



# Temperature-Driven Local Acclimatization of *Symbiodinium* Hosted by the Coral *Galaxea fascicularis* at Hainan Island, China

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The success of coral reef ecosystems largely depends on mutualistic symbiosis between scleractinian corals and the dinoflagellate photosymbiont *Symbiodinium* spp. However, further investigation is needed to elucidate the flexibility of coral-algae associations in response to environmental changes. In this study, we applied a molecular method (high-throughput internal transcribed spacer 2 region of ribosomal RNA gene amplicon sequencing) to explore diversity and flexibility of *Symbiodinium* associated with *Galaxea fascicularis*, an ecologically important scleractinian coral species collected at five locations around Hainan Island, South China Sea. The results revealed a high diversity of *Symbiodinium* subclades with C2r and D17 being dominant in *G. fascicularis*. Clade D *Symbiodinium* occurred most frequently in habitats where the annual average sea surface temperatures are the highest, suggesting that temperature is an important factor in determining *Symbiodinium* D abundance in *G. fascicularis*. The distribution of coral-*Symbiodinium* associations are possibly mediated by trade-off mechanisms which change the relative abundance of *Symbiodinium* clades/subclades under different environmental conditions. These findings provide further evidence that reef-building corals such as *G. fascicularis* can shuffle their symbionts to cope with environmental changes, and have implications for our understanding of the ecology of flexible coral-algal symbiosis.

**Keywords:** coral, *Symbiodinium*, symbiosis, diversity, flexibility

## INTRODUCTION

The success of coral reef ecosystems in oligotrophic ocean depends largely on mutualistic symbioses between reef-building corals and photosymbiotic algae of the genus *Symbiodinium* (zooxanthellae). *Symbiodinium* is comprised of nine phylogenetic clades (A–I), each containing multiple genetically distinct subclades or species (Baker, 2003; Pochon and Gates, 2010). Reef-building corals readily form associations with clades A–D *Symbiodinium*, but partnerships with clades F and G have also been reported (Lajeunesse, 2001; Baker, 2003; Lajeunesse et al., 2010a).

The functional and physiological diversity within *Symbiodinium* (Tchernov et al., 2004; Brading et al., 2011) strongly affects the response of coral holobionts to environmental disturbances. Heat tolerant *Symbiodinium* in clade D, for example, may strengthen the thermal tolerance of corals exposed to heat stress (Berkelmans and Van Oppen, 2006; Lajeunesse et al., 2009; Keshavmurthy et al., 2012). Therefore, reef-building corals that harbor multiple ecologically distinct *Symbiodinium* clades or types are expected to be flexible and have more opportunities to cope with environmental change (Baker, 2003; Little et al., 2004; Berkelmans and Van Oppen, 2006) or mediate their sensitivity to stress (Putnam et al., 2012).

Coral reefs are in serious decline worldwide as a result of global warming and anthropogenic activities (Pandolfi et al., 2011). However, it has been hypothesized that corals could adapt to environmental perturbations by either shuffling existing symbionts or switching to novel symbionts (Buddemeier and Fautin, 1993; Baker, 2003; Baker et al., 2004). Knowing whether corals can associate flexibly with a range of symbionts is a necessary prerequisite to test this hypothesis. Changes in the symbiont communities associated with scleractinian corals have been observed following disturbance and are presumed to be an important mechanism for acclimatization (Jones et al., 2008; Silverstein et al., 2015). For instance, an increase in *Symbiodinium* D1a has been previously reported following bleaching events in Pacific and Caribbean corals (Baker et al., 2004; Lajeunesse et al., 2009, 2010b). Moreover, flexible symbiosis in conspecific and congeneric corals has been shown to be related to both depth and geographical distribution (Sampayo et al., 2007; Lajeunesse et al., 2010a; Huang et al., 2011; Lien et al., 2013).

Progress in surveying *Symbiodinium* diversity and ecology has considerably improved our understanding of the flexibility of coral-algae symbiosis (e.g., Baker, 2003; Baird et al., 2007; Silverstein et al., 2012). However, most previous studies only considered the dominant *Symbiodinium* types because of the limitations of conventional screening approaches (e.g., Lajeunesse et al., 2010a; Zhou and Huang, 2011). More recently, as high-resolution methods including quantitative PCR and next-generation DNA sequencing have been increasingly used (e.g., Mieog et al., 2009; Arif et al., 2014; Boulotte et al., 2016; Ziegler et al., 2017), evidence for some corals hosting unusual or rare *Symbiodinium* is increasing. These less common symbionts have the potential to influence the whole holobiont function, including bleaching resilience (Mieog et al., 2009; Silverstein et al., 2012; Arif et al., 2014; Thomas et al., 2014; Cunning et al., 2015c; Boulotte et al., 2016). Therefore, coral-algal symbioses may be more flexible than previously thought and need to be investigated urgently to provide a better understanding of how flexibility in coral holobionts enables them to cope with environmental changes.

The coral species *Galaxea fascicularis* (Linnaeus, 1767) is broadly distributed in the Indo-Pacific region and is an ecologically important species in the South China Sea. Each generation of *G. fascicularis* acquires symbiotic algae horizontally and harbors multiple *Symbiodinium* clades or types, commonly

clades C and/or D (Lajeunesse et al., 2010a; Huang et al., 2011). Previous studies have shown that *Symbiodinium* associated with *G. fascicularis* is flexible with respect to both clades C and D at regional (Huang et al., 2011) and local scales (Zhou et al., 2012) in the South China Sea. However, coral-algal associations are also dependent on local physicochemical conditions (Lajeunesse et al., 2010a; Howells et al., 2012). Hainan Island is the largest island in the South China Sea and the coral reefs are affected by environmental conditions, such as coast and summer upwelling (Jing et al., 2015). In the present study, we investigated the *Symbiodinium* communities associated with *G. fascicularis* around Hainan Island using internal transcribed spacer 2 (ITS2) region of the ribosomal RNA gene amplicon sequencing to explore the diversity and flexibility of *Symbiodinium*. The result demonstrates that *G. fascicularis* at Hainan Island exhibits a high level of symbiont flexibility, and the changes in relative abundance of thermally tolerant *Symbiodinium* clade D associated with *G. fascicularis* are possibly driven by temperature. This finding implies that symbiont shuffling is likely a defensive mechanism of coral for local acclimatization to environmental changes.

## MATERIALS AND METHODS

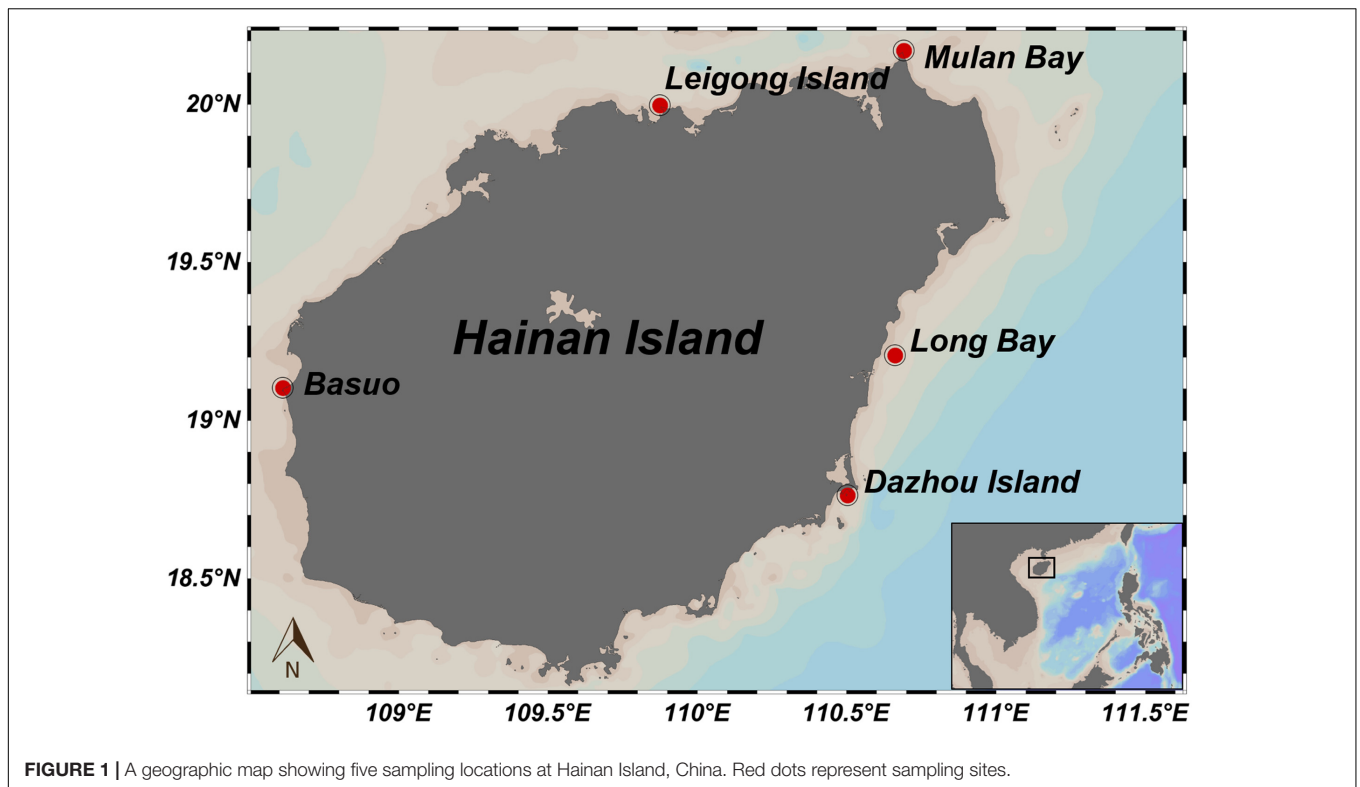
### Sample Collection

Samples of the scleractinian coral *G. fascicularis* were collected at depths between 2 and 4 m from the coast of Hainan Island in the South China Sea in September 2010 (Figure 1). At each location, six to seven colonies separated by at least 5 m were collected and fragments of approximately 4 cm<sup>2</sup> were picked and preserved in 95% ethyl alcohol at field temperature and stored at −20°C until DNA extraction took place.

### DNA Extraction and Amplicon Sequencing

Total DNA was extracted as described previously (Zhou et al., 2012). The quality and quantity of the DNA were determined with a NanoDrop spectrophotometer (Thermo Fisher Scientific, United States). Purified DNA samples were stored at −20°C for future use.

All samples were PCR amplified using a pair of barcoded *Symbiodinium*-specific primers: ITSintfor2 (5'-GAATTGCAGAACTCCGTG-3') and ITS2-reverse (5'-GGGATCCATATGCTTAAGTTCAGCGGGT-3') (Lajeunesse and Trench, 2000) targeting the ITS2 region of the ribosomal RNA gene for *Symbiodinium*. PCR amplification was carried out on a thermocycle controller (Bio-Rad, United States) with the following program: initial denaturing at 94°C for 5 min; 35 cycles at 94°C for 30 s, 51°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. All PCR products were purified using the Qiagen Agarose Gel DNA Purification Kit (Qiagen, China) and quantified with the NanoDrop spectrophotometer. All amplification products were mixed in equal amount followed by sequencing on an Illumina Miseq platform using the 2 × 300 bp mode at Novogene (Beijing,



China). The raw data were submitted to the NCBI Sequence Read Archive under accession number SRP066283.

### ITS2 Sequencing Data Processing

Overlapping paired-end reads were merged to obtain fragments using PEAR (Zhang et al., 2014). After de-multiplexing and quality control, a custom BLAST *Symbiodinium*-specific database of ITS2 types was downloaded (Arif et al., 2014), containing 408 ITS2 sequences. For each sample, datasets were randomly subsampled to 10,411 sequences (the lowest read number) which were subsequently searched against the database using BLASTn. Sequences were assigned to the ITS2 types that gave the highest identity in the BLASTn hits (Tong et al., 2017). The resulting counts of *Symbiodinium* ITS2 types were merged for downstream statistical analysis.

### Environmental Data

Aqua-MODIS sea surface temperature (SST) and chlorophyll *a* concentration (Chl *a*) with a spatial resolution of 4 km at each sampling location from January 2006 to December 2010 were obtained from NASA<sup>1</sup>.

### Statistical Analyses

The Shannon–Wiener ( $H'$ ) diversity index was calculated to assess the level of alpha-diversity across samples from different locations. One-way analysis of variance and *post hoc*

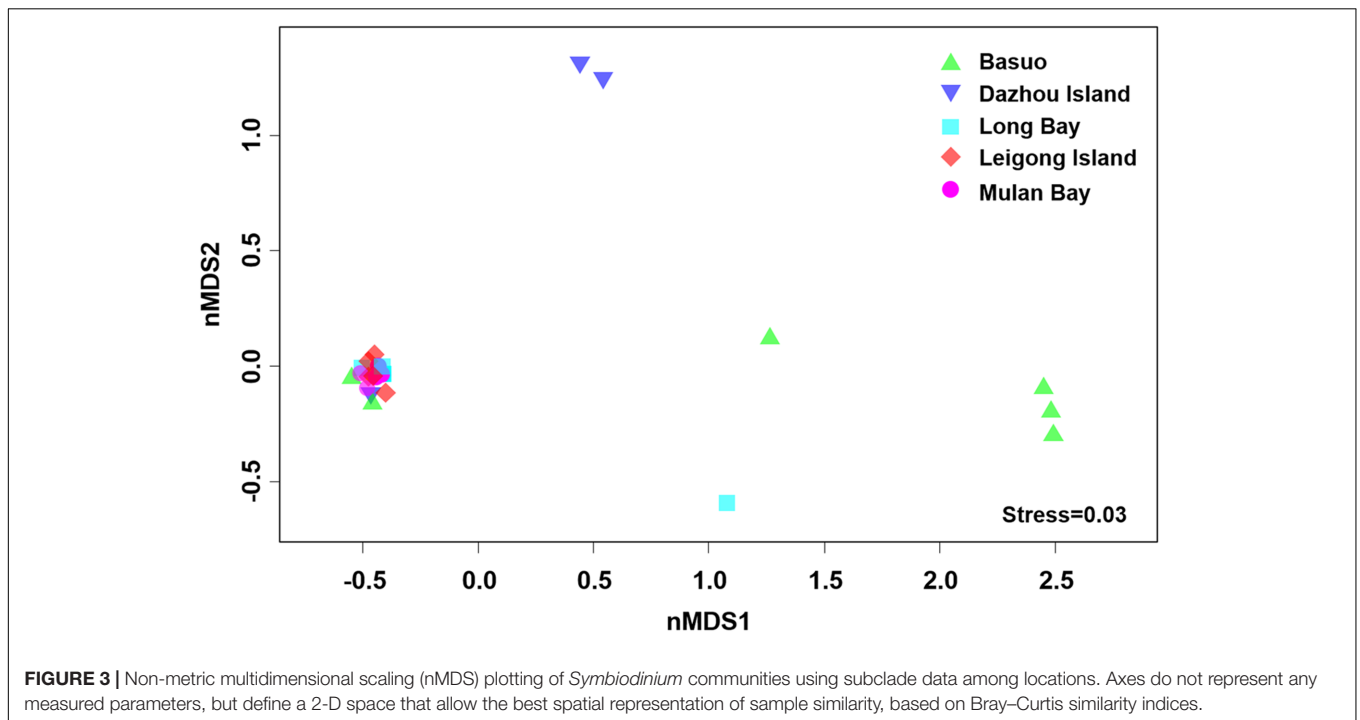
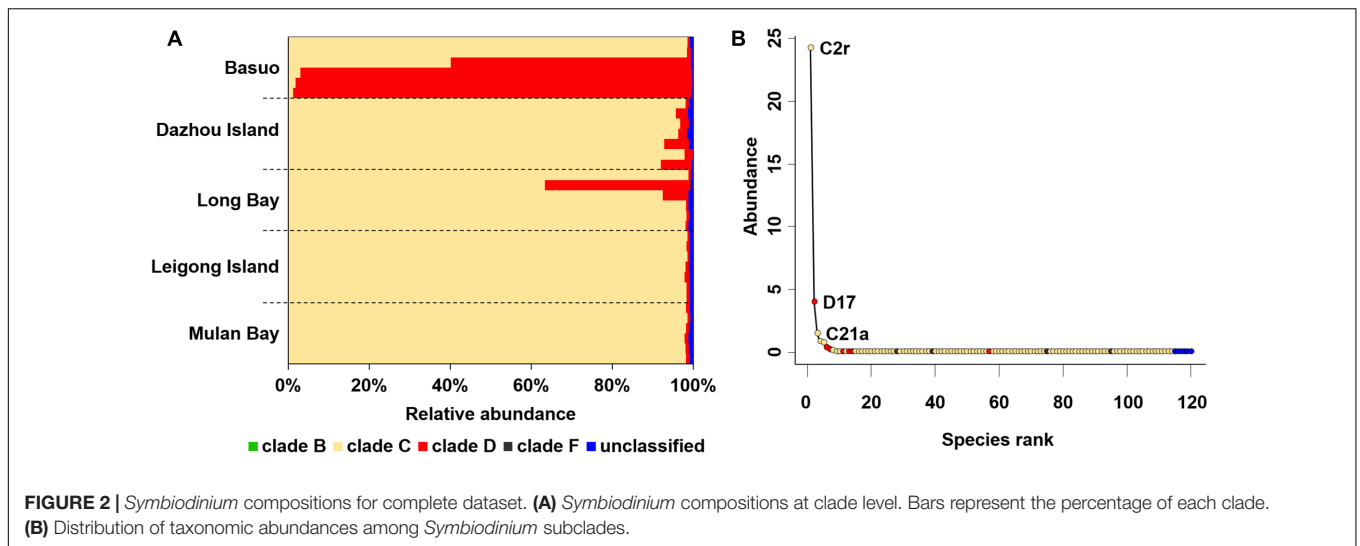
Tukey's HDS comparisons were conducted to test the significance of differences in diversity between sampling locations. The similarity of *Symbiodinium* assemblages was also characterized by non-metric multidimensional scaling (nMDS) using the Bray–Curtis distance metric after data transformation. Analysis of variance (ADONIS) was performed to test the significance of differences in *Symbiodinium* communities among different sampling locations. The significant relationship between environmental variables (SST and Chl *a*) and *Symbiodinium* community composition was assessed using Monte Carlo permutation methods. All statistical analysis were conducted using the vegan package (Oksanen et al., 2015) in the R software environment (R 3.1.2).

## RESULTS AND DISCUSSION

### *Symbiodinium* Community Diversity and Flexibility

In total, 997,760 qualified sequences were obtained from 32 samples (10,411–51,626 sequences per sample). A total of 119 *Symbiodinium* ITS2 subclades were assigned based on alignment with the ITS2 database at the 97% similarity level, covering clades B, C, D, and F. Overall, clade C comprised the highest proportion of sequences (averagely 85.6%), followed by clade D (averagely 13.6%) and then rare clades B and F (Figure 2A). C2r and D17 were the most dominant ITS2 subclades representing > 99% of the sequences for all samples. All individual colonies contained

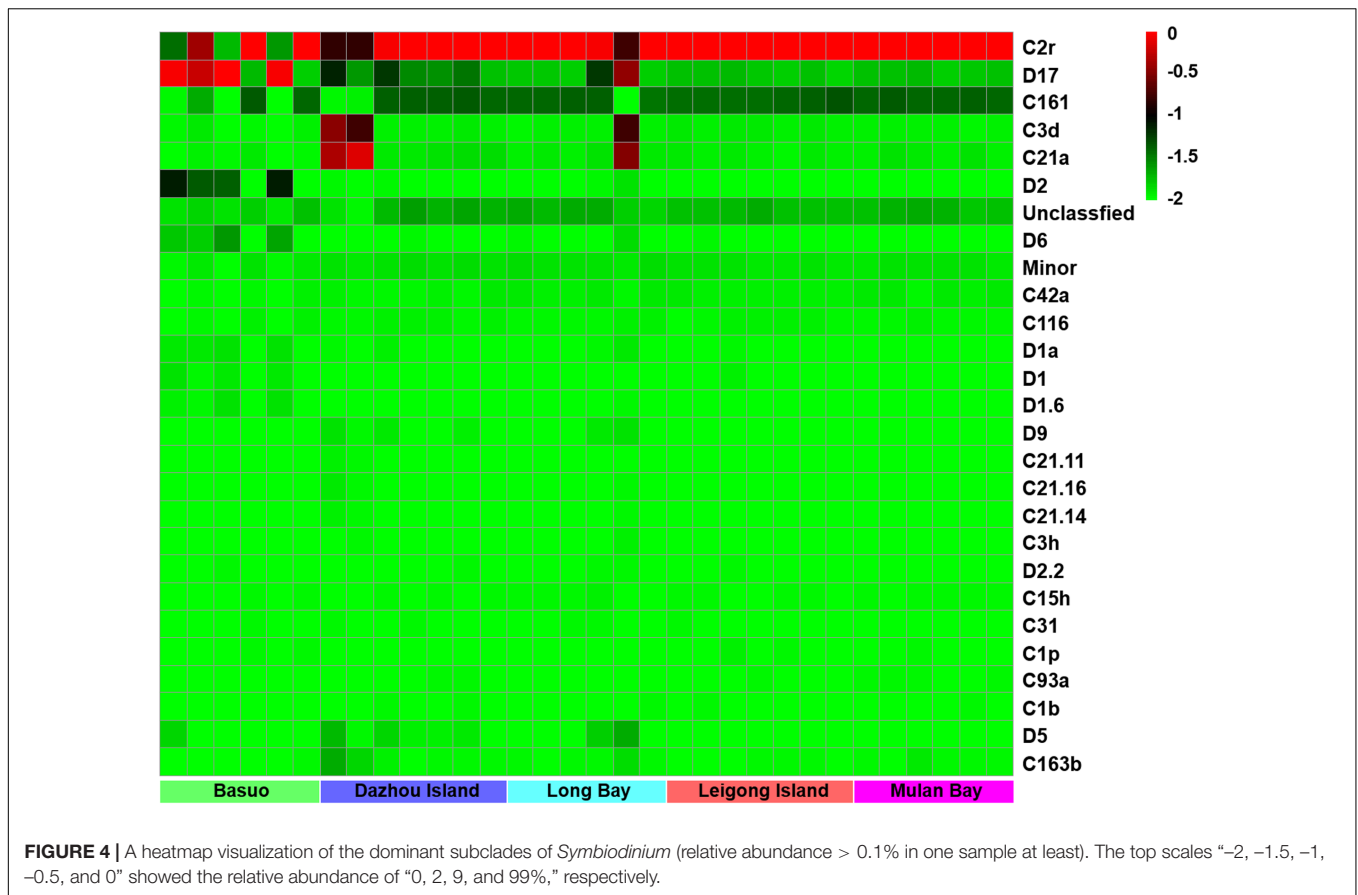
<sup>1</sup><https://modis.gsfc.nasa.gov/>



multiple *Symbiodinium* subclades belonging to different clades. Despite a high number of distinct *Symbiodinium* types, most of them had abundances lower than 0.1% (Figure 2B), indicating that rare subclades are present in heterogeneous *Symbiodinium* assemblages.

It is believed that low abundances of cryptic *Symbiodinium* have largely been overlooked by conventional screening techniques (Mieog et al., 2009). An increasing body of evidence shows that highly diverse rare taxa with important ecological roles are prevalent elsewhere (Lynch and Neufeld, 2015) and are being increasingly explored in reef-building corals (Silverstein et al., 2012; Green et al., 2014; Quigley et al., 2014; Kennedy

et al., 2015; Boulotte et al., 2016). The *Symbiodinium* types in clades B and F associated with *G. fascicularis* are unusual, which have rarely been reported from the South China Sea (Huang et al., 2011; Zhou and Huang, 2011; Zhou et al., 2012; Tong et al., 2017) or other regions (Lajeunesse et al., 2004, 2010a). The results presented here suggest that *G. fascicularis* exhibits a high cryptic diversity and flexibility in symbiotic associations. It has been shown that rare *Symbiodinium* types (e.g., type D1) have the potential to enable the coral host to resist heat stress through symbiont shuffling or switching (Lajeunesse et al., 2009; Silverstein et al., 2012, 2015; Bay et al., 2016; Boulotte et al., 2016). For example, *Acropora* can



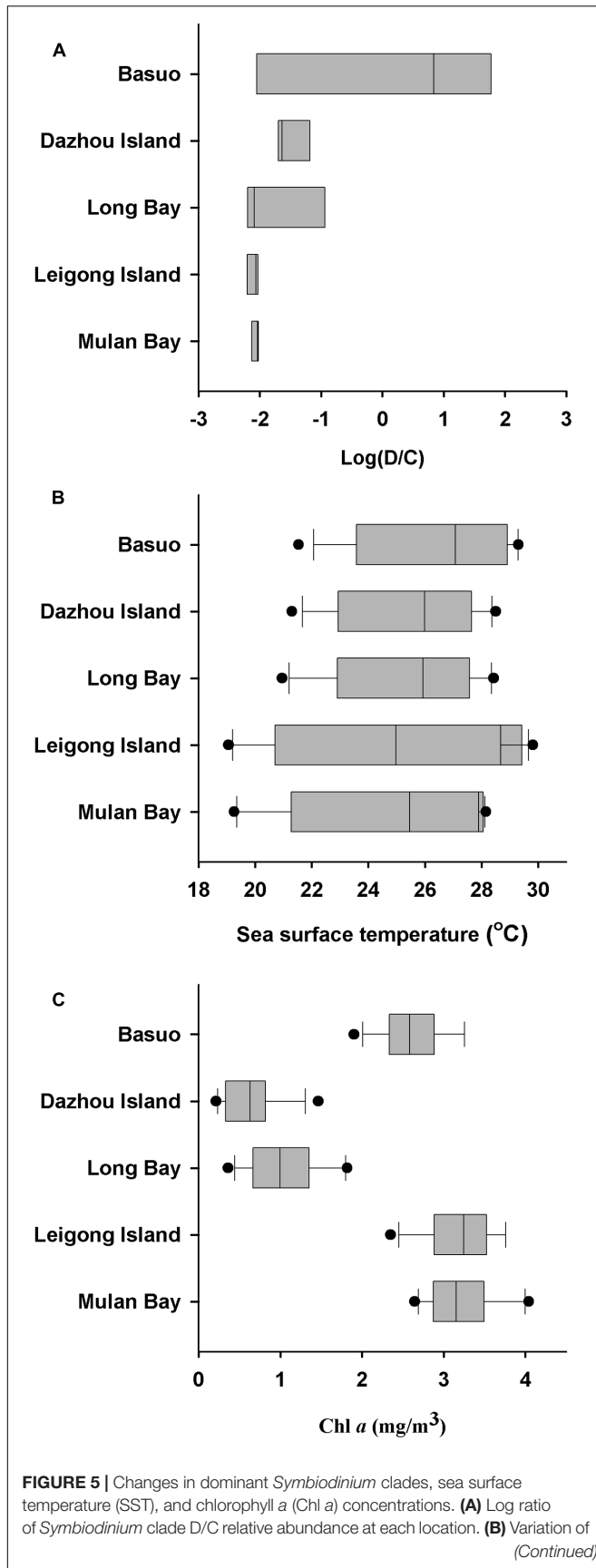
change its thermally tolerant symbiont abundance from rare to dominant in a response to heat stress (Berkelmans and Van Oppen, 2006). Community diversity and functional redundancy may contribute to the stability of community resistance and resilience (Oliver et al., 2015), which has been characterized in coral holobionts (Silverstein et al., 2012, 2015). Highly diverse and flexible *Symbiodinium* may facilitate the ability of *G. fascicularis* to survive successfully in various habitats they experience throughout the Indo-Pacific area. However, the real contribution of rare symbionts to the host coral and their ecological significance is still unclear and needs to be addressed in future (Lee et al., 2016).

The *G. fascicularis* holobiont can be viewed as a highly complex symbiotic system with the flexibility to associate with a wide range of *Symbiodinium* (Blackall et al., 2015). It has been suggested that the mode of transmission of symbionts can affect the flexibility of coral-algal symbiosis (Baker, 2003; Fabina et al., 2012). Therefore, horizontal transmission of endosymbionts in each generation may provide greater opportunities for *G. fascicularis* to obtain multiple symbionts from the external environment. However, emerging evidence shows that many corals can host multiple *Symbiodinium* subclades without correlation with the mode of transmission (van Oppen, 2004). In addition, other factors such as environmental variability, host recognition and maintenance mechanisms can also influence the flexibility of coral-algal associations (Baker,

2003; Rodriguez-Lanetty et al., 2006; Dunn and Weis, 2009). Moreover, the development of coral especially in early life stages has additional effects on symbiont acquisition and selection (Abrego et al., 2009; McIlroy and Coffroth, 2017; Zhou et al., 2017). For example, Abrego et al. (2009) demonstrated that the symbiont associations in juvenile *Acropora* are more flexible than those in adults.

### Temperature Drives the *Symbiodinium* Assemblages in *G. fascicularis*

No significant differences in the Shannon diversity index were detected among sampling locations (one-way ANOVA;  $F = 1.618, p = 0.198$ ). However, there were significant differences in the *Symbiodinium* assemblages between sampling locations (Figure 3; ADONIS,  $p = 0.01$ ), demonstrating that coral-algal symbiosis is highly flexible around Hainan Island. Importantly, these differences were attributed to changes in the relative abundance of existing *Symbiodinium* types in individuals. Of six colonies of *G. fascicularis* at Basuo, four were dominated by clade D with sparse clade C, whereas the others contained abundant clade C with rare clade D. At subclade level, D17 dominated at Basuo, whereas C2r dominated in other locations (Figure 4). The abundance of each of the D1a, D2, and D6 subclades was higher at Basuo than at other locations, indicating that these holobionts are likely to be locally adapted through shifts in symbiont community composition.

**FIGURE 5 |** Continued

SST at each location. **(C)** Variation of Chl *a* concentrations at each location. Average monthly satellite measurements (SST and Chl *a*) from January 2006 to December 2010 were acquired from the Giovanni online data system, which is maintained by the NASA Goddard Earth Sciences Data and Information Services Center. Each dot represents the monthly average  $\pm$  SD. Boxplots showing median, first and third quartiles, and maximum and minimum seasonal ranges by location.

Sea surface temperatures and Chl *a* concentrations from 2006 to 2010 at each location were employed to investigate the relationships between environmental conditions and *Symbiodinium* communities (Figure 5). Monthly average SSTs at Mulan Bay, Long Bay and Dazhou Island decreased sharply in July, possibly due to the Qiongdong Upwelling in summer (Jing et al., 2015). Of the sample locations, Leigong Island experienced the largest annual fluctuation in monthly average SST ( $\sim 11^\circ\text{C}$ ). Annual average SSTs were highest at Basuo ( $26.4^\circ\text{C}$ ), followed by Dazhou Island ( $25.5^\circ\text{C}$ ), Long Bay ( $25.3^\circ\text{C}$ ), Leigong Island ( $24.7^\circ\text{C}$ ), and Mulan Bay ( $24.5^\circ\text{C}$ ). The monthly average Chl *a* concentrations of all the locations showed little variation throughout the year, but Long Bay and Dazhou Island had lower yearly average Chl *a* concentrations. *Symbiodinium* communities at Basuo was significantly correlated with spring average SSTs (Monte Carlo permutation test;  $p < 0.05$ ), but there were no significant differences between *Symbiodinium* communities and Chl *a* concentrations (Monte Carlo permutation test;  $p > 0.05$ ). *G. fascicularis* had a high specificity for *Symbiodinium* clade D at Basuo where annual average SSTs were the highest (Figure 5). In contrast, Chl *a* values at all locations did not show any patterns or trends consistent with observed *Symbiodinium* distribution patterns.

It is well-known that *Symbiodinium* clade D occurs more frequently in areas with high SST and high turbidity (e.g., Lajeunesse et al., 2010a; Keshavmurthy et al., 2012). Furthermore, it is evident that heat tolerant *Symbiodinium* can confer thermal tolerance to its host coral but at a cost of reduced growth rate, a decline in reproduction and increased susceptibility to disease (e.g., Little et al., 2004; Berkelmans and Van Oppen, 2006; Silverstein et al., 2015). It has been suggested that symbiont compositions may be regulated to maintain optimal benefit to the host in a given environment (Cunning et al., 2015a,b). Such trade-off mechanisms may depend on both biotic (e.g., host species, host ontogeny, and symbiont competition) and abiotic (e.g., temperature, light, and nutrients) factors, and may also involve stochastic processes and selective pressures. In the present study, relative high abundances of *Symbiodinium* clade D at Basuo where the annual average SST is the highest are possibly mediated by a cost-benefit trade-off by the coral *G. fascicularis*. These observations agree with our previous investigations (Huang et al., 2011; Tong et al., 2017), which reported that clade D in *G. fascicularis* was more prevalent in tropical locations than in subtropical locations from the South China Sea, which might be attributed to the latitudinal temperature gradients. It is known that temperature has a profound influence on the ecological structure of coral communities (Brown et al., 2004). Previous studies also found that temperature is the main

determinant to the geographic distribution of *Symbiodinium* in both conspecific corals (Lajeunesse et al., 2010a) and local adaptation (Howells et al., 2012). We suggest that temperature is the main environmental factor influencing the spatial variability of *Symbiodinium* assemblages in *G. fascicularis* around Hainan Island. However, other undetermined environmental factors, such as light intensity, and nutrient levels may also contribute to the biogeographical patterns of host-*Symbiodinium* associations (Baker, 2003), which can be investigated thoroughly in the future.

A better understanding of the spatial patterns will allow us to predict how corals will respond to environmental change over time (Dunne et al., 2004). In the present study, it may also reflect the capacity of the coral *G. fascicularis* to respond to environmental disturbances (e.g., thermal bleaching) by shuffling its internal symbionts. Some coral-algal associations remain remarkably stable over time (Thornhill et al., 2009; Williams et al., 2015) or revert to their original status after thermal bleaching (Lajeunesse et al., 2010b), which can be explained by the trade-off mechanism (Cunning et al., 2015b). More recently, it has been shown that symbiont shuffling in reef-building corals is attributed to the magnitude of the disturbance and the recovery conditions (Cunning et al., 2015a; Silverstein et al., 2015). The combinations of long-term, *in situ* field observations and elaborate laboratory experiments may provide more supporting evidence for symbiont shuffling in a better understanding of how coral will adapt to future climate changes. With the advances in 'omic' technologies, it is becoming feasible to elucidate the molecular mechanism of local acclimation and symbiont shuffling by analyzing biochemical complementarity of the symbiotic partners (e.g., Lin et al., 2015).

## CONCLUSION

This study characterized the geographic patterns of host-*Symbiodinium* associations in an ecologically important

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scleractinian coral *G. fascicularis* using high-throughput sequencing of ITS2 amplicons. We confirmed that *G. fascicularis* at Hainan Island exhibits a high level of symbiont flexibility, with the thermally tolerant *Symbiodinium* types in clade D being prevalent and highly abundant at locations with the highest annual average SSTs. These findings suggest that symbiont shuffling has the potential to serve as a trade-off mechanism for local acclimatization in *G. fascicularis*. The present study provides a better understanding of *Symbiodinium* diversity and distribution, which is important to predict the persistence of coral-algal associations in the presence of increasing environmental perturbations such as global warming.

## AUTHOR CONTRIBUTIONS

GZ, SL, P-YQ, and HH designed the study. YL collected samples. HT, LJ, YZ, XL, and MG performed experiments. GZ and LC analyzed data. GZ wrote the paper. All authors revised and approved the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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