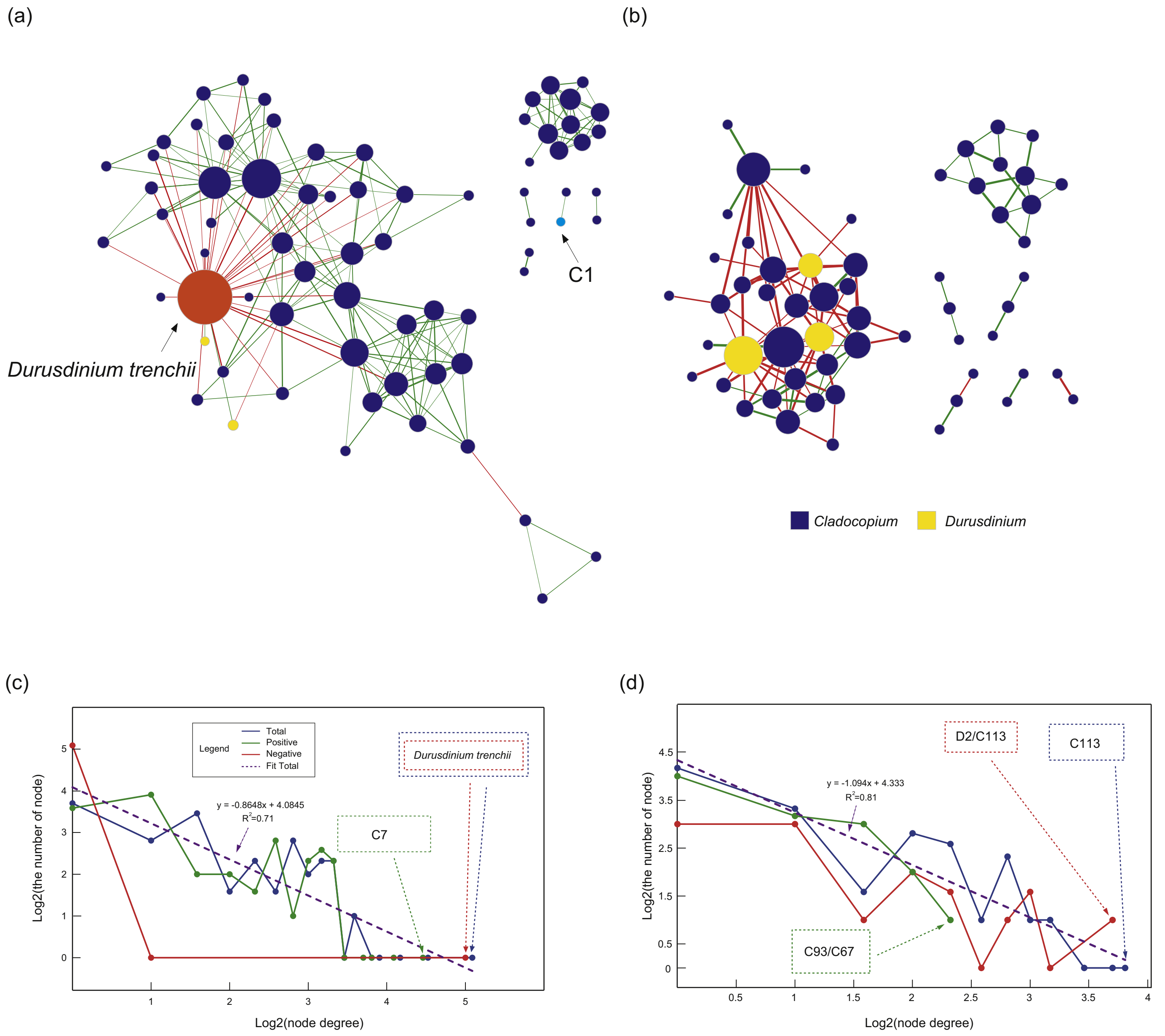

Fig. 7. Network analysis revealing the interactions among the Symbiodiniaceae sub-clade. (a) Symbiodiniaceae ITS2 sub-clades network analysis of the tropical symbionts. The size of the node is proportional to the abundance. Node color corresponds to genera taxonomic classification. Edge color represents co-occurrence (green) and co-exclusive (red) relationships, and the width of edge is equivalent to the correlation values. (b) Symbiodiniaceae ITS2 sub-clades network analysis of the subtropical symbionts. Node degree distribution of overall, co-occurrence, and co-exclusion relationships in the (c) tropical and (d) subtropical Symbiodiniaceae sub-clades (These are well-fit by a power law with slope −0.9 and −1.1, dotted purple regression line, adjusted $R^2 = 0.71$ and $R^2 = 0.81$, respectively). The top three most connected hubs as indicated in callouts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sea surface current in the SCS. Monsoons are the main factor driving the change in sea surface currents in the SCS (Liu et al., 2014). In summer and autumn (time of our sampling), the prevailing southwest monsoon drives the northward movement of the surface current in the SCS (flow velocity is 0.5–1.0 knot per hour; Fig. 3a) (Zhao et al., 2017), providing the basic conditions for the northward spread of *D. trenchii* from the warm water zone in the low latitude coral habitats. Notably, the relative abundance of *D. trenchii* in W2 was higher than that in W3 (Fig. 3f), which are distributed in the northern and southern Xisha Islands, respectively, indicating that the number of *D. trenchii* was recharged when the sea current flowed through hot large-scale coral habitats. Additionally, *D. trenchii* was identified in sea water from Daya Bay(relative abundance: 4%, Fig. 3f), but was not detected in *G. fascicularis*, even in the rare Symbiodiniaceae community (Fig. 3f, Additional file 8: Table S7). Previous studies also revealed no symbiotic relationship between coral and *D. trenchii* (Chen et al., 2019; Tong et al., 2017). This suggests that *D. trenchii* in subtropical sea water originates from the low latitude coral habitat, and the monsoon-driven current transports them to the north of the SCS. Symbiodiniaceae can spread for long distances by establishing symbiotic or epiphytic relationships with coral larvae, but this mode also relies on the sea current (Baums et al., 2014; Thornhill et al., 2017).

In summary, *D. trenchii* may have long lifespans originating from the low latitude warm water zone. Monsoon-driven current may promote the dispersal of *D. trenchii* in the SCS.

4.2. Low SST prevented D. trenchii from establishing stable symbioses with coral in the subtropical SCS

Durusdinium trenchii accounts for a high proportion of the community in *G. fascicularis* habitats in the tropical SCS, but there were not stable symbioses between *D. trenchii* and *G. fascicularis* in the subtropical Daya Bay (Fig. 3b–e). In previous studies, *D. trenchii* was shown to be a “generalist” species among *Durusdinium* (LaJeunesse et al., 2010; Pettay et al., 2015), which adapt to high temperature and turbidity fluctuations in marginal reef environments better than other Symbiodiniaceae species (LaJeunesse et al., 2010; LaJeunesse et al., 2014; LaJeunesse et al., 2018). Many studies also have shown that the abundance of *D. trenchii* is increased significantly in corals subjected to heat-based physiological trauma, and it is considered unlikely for this to be reversed after the host recovers from bleaching (Boulotte et al., 2016; LaJeunesse et al., 2016; Pettay et al., 2015). Therefore, sustained high SST or thermal bleaching may improve the success rate of establishing symbioses between *D. trenchii* and its coral hosts.

It's worth noting that *Galaxea fascicularis* form stable symbioses with cold-tolerant C1 in Daya Bay (Fig. 3b), which established wider symbioses with *D. trenchii* in tropical coral habitats from the SCS (Fig. 3c–e). Members of *Durusdinium* as rare Symbiodiniaceae were detected in corals from subtropical habitats, which were generally dominated by C1 lineage members with cold tolerance (Chen et al., 2019; Palmas et al., 2015; Reimer et al., 2006). The average SST in the subtropical Daya Bay was significantly lower than that in the tropical coral habitats and presented stronger fluctuations (SNK test, $p < 0.05$; Fig. 2). The field-measured SST data show that DY had an extremely low temperature of 14 °C for 19 days, reaching the lethal SST level for corals (Chen et al., 2009; Huang et al., 2018). Versatile *D. trenchii* was identified in the seawater of Daya Bay (Fig. 3f), providing a potential source of Symbiodiniaceae to coral (LaJeunesse et al., 2010; Pettay et al., 2015), but the extreme seasonal low temperature was not suitable for heat-tolerant *D. trenchii* to establish stable symbioses with the coral host.

The effect of low temperatures on the successful establishment of symbiosis between *D. trenchii* and corals may be reflected by competition between symbionts. Coral habitats are also distributed in the Leizhou Peninsula and Hongkong in the subtropical region of the SCS, and the coral hosts living there display more symbioses with C1 symbionts (Chen et al., 2019; Liu et al., 2012; Ng and Ang, 2016). Like *D. trenchii*, heat-sensitive C1 is a host-generalist symbiont (LaJeunesse et al., 2018; Thornhill et al., 2014). Moreover, C1 is considered to have a high photosynthetic efficiency and is adapted to low SST and rich nitrogen-acquisition environments (Baker et al., 2013). Furthermore, C1 was observed to be a dominant symbiotic sub-clade even in the cold and temperate communities of Okinawa and Jeju Island (Palmas et al., 2015; Reimer et al., 2006). Because some subtropical and temperate coral habitats are affected by low SST, corals must establish symbiosis with Symbiodiniaceae with high photosynthesis efficiency to survive and secrete calcium carbonate (Ng and Ang, 2016). Therefore, heat-tolerant *D. trenchii* may be less competitive than C1 in the subtropical regions of the SCS. Accordingly, temperature is one of the key factors preventing *D. trenchii* from establishing a stable symbiosis with coral in the subtropical SCS.

4.3. Geographical isolation and SST affect the genetic variation of D. trenchii

The results of STRUCTURE analysis showed that our *D. trenchii* MLG dataset was divided into three cluster segregated by geography, including SCS, Palua, and Thailand (Fig. 5a). As expected, obvious genetic variations were observed among *D. trenchii* populations from SCS, Palua and Thailand, as these regions are separated from each other by large oceanographic barriers (Fig. 1a; Yu et al., 2010). The SCS is the largest semi-closed marginal sea in the western Pacific (Yu, 2000) and lacks seawater exchange channels with the Indian and Pacific Oceans (Yu et al., 2010). Therefore, there is limited connectivity between coral or Symbiodiniaceae populations in the SCS and surrounding seas. The Mantel test results also revealed a significant positive correlation between genetic variation Φ_{PT} (Fst analogue) and geographical distance of the *D. trenchii* population ($R^2 = 0.1729$, $p = 0.004 < 0.05$, Fig. 6). Interestingly, analysis of biophysical dispersal models indicated that present-day ocean currents lead to the transport of coral larvae from the Nansha Islands (low latitude coral habitats in the SCS) into the Coral Triangle Region via the Sulu Sea, which contribute to high levels of diversity in the Coral Triangle (Kool et al., 2011). This suggests that there is potential connectivity of Symbiodiniaceae associated with coral larvae between the Nansha Islands and Coral Triangle. The proportion of yellow and red genetic clusters of *D. trenchii* in the low latitude regions was higher than that in the relatively high latitude regions from the SCS (Fig. 5), even at sites where the two genetic clusters cooccurred, and most samples were not assigned to one or the other, indicating that recombination had occurred.

Notably, the three clusters were further split into a total of four clusters at high resolution (Fig. 5b), which best reflected the number of populations. Most samples of *D. trenchii* were separated by yellow and red genetic clusters in the SCS. The red genetic cluster was composed nearly entirely of samples from Xisha Islands and Luhuitou, whereas more samples from Nansha Islands were assigned to the yellow genetic cluster. *Durusdinium trenchii* were identified in the W3 and W4, indicating no physical barriers between Nansha Islands and Xisha-Luhuitou. This genetic structure pattern was consistent with the SST. The SST has important effects on the distribution and prevalence of Symbiodiniaceae (Pettay and Lajeunesse, 2013). There were significant differences in the SST among the Nansha Islands, Xisha Islands, and Luhuitou (SNK test, $p < 0.05$; Fig. 2b), which is consistent with the result of spatial clustering of the k-means algorithm run on the monthly means of SST data calculated from weekly FilledSST data in the SCS (Zuo et al., 2015). In addition, the results of Mantal test indicated that the SST (average SST: $R^2 = 0.3671$; SST variation: $R^2 = 0.4352$) has larger effects on genetic variation than did geographic isolation ($R^2 = 0.1729$; Fig. 6). Therefore, different SSTs among coral habitats may screen *D. trenchii* genotypes adapted to the local environment, leading to obvious genetic variation between the Nansha Islands and Xisha-Luhuitou. Pettay and Lajeunesse (2013) also found that the SST can screen for *Durusdinium glynii*, which is phylogenetically closely related to *D. trenchii*, resulting in the retention of genotypes that can adapt to local environments. However, there were significant difference in the SST between Xisha Islands and Luhuitou (SNK test, $p < 0.05$; Fig. 2b), but there is some geographic isolation between two regions, which may explain why no significant differences in genetic clusters were observed between these regions (Fig. 5b). The high relative abundance of *D. trenchii* in the seawater (W2) between Xisha Islands and Luhuitou suggested high connectivity between these two coral habitats (Fig. 3a and f).

Accordingly, the genetic structure and variation of *D. trenchii* may be affected by geographical isolation and SST differences around the SCS. There are huge geographical barriers among the SCS, Palau, and Thailand, which may lead to the strong genetic structure of *D. trenchii* population among these regions. The genetic variations in *D. trenchii* inside the SCS may be shaped by the SST, and there are two potential local adaptive genetic clusters in the Nansha Islands and Xisha-Luhuitou regions, respectively.

4.4. Durusdinium trenchii may maintain the stability of symbioses by inhibiting potential parasitic Symbiodiniaceae sub-clades

The network inference showed that *D. trenchii* plays an important role in construction of the interaction network of tropical Symbiodiniaceae communities (Fig. 7a), whereas C1, as the dominant sub-clade in the subtropical coral habitat, did not participate in regulating Symbiodiniaceae cooperation and competition (Fig. 7b). Many interaction sub-clades have been identified in our ITS2 reads dataset (tropical sub-clades:71; subtropical sub-clades:58, Additional file 8:

Table S7), most of which belonged to background rare symbionts. A recent study has shown that a rare symbiont assemblage, which is comparable to the rare biosphere reported for bacteria in microbial communities in the ocean (Pedrós-Alió, 2012) and human gut (Arumugam et al., 2011), has significantly contributed to Symbiodiniaceae diversity (Ziegler et al., 2018). Previous network theoretic modelling predicted that both elevated symbiont diversity and sub-clades occurring at low abundance, which provide redundant or complementary symbiotic function, can significantly increase community stability in response to environmental changes (Fabina et al., 2013). Moreover, the functional role of Symbiodiniaceae in the holobiont is not determined by a single individual taxon but rather by assemblages of symbionts (Ziegler et al., 2018). Therefore, the regulation of *D. trenchii* on Symbiodiniaceae microbial biosphere was closely related to the stability and resilience of coral-Symbiodiniaceae symbioses to respond to climate change.

However, rare Symbiodiniaceae members associate in various manner with coral host (Amend et al., 2012; Baker et al., 2018; Kirk et al., 2013; Knowlton and Rohwer, 2003), which may include non-mutualistic species and free-living non-symbiotic species. *Durusdinium trenchii* is a key species in the Symbiodiniaceae interaction network in the tropical SCS and contributes the greatest number of co-exclusion relationships ($n = 34$, 47.1%). This suggests that *D. trenchii* has strong competitiveness and can effectively inhibit opportunistic Symbiodiniaceae sub-clades. Some Symbiodiniaceae species or sub-clades (*Symbiodinium fitti* and C7) may be changed from a mutualistic to a parasitic state by climate change to sequester more resources for their own growth, thus parasitizing their hosts for nutrition(Baker et al., 2018). The potential parasitic Symbiodiniaceae C7 as a key sub-clade was detected in the network inference (Fig. 7c), which showed the greatest number of co-occurrence degrees ($n = 23$, 6.1%). However, there is a significant co-exclusion relationship between *D. trenchii* and C7 (Spearman's rho $= -0.807$, $p < 0.05$; Pearson's rho $= -0.738$, $p < 0.05$; see Additional file 9: Table S8), indicating considerable competition and inhibition of interactions or opposite environmental forces between them. Overall, *D. trenchii* as a dominant Symbiodiniaceae species can affect the rare symbiont community, which may improve the stability of Symbiodiniaceae communities by inhibiting potential parasitic Symbiodiniaceae sub-clades.

4.5. Heat-tolerant D. trenchii may help corals adapt to climate change in the SCS

The SCS is a critical region for coral reef connectivity in the Indian and Pacific oceans and borders the low-latitude coral reefs of Thailand, Indonesia, and Palau and the high-latitude coral reefs of Japan and Korea (Spalding, 2001; Yu, 2012; Wang and Li, 2009). Accordingly, the health of coral-algal symbioses in the SCS affects the stability of the coral reef ecosystem throughout the region. Geochemistry, satellite remote sensing, and field measurement data indicate that SSTs in the SCS continue to rise (Nie et al., 1999; Yu, 2000; Yu, 2012; Yu et al., 2004), which has led to continued degradation of coral reef ecosystems in this region (Yu, 2012). High SSTs also provide favourable environmental conditions for the generalist symbiont *D. trenchii* (LaJeunesse et al., 2010), which can establish symbioses with more hosts and thus strengthen the heat tolerance of its coral hosts, enabling them to better respond to climate change in the future. Moreover, *D. trenchii* exhibit patterns of cell proliferation and spreading, which can compensate for losses in coral's normal symbiont population (Lee et al., 2016). A recent study showed that the endangered *Orbicella faveolata,* which had survived a bleaching event, was dominated by *D. trenchii* in the Florida Keys (Manzello et al., 2018).

Durusdinium trenchii with a long lifespan is widely distributed in seawater from the SCS, which may assist corals in obtaining heat-tolerant symbionts in the larval stage (Thornhill et al., 2017; van Oppen and Blackall, 2019), and potentially recolonize corals and restart carbon fixation following mass bleaching (Lee et al., 2016). In addition, *D. trenchii* have high genetic diversity (Fig. 4) and produce potential local genotypes (Fig. 5) through the effects of environmental factors (i.e., SST and sea current) across coral habitats in the SCS, avoiding the negative ecological effects caused by *D. trenchii* with a single genotype associated with the coral host (LaJeunesse et al., 2016; Pettay et al., 2015). Unexpectedly, *D. trenchii* can affect the rare Symbiodiniaceae community, may inhibit potential parasitic symbionts, and may further improve the ability of the holobiont to cope with environmental disturbance. Accordingly, heat-tolerant *D. trenchii* likely assists corals in their adaption to climate change in the SCS.

5. Conclusion

Durusdinium trenchii is widely distributed in the seawater across 13° latitudes in the SCS, suggesting that its heat-tolerant characteristics enables its long lifespan. The monsoon-driven current provides conditions that allow the expansion of *D. trenchii* originating from heat low latitude coral habitats, which may lead to the presence of *D. trenchii* in subtropical Daya Bay. However, the seasonal low SST prevented *D. trenchii* from establishing stable symbioses with local corals in the subtropical SCS. In addition, SST is a main factor affecting *D. trenchii* genetic variation among tropical coral habitats inside the SCS. Two regional genetic clusters were divided from Nansha Islands and Xisha-Luhuitou, indicating different *D. trenchii* local genotypes in the SCS. There are large oceanographic barriers among the SCS and surrounding ocean, which may directly lead to significant genetic variation of *D. trenchii* among the SCS, Palau, and Thailand. Interestingly, the network modelling inference suggested that *D. trenchii* can affect the rare symbiont community, which may improve the stability of Symbiodiniaceae communities by inhibiting potential parasitic Symbiodiniaceae sub-clades. Our study provides insight into the dispersal, genetic variation, environmental response, and symbionts interaction network regulation mechanism of heat-tolerant *D. trenchii* in the Indo-Pacific region. This suggests that *D. trenchii* with high genetic diversity can assist coral in adapting to climate change in the future.

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Author contributions

KY and BC deigned the research, ZQ, JL, GW, HS, XH, LJ contributed the materials, BC and QW performed the research, BC analysed the data and drawn all pictures, BC and KY wrote the manuscript. All authors reviewed the paper.

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CRediT authorship contribution statement

Biao Chen: Conceptualization, Methodology, Resources, Data curation, Visualization, Validation, Writing - original draft. **Kefu Yu:** Conceptualization, Resources, Methodology, Validation, Writing - review & editing, Project administration, Funding acquisition. **Zhenjun Qin:** Software, Formal analysis, Visualization, Writing - review & editing. **Jiayuan Liang:** Project administration, Resources, Investigation, Writing - review & editing. **Guanghua Wang:** Resources, Investigation, Writing - review & editing. **Xueyong Huang:** Resources, Investigation, Writing - review & editing. **Qian Wu:** Resources, Investigation, Writing - review & editing. **Leilei Jiang:** Data curation, Writing - review & editing.

Declaration of competing interest

We declare we have no competing interests.

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