

## Research Article

## Low genetic diversity of symbiotic dinoflagellates (*Symbiodinium*) in scleractinian corals from tropical reefs in southern Hainan Island, China

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**Abstract** Endosymbiotic dinoflagellates in the genus *Symbiodinium* are among the most abundant and important group of photosynthetic protists found in coral reef ecosystems. In order to further characterize this diversity and compare with other regions of the Pacific, samples from 44 species of scleractinian corals representing 20 genera and 9 families, were collected from tropical reefs in southern Hainan Island, China. Denaturing gradient gel electrophoresis fingerprinting of the ribosomal internal transcribed spacer 2 identified 11 genetically distinct *Symbiodinium* types that have been reported previously. The majority of reef-building coral species (88.6%) harbored only one subcladal type of symbiont, dominated by host-generalist C1 and C3, and was influenced little by the host's apparent mode of symbiont acquisition. Some species harbored more than one clade of *Symbiodinium* (clades C, D) concurrently. Although geographically isolated from the rest of the Pacific, the symbiont diversity in southern Hainan Island was relatively low and similar to both the Great Barrier Reef and Hawaii symbiont assemblages (dominated by clade C *Symbiodinium*). These results indicate that a specialist symbiont is not a prerequisite for existence in remote and isolated areas, but additional work in other geographic regions is necessary to test this idea.

**Key words** coral, dinoflagellate, diversity, South China Sea, *Symbiodinium*.

Dinoflagellates in the genus *Symbiodinium* (also called zooxanthellae) are found in symbiosis with a diverse range of marine invertebrate taxa and some protists (reviewed in Baker, 2003; Finney et al., 2010). Although originally thought to be a single dinoflagellate species, *Symbiodinium microadriaticum* (Freudenthal, 1962), studies based on biochemical, morphological, physiological, and molecular data have gradually established that the genus *Symbiodinium* is a diverse assemblage (Rowan, 2004; LaJeunesse et al., 2010a). The genus *Symbiodinium* is now recognized to comprise nine divergent lineages (clades A–I) based on analyses of nuclear ribosomal DNA (LaJeunesse, 2002; LaJeunesse et al., 2004a; Pochon & Gates, 2010) and chloroplast 23S rDNA (Santos et al., 2002). Six of these clades (A–D, F, and G) are known to form associations with scleractinian corals, but predominantly with those of clades A–D (Baker, 2003). Each of these clades contains multiple subcladal lineages or types that show geographic and host specificity using by more variable molecular markers such as ITS rDNA (Sampayo et al., 2009).

An increasing number of studies have focussed on the biogeographic distribution of *Symbiodinium* (e.g. LaJeunesse, 2002; LaJeunesse et al., 2003, 2004a; Chen et al., 2005; van Oppen et al., 2005). This understanding may provide insights into the future responses of reef corals to environmental change (Buddemeier & Fautin, 1993; Baker et al., 2004; LaJeunesse et al., 2010a). As environmental factors may vary enormously from spatial and temporal scales, regional differences in *Symbiodinium* diversity within a single host or among multiple species are therefore important (LaJeunesse et al., 2010a). For example, *Symbiodinium* A and B are much more common in Caribbean scleractinians compared to the Indo-Pacific, where clade C *Symbiodinium* prevail. Moreover, the *Symbiodinium* in the same host species are varied among different environments. For example, the Indo-Pacific coral *Plesiastrea versipora* (Lamarck, 1816) hosts clade C at low latitudes and clade B at the latitudinal margins of its distribution (Rodriguez-Lanetty et al., 2001). It is believed that more research into regions of the Indo-Pacific will uncover more symbiont diversity (Baker & Rowan, 1997; LaJeunesse et al., 2003).

Considerable research on *Symbiodinium* diversity and distribution has been carried out in the Caribbean

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and Central and Eastern Pacific (LaJeunesse, 2002; LaJeunesse et al., 2003, 2004a, 2004b), but little has been done in the South China Sea (SCS), Western Pacific, one of the largest marginal seas. The SCS is rich in coral cover and diversity and has recently been shown in decline (Huang, 2005). Several studies have reported the *Symbiodinium* diversity in reef-building corals in the SCS (Huang et al., 2006, 2011; Dong et al., 2009). Dong et al. (2008) reported the diversity of *Symbiodinium* in Luhuitou Bay (Sanya, China) by polymerase chain reaction (PCR) restriction fragment length polymorphism. Unfortunately, the method mainly used in previous studies was only at the cladal level and could not provide more details about the ecological aspects (Sampayo et al., 2009). Additionally, it is not known whether the *Symbiodinium* communities in this isolated area are different from other regions of the Pacific.

In the present study, denaturing gradient gel electrophoresis (DGGE) and sequencing of the internal transcribed spacer 2 (ITS2) region were used to determine the diversity and community structure of *Symbiodinium* associated with reef-building corals from four reefs in southern Hainan Island, SCS. We also compared our findings to the data available for these coral species in other parts of the Pacific, including the Great Barrier Reef (GBR), Okinawa, and Hawaii (LaJeunesse et al., 2003, 2004a, 2004b), to corroborate hypothesized biogeographic patterns in the distribution of *Symbiodinium* diversity, and to determine whether or not an exception occurred.

## 1 Material and methods

### 1.1 Sample collection

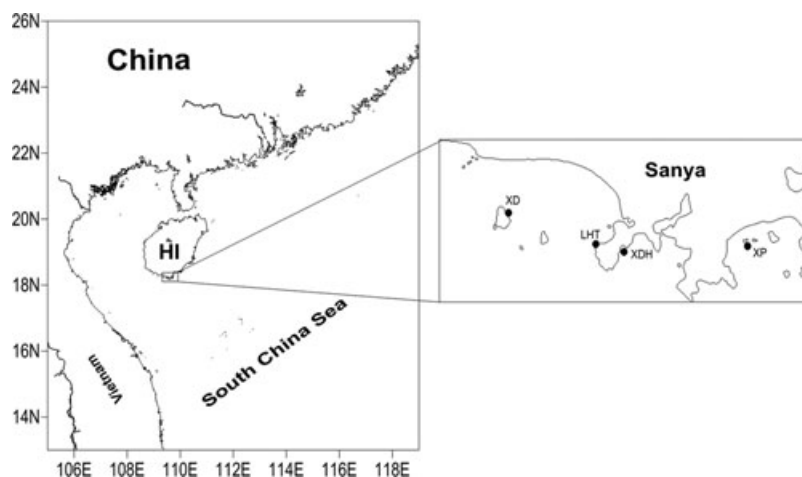
The sampling for this study was carried out at Sanya, on the southernmost coast of Hainan Island,

northern SCS. Luhuitou Bay (LHT), Ximao Island (XD), Xipai Island (XP), and Xiaodonghai Bay (XDH) are part of Sanya National Coral Reefs Nature Reserve, established in 1990. Small fragments (approximately 5 cm<sup>2</sup>) of reef corals were collected at a depth of 1–10 m with hammer and chisel using scuba diving at four locations in Sanya in October 2008 (Fig. 1). All specimens were preserved in 100% EtOH until further processing.

### 1.2 DNA extraction and ITS2-DGGE fingerprinting

Total DNA was extracted using the protocol described in Huang et al. (2006). The *Symbiodinium* ITS2 region was analyzed using DGGE in combination with DNA sequencing of dominant bands. The ITS2 was amplified using the primers ITS2 clamp (5'-CGCCCGCCGCGCCCCGCGCCCGTCCCCGCGCCCCCGCCCGGGATCCATATGCTTAAGTTCAGCGGGT-3') and ITSintfor2 (5'-GAATTGCAGAACTCCGTG-3') with a touch-down PCR protocol following LaJeunesse (2002). The PCR products were loaded on 8% polyacrylamide gels (acrylamide : N, N'-methylenebisacrylamide 37:1) with a denaturant gradient of 30%–60% (where 100% is defined as 7 mol/L urea and 40% V/V formamide). Electrophoresis was carried out with the DCode System (Bio-Rad, Hercules, CA, USA) using 0.5 × Tris-Acetate-EDTA (TAE) buffer at 60 °C for 14 h at 100 V. Gels were stained for 30 min in SYBR Green nucleic acid gel stain (Molecular Probes, Eugene, OR, USA) and photographed with ultraviolet transillumination.

Distinct ITS2-DGGE profiles (fingerprints) were characterized by sequencing of the dominant bands. The most prominent DGGE bands were excised from



**Fig. 1.** Maps of coral reefs sampled in this study, downloaded from <http://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html> and produced using Surfer 9. HI, Hainan Island; LHT, Luhuitou Bay; XD, Ximao Island; XDH, Xiaodonghai Bay; XP, Xipai Island.

the gel, eluted in DNAase free water overnight, and re-amplified with a reverse primer lacking the GC-clamp under the conditions described above. Sequencing was carried out using an ABI3730 DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA). The DGGE fingerprints and their corresponding sequences were compared against a reference database from diversity analyses carried out at other Indo-Pacific locations; *Symbiodinium* ITS2 sequences were named as in previous studies (LaJeunesse et al., 2003, 2004a, 2004b). The capital letter of these taxonomic designators refers to the particular clade of *Symbiodinium* and the numbers and lowercase letters refer to bands diagnostic of the symbiont's ITS2-DGGE fingerprint (LaJeunesse et al., 2003).

### 1.3 Phylogenetic analyses

Chromatograms were checked and edited, and sequences were blasted against previously recorded *Symbiodinium* types on GenBank (<http://www.ncbi.nlm.nih.gov>). The ITS2 sequences generated in this study and previous studies were aligned using CLUSTAL W (Thompson et al., 1994). A phylogenetic reconstruction based on maximum parsimony (MP) and neighbor joining (NJ) was carried out using MEGA version 4.0 (Kumar et al., 2008). Sister lineages to clade C represented by F1 (*Symbiodinium kawagutii* AF333517) and F2 (AF333516) in clade F were used as outgroups. A bootstrap resampling was carried out for 500 replicates to assess relative branch support. A Bayesian tree estimation method was also employed using the program MrBayes version 3.1 (Huelsenbeck & Ronquist, 2001).

## 2 Results

A total of 11 distinctive *Symbiodinium* types were characterized by PCR-DGGE fingerprinting and sequencing of the ITS2 region, from 44 host species representing 20 genera (see Table 1). *Symbiodinium* type D1 and *Symbiodinium trenchi* (formerly D1a) were the two representatives of clade D. Several scleractinian hosts (11.4%), including *Acropora cerealis* (Dana, 1846), *A. microclados* (Ehrenberg, 1834), *Montipora grisea* (Bernard, 1897), *Galaxea fascicularis* (Linnaeus, 1767) and *Pocillopora damicornis* (Linnaeus, 1758) possessed type D1 or *S. trenchi*. The remaining nine types were members of clade C, which hosted by the vast majority of corals (88.6%). Among the sampled corals, *A. microclados*, *A. cerealis*, *P. damicornis* and *G. fascicularis* harbored clades C or D or simultaneously within a single colony ( $n = 3$  of 80; Table 1). All *Symbiodinium* types have been previously recorded in

other regions of the Pacific including the GBR, Okinawa, and Hawaii (LaJeunesse et al., 2003, 2004a, 2004b). Figure 2 shows the typical band profiles obtained, labelled with their ITS2 type (LaJeunesse, 2002; LaJeunesse et al., 2003). Heteroduplexes were usually produced from the DGGE analysis due to mismatching of variants during the PCR process (LaJeunesse et al., 2003). The ITS2 phylogeny inferred from MP is shown for *Symbiodinium* types in clade C, as there was divergence among clade C symbionts (Fig. 3). Maximum parsimony, NJ, and Bayesian inference methods yielded very similar trees (data not shown). C3 is the most basal among clade C sequences (c.f. LaJeunesse, 2002). Some branch connections within clade C varied slightly.

*Symbiodinium* community diversity at XP was relatively high with 0.67 symbiont types/host species compared to 0.53 (8 types in 15 species), 0.43 symbiont types/host species (7 types in 16 species), and 0.42 symbiont types/host species (10 types in 24 species) at XDH, XD, and LHT, respectively. At four locations in Sanya, the majority of host species sampled contained populations with single and same symbiont type. In some cases, host-symbiont associations surveyed from each location in Sanya were different. For instance, *P. damicornis* hosted type C1c at XD, but associated with D1 at both LHT and XDH.

Collectively, members of clade C were ecologically dominant in both reefs and found in numerous host taxa occurring at all locations in Sanya. *Symbiodinium* types C3, C1, C1c and C21a were the most prevalent among host species, whereas numerous others were host-specific and/or rare (Fig. 4). Examples include C17 with *M. truncata* (Zou, Song & Ma 1975), C31 with *M. mollis* (Bernard, 1897), and C3u with *Diploastrea heliophora* (Lamarck, 1816). These patterns were similar to the *Symbiodinium* community structure in the central and western Pacific (Table 1). The pandemic *Symbiodinium* type C3 associated with 21 species in Sanya; type C1, also a "host-generalist", was found in seven host species. Most coral species harbor the same symbiont type or a closely-related symbiont lineage across their range, including the present study sites, the central GBR, and the west Pacific (Table 1). However, some coral species associated with divergent types; for example, *M. mollis* harbored C58 in Okinawa (LaJeunesse et al., 2004b) but hosted C31 in Sanya.

## 3 Discussion

This is the first study to examine the diversity and community structure of *Symbiodinium* spp. populations

**Table 1** *Symbiodinium* types detected in host species from Luhuitou Bay (LHT), Ximao Island (XD), Xipai Island (XP), and Xiaodonghai Bay (XDH) in Sanya (this study) compared to those from Okinawa, Hawaii, and the Great Barrier Reef (GBR) (LaJeunesse et al., 2003, 2004a, 2004b)

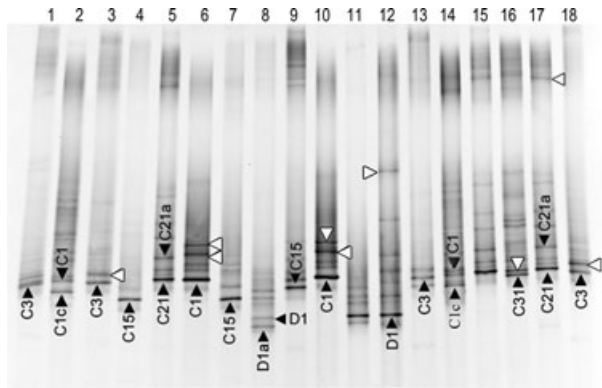
| Host species                               | LHT                | XD             | XP   | XDH   | Okinawa   | GBR, Hawaii      |
|--|--------------------|----------------|------|-------|-----------|------------------|
| <b>Acroporidae</b>                         |                    |                |      |       |           |                  |
| <i>Acropora valida</i> (h: 2) <sup>†</sup> | C3(2) <sup>‡</sup> | –              | –    | –     | C3i, C3   | C3               |
| <i>Acropora nasuta</i> (h: 1)              | –                  | C3             | –    | –     | –         | C3k, C3i, C3     |
| <i>Acropora hyacinthus</i> (h: 1)          | C3                 | –              | –    | –     | –         | C3k, C3          |
| <i>Acropora microclados</i> (h: 3)         | C3, C3/D1a         | –              | –    | C3    | –         | C3               |
| <i>Acropora digitifera</i> (h: 1)          | –                  | C3             | –    | –     | C3        | C3k, C3i         |
| <i>Acropora cerealis</i> (h: 1)            | –                  | –              | –    | C3/D1 | C3        | C1, C3k, C3i, C3 |
| <i>Acropora anthocercis</i> (h: 1)         | –                  | –              | –    | C3    | –         | –                |
| <i>Acropora abrotanoides</i> (h: 2)        | C3(2)              | –              | –    | –     | –         | –                |
| <i>Acropora intermedia</i> (h: 1)          | –                  | –              | C3   | –     | –         | –                |
| <i>Acropora formosa</i> (h: 1)             | –                  | –              | –    | C3    | –         | C3               |
| <i>Acropora humilis</i> (h: 1)             | C3                 | –              | –    | –     | –         | C3, C3k, C3i     |
| <i>Astreopora myriophthalma</i> (h: 2)     | C1(2)              | –              | –    | –     | C1        | –                |
| <i>Montipora mollis</i> (v: 2)             | C31(2)             | –              | –    | –     | C58       | –                |
| <i>Montipora grisea</i> (v: 1)             | –                  | D1a            | –    | –     | –         | C61              |
| <i>Montipora truncata</i> (2)              | C17                | –              | –    | C17   | –         | –                |
| <i>Montipora turgescens</i> (v: 1)         | C1c                | –              | –    | –     | –         | –                |
| <i>Pavona decussata</i> (h: 1)             | C1                 | –              | –    | –     | –         | –                |
| <b>Dendrophylliidae</b>                    |                    |                |      |       |           |                  |
| <i>Turbinaria mesenterina</i> (h: 1)       | –                  | –              | C1   | –     | –         | –                |
| <b>Faviidae</b>                            |                    |                |      |       |           |                  |
| <i>Cyphastrea serailia</i> (h: 1)          | C1                 | –              | –    | –     | –         | C1, C1i, C3      |
| <i>Diploastrea heliophora</i> (3)          | C3u                | C3u            | –    | C3u   | –         | C3               |
| <i>Favia speciosa</i> (h: 1)               | –                  | –              | –    | C3    | –         | C1               |
| <i>Favia matthaii</i> (h: 1)               | –                  | C1c            | –    | –     | C3        | –                |
| <i>Favia veroni</i> (3)                    | C3                 | C3             | C3   | –     | –         | –                |
| <i>Favites pentagona</i> (2)               | C21a               | –              | –    | C21a  | –         | –                |
| <i>Goniastrea aspera</i> (h/v: 1)          | C3                 | –              | –    | –     | –         | C3               |
| <i>Leptoria phrygia</i> (v: 1)             | –                  | –              | –    | C3    | –         | –                |
| <i>Leptastrea purpurea</i> (h: 1)          | –                  | C3             | –    | –     | –         | C1, C1b, C1f     |
| <i>Platygyra daedalea</i> (h: 7)           | C3                 | C3(4)          | C3   | C15   | –         | C3h, C3          |
| <i>Platygyra sinensis</i> (h: 2)           | –                  | C21a           | –    | C3    | –         | –                |
| <i>Platygyra lamellina</i> (h: 1)          | –                  | C3             | –    | –     | –         | C3               |
| <i>Platygyra crosslandi</i> (1)            | –                  | C3             | –    | –     | –         | –                |
| <i>Platygyra pini</i> (h: 2)               | –                  | –              | –    | C3(2) | –         | C3, C3h          |
| <b>Merulinidae</b>                         |                    |                |      |       |           |                  |
| <i>Hydnophora exesa</i> (2)                | C21a               | C21a           | –    | –     | C21a, C27 | C3, C3h          |
| <i>Hydnophora contignatio</i> (1)          | C21a               | –              | –    | –     | –         | –                |
| <b>Mussidae</b>                            |                    |                |      |       |           |                  |
| <i>Symphyllia radians</i> (h: 1)           | –                  | –              | C21a | –     | C21a      | C3, C40          |
| <i>Symphyllia agaricia</i> (1)             | C1d                | –              | –    | –     | –         | C3               |
| <b>Oculinidae</b>                          |                    |                |      |       |           |                  |
| <i>Galaxea fascicularis</i> (h: 5)         | –                  | D1a(3), D1a/C1 | D1a  | –     | C21a      | C1, D1a          |
| <b>Pocilloporidae</b>                      |                    |                |      |       |           |                  |
| <i>Pocillopora damicornis</i> (v: 4)       | D1                 | C1c            | –    | D1(2) | C1c       | C1j, C1c, C1d    |
| <i>Pocillopora verrucosa</i> (v: 5)        | C1c                | C1c(2)         | C1c  | C1c   | –         | C1c              |
| <b>Poritidae</b>                           |                    |                |      |       |           |                  |
| <i>Goniopora duofasciata</i> (2)           | C1(2)              | –              | –    | –     | –         | –                |
| <i>Goniopora planulata</i> (h: 1)          | C3                 | –              | –    | –     | –         | –                |
| <i>Porites lutea</i> (v: 3)                | –                  | C15(2)         | C15  | –     | C15       | –                |
| <b>Siderastreidae</b>                      |                    |                |      |       |           |                  |
| <i>Psammocora contigua</i> (2)             | C1                 | –              | –    | C1    | –         | C1               |
| <i>Psammocora digitata</i> (1)             | –                  | –              | C1c  | –     | –         | C1               |

<sup>†</sup>Numbers in parentheses indicate the number of colonies independently sampled. <sup>‡</sup>“Types” separated by solidus were identified together in the same sample. Numbers in parentheses indicate the number of colonies in which a symbiont was found. –, Not found; v, Vertical transmitter; h, Horizontal transmitter (Richmond & Hunter, 1990; Baird et al., 2009).

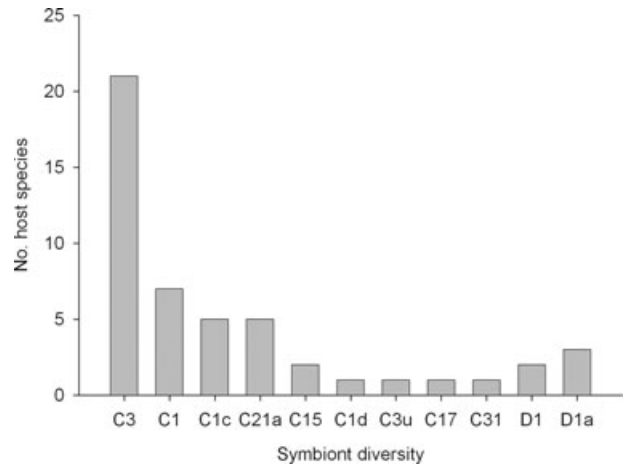
over common reef-building coral assemblage from the SCS at the subcladal level. These data indicate that symbiont diversity is relatively low and the pattern of associations in southern Hainan Island, SCS, is similar to other parts of the Pacific, although, as one of the largest marginal sea in the Pacific, its geography is distinctive.

### 3.1 Diversity of *Symbiodinium* in Sanya

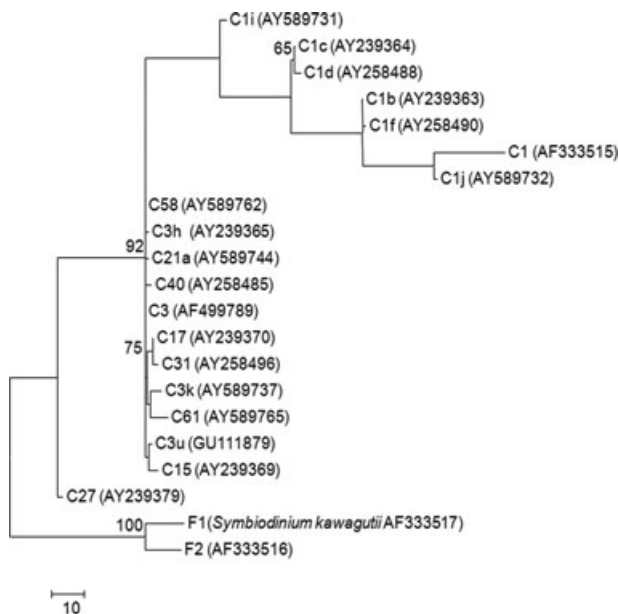
The dominance of clade C *Symbiodinium* types in Sanya in the present study is consistent with their prevalence in cnidarian symbiosis in the Indo-Pacific (Baker, 2003; LaJeunesse et al., 2003, 2004a, 2004b), as well as corals in LHT of Hainan Island, the Xisha



**Fig. 2.** Negative image of typical polymerase chain reaction–denaturing gradient gel electrophoresis profiles indicating presence of more than one symbiont type. Black arrowheads indicate the prominent bands diagnostic of a particular profile that were excised and sequenced. Bands in the upper part of a profile are heteroduplexes that form during polymerase chain reactions (white arrowheads). Each *Symbiodinium* is named according to its internal transcribed spacer 2 sequence.



**Fig. 4.** Prevalence of *Symbiodinium* types identified by polymerase chain reaction–denaturing gradient gel electrophoresis from 44 different host species in Sanya (Hainan Island, South China Sea, China).



**Fig. 3.** Phylogenetic reconstruction of internal transcribed spacer 2 genes from different symbionts within clade C detected in the reef-building corals from this study and previous studies (LaJeunesse et al., 2003, 2004a, 2004b) using maximum parsimony. Types F1 and F2 were used as outgroups. Corresponding GenBank Accession Numbers are provided next to each symbiont type.

Islands, and Zhubi Reef of the Nansha Islands from the SCS (Huang et al., 2006; Dong et al., 2008, 2009). *Symbiodinium* types C3, C1c (containing C1) and C21a were the most prevalent among host species in the present study and occupied tissues from a wide range of host taxa as generalists across the Pacific (Fig. 4, LaJeunesse et al., 2003, 2004a). These *Symbiodinium* types within

clade C are thought to have evolved from pandemic generalist types C1 and C3 that are distributed globally, and appear to represent the most ecologically important group in the Indo-Pacific (Baker, 2003; LaJeunesse et al., 2004a; LaJeunesse, 2005).

The patterns of symbiont distribution are influenced by the mode of symbiont acquisition (Barneah et al., 2004; Thornhill et al., 2006a; Stat et al., 2008). Symbiosis in corals that acquire symbionts directly from their parent (vertical transmission) are thought to be more specific and stable compared to hosts that acquire their symbionts from the extra environment (horizontal transmission) (LaJeunesse et al., 2008; Stat et al., 2008; Baird et al., 2009). At Sanya, approximately 76% of the coral species sampled (with known transmission strategies) acquire symbionts by horizontal transmission and thus have the opportunity to associate with a range of symbionts available from environmental sources. There is a strong host specificity reflected by many of the horizontal strategists associating with the pandemic host-generalist types C1 or C3. LaJeunesse et al. (2004a) showed that coral hosts from Hawaii harbored a high number of unique symbiont types, perhaps due to the predominance of coral hosts that are vertical transmitters in Hawaiian reefs. Nevertheless, van Oppen (2004) found that the mode of symbiont transmission did not affect symbiont diversity in acroporid corals. However, these results have to be carefully interpreted since it is derived methodological issues. Further study is required on the unambiguous relationships between symbiont diversity and the transmission mode of host corals.

Despite the distinct environment and geography of the SCS, there were no specialist symbionts and

lower genetic diversity of *Symbiodinium* in Sanya relative to other regions of the Pacific, including the GBR and Hawaii (LaJeunesse et al., 2003, 2004a, 2004b). Several possible interpretations for the low diversity are given below. First, the coral sampled from Sanya has a high proportion of host taxa in the genera *Acropora* and *Platygyra* corals that acquire their symbionts from the extra environment and may have common symbionts. Secondly, the low sample sizes and restricted sampling of coral diversity in the current study could underestimate the real diversity of *Symbiodinium* in this region. Finally, it is worthwhile noting that there is potential for additional diversity if more bands (such as faint DGGE bands) in DGGE profiles were sequenced.

### 3.2 Biogeographical distribution of *Symbiodinium*

Previous studies indicate that various *Symbiodinium* show differences in geographic distribution (LaJeunesse, 2002; LaJeunesse et al., 2003, 2010a). At the cladal level, the Pacific hosts associate predominantly with *Symbiodinium* clade C, whereas in the Caribbean, symbiosis involving *Symbiodinium* clades A and B is more common (LaJeunesse, 2002).

In contrast to the Caribbean, only symbiont types belonging to clades C and D were found in scleractinian corals from the SCS, western Pacific. A comparison of symbionts from Sanya with the same host species from the GBR, Hawaii, and Okinawa indicates that the Central Pacific province contains many types not found in the western Pacific (Table 1) (LaJeunesse et al., 2003, 2004a, 2004b). The connectivity and short geographic distance between the SCS and Okinawa may partly explain the similar dominance of *Symbiodinium* in both regions. However, type C27 is widely distributed throughout the Pacific, including Hawaii and Okinawa, but is rare in the southern GBR and absent in Sanya (LaJeunesse et al., 2003, 2004b). This example indicates that some symbiont types may have more restricted geographic distribution and are possibly endemic (Finney et al., 2010; LaJeunesse et al., 2010a). Furthermore, it is important to note that the diversity of *Symbiodinium* in the SCS is not well characterized as well as in the entire ocean environment.

Patterns of association between coral and *Symbiodinium* can vary across scales, ranging from local scales to wide geographic regions (Rodríguez-Lanetty et al., 2001; van Oppen et al., 2005; Huang et al., 2011). Recent population genetic studies indicate a high degree of endemism and limited gene flow among reefs (Howells et al., 2009; Thornhill et al., 2009). Relatively high overlap in host-symbiont partnerships can be seen among Sanya in the present study and Hawaii, the GBR, and

Okinawa. For example, *A. cerealis* hosts *Symbiodinium* type C3 in the central GBR, Okinawa, and Sanya. Importantly, most corals appear to associate stably with a particular symbiont regardless of external environmental factors (Thornhill et al., 2006b; Sampayo et al., 2008). However, there are some exceptions, for example, coral *A. cerealis* hosts *Symbiodinium* types C1, C3w, C3i, and C3k at the GBR, only type C3 at Okinawa (LaJeunesse et al., 2004b), but simultaneously associates with C3 and D1 in Sanya in the present study. Silverstein et al. (2011) recently reported that novel symbiont types were found in reef corals of Western Australia. Realistically, more detailed information regarding *Symbiodinium* diversity in uncovered regions, especially in the western Pacific, are vital to our understanding of the ecology and biogeography of *Symbiodinium*.

### 3.3 Ecological implications

The diversity of *Symbiodinium* may contribute to the resilience of symbiosis in the face of changes in environmental conditions (Baker et al., 2004; Berkelmans & van Oppen, 2006; LaJeunesse et al., 2010a). Indeed, distinct *Symbiodinium* types have been linked to differences in holobiont (coral and symbionts) performances such as thermal resistance, disease susceptibility, and photophysiology (Jones et al., 2008; Sampayo et al., 2008). The members of *Symbiodinium* D, presumed to be a stress-tolerant clade, are reported to predominate in corals from environments of high temperature and salinity, and significant terrestrial impacts such as turbidity (Toller et al., 2001; LaJeunesse et al., 2003; Rowan, 2004; Thornhill et al., 2006b). Most recently, LaJeunesse et al. (2010b) found that corals in the genus *Pocillopora* harboring *Symbiodinium* C1b-c were bleached in the Gulf of California during an extreme cold-water event, whereas colonies contained *Symbiodinium* D1 were mostly unaffected. In this survey, the ecologically dominant corals *G. fascicularis* and *P. damicornis* associate with clade D *Symbiodinium*, which might partly explain the levels of resistance and resilience, respectively, we observed in Sanya (Huang et al., 2006, unpublished data). Interestingly, there is increasing evidence to suggest that certain clade C *Symbiodinium* types are also thermally tolerant (Jones et al., 2008; Sampayo et al., 2008). For instance, the coral *Porites lutea* (Milne Edwards & Haime, 1851) possessed C15 in Sanya. Like other species from the genus *Porites*, these are among the most tolerant corals worldwide (LaJeunesse et al., 2003). However, it has been speculated how coral-symbiont partnerships will fare with changes in their environment, either by symbiont shuffling (Buddemeier & Fautin, 1993; Berkelmans & van Oppen, 2006; Jones et al., 2008) or by differential

mortality of holobiont combinations (Sampayo et al., 2008).

#### 4 Conclusions

In the present study, within scleractinian corals sampled from Sanya in the SCS, 11 distinct symbiont types belonging to *Symbiodinium* clades C and D were identified, but no novel types detected. Reef-building corals almost exclusively hosted *Symbiodinium* belonging to clade C and dominated by C1 and C3; fewer corals possessed *Symbiodinium* type D1 or *S. trenchi*. Furthermore, we found that the symbiont diversity in southern Hainan Island was similar, and relatively low, compared with both the GBR and Hawaii symbiont assemblages. The results of this study indicate that reef corals in Sanya may be more vulnerable to a changing environment than in other regions of the Pacific, due to the low diversity of *Symbiodinium*. These data make a fundamental and important contribution to our understanding of the biogeography and ecology of *Symbiodinium* across the Pacific.

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