

Diversity of symbiotic algae of the genus *Symbiodinium* in scleractinian corals of the Xisha Islands in the South China Sea

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Abstract Symbiotic algae (*Symbiodinium* sp.) in scleractinian corals are important in understanding how coral reefs will respond to global climate change. The present paper reports on the diversity of *Symbiodinium* sp. in 48 scleractinian coral species from 25 genera and 10 families sampled from the Xisha Islands in the South China Sea, which were identified with the use of restriction fragment length polymorphism (RFLP) of the nuclear ribosomal DNA large subunit gene (rDNA). The results showed that: (i) *Symbiodinium* Clade C was the dominant zooxanthellae in scleractinian corals in the Xisha Islands; (ii) *Symbiodinium* Clade D was found in the corals *Montipora aequituberculata*, *Galaxea fascicularis*, and *Plerogyra sinuosa*; and (iii) both *Symbiodinium* Clades C and D were found simultaneously in *Montipora digitata*, *Psammocora contigua*, and *Galaxea fascicularis*. A poor capacity for symbiosis polymorphism, as uncovered by RFLP, in the Xisha Islands indicates that the scleractinian corals have low adaptability to environmental changes. Further studies are needed to investigate zooxanthellae diversity using other molecular markers.

Key words global changes, restriction fragment length polymorphism (RFLP), scleractinian coral, South China Sea, symbiotic algae (*Symbiodinium* sp.), Xisha Islands.

The increased global frequency and severity of coral bleaching events indicate that the coral reef ecosystem is fast approaching a critical survival threshold (Hoegh-Guldberg, 1999). Until recently, both the genetic and functional diversity of symbiotic algae indicated that symbiotic algae have a key role in contributing to our understanding of coral reef bleaching episodes (Baker, 2001). Therefore, research on diversity, biogeography, and ecology of *Symbiodinium* sp. in coral reefs has been undertaken. It is difficult to culture these microalgae to obtain morphological descriptions (Rowan & Powers, 1991a), so molecular DNA techniques have been used to qualify, classify, and study the distribution of the *Symbiodinium* genus (reviewed in Baker, 2003; Coffroth & Santos, 2005; Pochon et al., 2006). *Symbiodinium* sp. have been classified into four groups (A, B, C, D) on the basis of polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) patterns of 18S and 28S ribosomal (r) DNA (Rowan & Powers, 1991a; Chen et al., 2005). For example, 28S rDNA was used to investigate the diversity of symbiotic

algae of scleractinian corals living in the sea area around Taiwan Island, with results indicating that most of the *Symbiodinium* sp. within the scleractinian corals consisted of *Symbiodinium* Clade C, except eight species from Clade D (Chen et al., 2005). Phylogenetic analyses based on 28S rDNA showed that there were four major clades of zooxanthellae (i.e. Clades A, B, C, and D of *Symbiodinium*), which is in accord with data inferred from RFLP patterns (Chen et al., 2005).

Studies on biogeographical distributions of symbiotic algae in scleractinian corals have been conducted worldwide (Baker, 2003; Coffroth & Santos, 2005). For example, *Symbiodinium* Clades A, B, and C are common in scleractinian corals in the Caribbean oceans (LaJeunesse, 2002). Previous studies have shown that in the Indo-Pacific oceans, scleractinian corals predominantly harbor *Symbiodinium* Clade C and occasionally *Symbiodinium* Clade D (Baker, 2003). However, only a few studies have been performed on the diversity of symbiotic algae within scleractinian corals in the South China Sea. For example, Chen et al. (2003) examined the clade identification of *Symbiodinium* sp. sampled from the scleractinian coral *Oulastrea crispata* in the Sanya fringing reef of the South China Sea and Huang et al. (2006) investigated the diversity of symbiotic algae in 10 host scleractinian coral species in the Zhubi Coral Reef of the Nansha Islands.

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In the present study, 48 scleractinian coral species were sampled from the Xisha Islands, which are typical of islands within the tropical zone of the South China Sea, and symbiont diversity was examined using RFLP of 28S rDNA.

1 Material and methods

1.1 Study area

The Xisha Islands, located 330 km south-east of Hainan Island, make up the largest archipelago in the South China Sea, with an open water area of approximately 5×10^5 km². Based on data from the Yongxing Island Observatory, the sea surface temperature is highest between May and September, with a monthly average temperature ≥ 29 °C. From January to April, the sea surface temperature increases gradually from a minimum of 24 °C, whereas from October to December the sea surface temperature decreases gradually to a minimum of 24.5 °C. The salinity of the Xisha Islands is relatively high, beyond 33.4 p.p.t.

1.2 Coral collection and coral tissue preparation

Coral samples were collected at 0–35 m below the sea surface in the Xisha Islands in May 2005 and May 2006 using SCUBA diving techniques. Fragments of coral tissue were placed in labeled bags and preserved in 95% (v/v) ethanol for DNA extraction.

1.3 DNA isolation

Zooxanthellae were isolated using a modification of the methods described by Rowan and Powers (1991b) and Chen et al. (2003). For isolation from skeletonized material, tissue was homogenized in zooxanthellae buffer (ZB). The resulting homogenates were filtered through 125- μ m mesh to remove large pieces of animal tissue before recovering the zooxanthellae by centrifugation at 10 000 g for 1 min. The brown cell pellets were washed three to five times with 10-mL aliquots of ZB and once in DNA isolation buffer (DNAB; 0.4 mol/L NaCl and 50 mmol/L EDTA, pH 8.0). The cell pellets were then resuspended in 0.75 mL DNAB containing 1% sodium dodecyl sulfate (SDS) and heated to 65 °C for 30 min. Proteinase K was then added to a final concentration of 0.5 mg/mL prior to incubation at 50 °C for at least 6 h. Subsequently, samples were extracted with phenol–chloroform and DNA was precipitated with absolute ethanol and 0.3 mol/L sodium acetate overnight at –20 °C. Precipitated DNA was resuspended in 100- μ L aliquots of water and stored at –20 °C.

1.4 PCR amplification

An approximate 520-bp fragment corresponding to the 5' end of the nuclear encoded 28S rDNA gene of symbiotic algae was amplified using PCR. Amplifications were performed with 0.05 mmol/L oligonucleotide primers 28SzooxF (5'-CCTCAGTAATGGCGAATGAAC-3') and 28SzooxR (5'-CCTTGGTCCGTGTTTCAAGA-3') and 20 ng total DNA in 50 μ L PCR buffer (1 \times PCR buffer (Mg²⁺ free)), dimethylsulphoxide (DMSO), 3 mmol/L MgCl₂, 0.1 mmol/L dNTPs, and 0.5 U *Taq* DNA polymerase (Promega, Madison, WI, USA). The PCR amplification conditions consisted of an initial 3 min denaturation at 95 °C, followed by a cycling profile of 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 2 min for 30 cycles, with a final 10 min extension at 72 °C. The PCR products were electrophoresed in a 1% agarose gel, using a 1 \times TAE buffer (40 mmol/L Tris–acetic acid, 2 mmol/L EDTA, pH 8.5) to assess the yield. The amplified DNA was extracted once with chloroform, precipitated with ethanol at –20 °C and then resuspended in a TE buffer (10 mmol/L Tris-HCL, 1 mmol/L EDTA, pH 8.0).

1.5 RFLP

For RFLP analysis, the PCR products were digested using *Rsa*I for 12 h. Restriction fragments were examined by electrophoresis on 2% low melting temperature agarose (Bio Whittaker Molecular Applications, Rockland, ME, USA) mixed with 1% agarose (FMC BioProduct, Rockland, ME, USA).

2 Results

Products of approximately 520 bp were yielded from all 48 coral species with the PCR amplification of symbiotic algae partial 28S rDNA genes.

Two RFLP patterns were observed, as described by Chen et al. (2005). *Symbiodinium* Clade C was identified with an RFLP pattern of 320/200 bp for *Rsa*I, whereas *Symbiodinium* Clade D was identified with an RFLP pattern of 220/200/100 bp for *Rsa*I. According to the standard electrophoresis figure (Fig. 1), the symbiotic algae of the genus *Symbiodinium* in scleractinian corals collected from the Xisha Islands in the South China Sea were identified at the clade level. A summary of coral species, living depth, and *Symbiodinium* clades is given in Table 1. The results showed that *Symbiodinium* Clade C was found in all coral species sampled from the Xisha Islands in the South China Sea. *Symbiodinium* Clade D was found in three coral species, namely *Montipora aequituberculata*, *Galaxea fascicularis*, and *Plerogyra sinuosa*; whereas

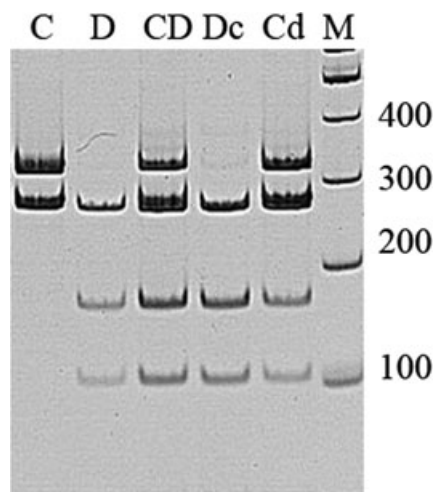


Fig. 1. Standard electrophoresis of *RsaI* restriction fragment length polymorphism analyses of partial 28S ribosomal (r) DNA genes. C, *Symbiodinium* Clade C; D, *Symbiodinium* Clade D; CD, Dc, and Cd, a mixture of *Symbiodinium* Clades C and D; M, DNA ladder.

the two variant genotypes *Symbiodinium* Clades D and C occurred simultaneously in colonies of the scleractinian coral *Montipora digitata*, *Psammocora contigua*, and *Galaxea fascicularis* sampled in the present study.

3 Discussion

3.1 Symbiont diversity of scleractinian corals in the South China Sea

Species diversity of scleractinian corals in the Xisha Islands of the South China Sea has been investigated by Zou since 1975, resulting in the identification of 127 coral species from 38 genera in the Xisha Islands (Zou, 2001). However, the diversity of the zooxanthellae has received considerably less attention than their scleractinian coral hosts because of difficulties in distinguishing species based on morphological characteristics. More recently, the reliable identification of *Symbiodinium* sp. has been enabled by RFLP analysis or sequence-based phylogenetic reconstruction of rDNA genes (Rowan & Powers, 1991a, b; Rowan et al., 1997; Carlos et al., 1999; Darius et al., 2000; Chen et al., 2005).

In the present study, all 48 coral species examined were found to harbor *Symbiodinium* Clade C. Dong et al. (2008a) examined the diversity of symbiotic algae in 22 species of scleractinian corals from 14 genera and eight families in the Luhuitou fringing reef of Sanya, and found that 18 species formed an association with *Symbiodinium* Clade C (Table 2). In Dongshan bay in Fujian

Table 1 Taxonomic information, colonies, *Symbiodinium* clades, and living depth of zooxanthellae isolated from scleractinian corals collected in the Xisha Islands of the South China Sea

Scleractinian coral	<i>n</i>	Depth (m)	Clade
Family Pocilloporidae			
<i>Pocillpora meandrina</i> Dana	5	5–10	C
<i>Pocillpora damicornis</i> Linnaeus	4	15–20	C
<i>Pocillpora verrucosa</i> Ellis & Solander	1	0–5	C
<i>Seriatorpora hystrix</i> Dana	1	15–20	C
<i>Stylophora pistillata</i> Esper	3	15–20	C
Family Acroporidae			
<i>Montipora monasteriata</i> Forskal	3	15–20	C
<i>Montipora aequituberculata</i> Bernard	3	5–10	C; D
<i>Montipora tuberculosa</i> Lamarck	3	25–30	C
<i>Montipora digitata</i> Dana	2	5–10	C; C and D
<i>Montipora Foliosa</i> Pallas	3	5–10	C
<i>Montastrea annuligera</i> Edwards & Haime	1	15–20	C
<i>Astropora listeri</i> Bernard	2	25–30	C
<i>Acropora robusta</i> Dana	3	0–5	C
<i>Acropora pulchra</i> Brook	3	0–5	C
<i>Acropora digitifera</i> Dana	2	0–5	C
<i>Acropora secale</i> Studer	2	0–5	C
<i>Acropora elseyi</i> Brook	1	0–5	C
<i>Acropora nasuta</i> Dana	3	0–5	C
<i>Acropora acuminata</i> Verrill	3	5–10	C
<i>Acropora cerealis</i> Dana	3	5–10	C
<i>Acropora microphthalma</i> Verrill	1	0–5	C
<i>Acropora muricata</i> Dana	2	0–5	C
<i>Acropora subulata</i> Dana	1	5–10	C
<i>Acropora abrotanoides</i> Lamarck	3	5–10	C
Family Fungiidae			
<i>Fungia scabra</i> Duederlein	3	15–20	C
<i>Fungia granulose</i> Klunzinger	3	25–30	C
<i>Herpolitha limax</i> Esper	2	15–20	C
Family Siderastreaeidae			
<i>Psammocora contigua</i> Esper	3	15–20	C and D
Family Agariciidae			
<i>Pavona varians</i> Verrill	2	5–10	C
<i>Leptoseris explanata</i> Yabe & Sugiyama	2	35	C
<i>Pachyseris rugosa</i> Lamarck	1	5–10	C
Family Oculinidae			
<i>Galaxea paucisepta</i> Claereboudt	3	0–5	C
<i>Galaxea fascicularis</i> Linnaeus	15	0–35	C; D; C and D
<i>Galaxea horrescens</i> Dana	3	15–20	C
<i>Hydnophora contignatio</i> Forskal	2	0–5	C
<i>Hydnophora rigida</i> Dana	1	0–5	C
<i>Merulina scabricula</i> Dana	3	0–5	C
Family Faviidae			
<i>Favia fava</i> Forskal	1	0–5	C
<i>Echinopora lamellose</i> Esper	1	0–5	C
<i>Platygyra pini</i> Chevalier	1	5–10	C
<i>Platygyra daedalea</i> Ellis & Solander	1	5–10	C
<i>Leptastrea purpurea</i> Dana		0–5	C
Family Mussidae			
<i>Lobophyllia hemprichii</i> Ehrenberg	2	25–30	C
Family Pectiniidae			
<i>Pectinia alvicornis</i> Kent	2	0–5	C
Family Caryophylliidae			
<i>Euphyllia ancora</i> Veron & Pichon	2	25–30	C
<i>Euphyllia glabrescens</i> Chamisso & Eysenhardt	3	25–30	C
<i>Plerogyra sinuosa</i> Dana	3	35	C; D
<i>Physogyra lichtensteini</i> Edwards & Haime	1	15–20	C

Table 2 Published reports of *Symbiodinium* clades from scleractinian corals in the Indo-Pacific oceans

Location	Clade A	Clade B	Clade C	Clade D	Multiple clades	References
Zamami Island, Okinawa, Japan	0	0	64	1	1	LaJeunesse et al., 2004b
Cheju Island, Korea	1	0	1	0	0	Rodriguez-Lanetty et al., 2002
Penghu Island and Kenting, Taiwan, China	0	0	45	2	6	Chen et al., 2005
Dongshan Island, China	0	0	3	0	0	Dong et al., 2008a
Luhuitou fringing reef, China	0	0	18	2	2	Dong et al., 2008b
Xisha Islands, China	0	0	48	3	3	Present study
Zhubi coral reef of the Nansha Islands, China	0	0	7	1	1	Huang et al., 2006
Central Great Barrier Reef, Australia	0	0	118	6	4	LaJeunesse et al., 2004b
Southern Great Barrier Reef, Australia	0	0	71	2	1	LaJeunesse et al., 2003
Hawaiian Islands, USA	0	0	23	2	0	LaJeunesse et al., 2004a
Kenya	1	0	4	3	1	Visram & Douglas, 2006

province, Dong et al. (2008b) investigated the molecular taxonomy and diversity of symbiotic algae in three dominant scleractinian corals and concluded that all coral species harbored *Symbiodinium* Clade C on the basis of RFLP patterns of the nuclear large-subunit rDNA. Moreover, sequencing of PCR-amplified fragments of internal transcribed spacer (ITS) rDNA genes showed that the Clade C sequences were clustered with a known group *Symbiodinium* Subclade C1 (Table 2; Dong et al., 2008b). Therefore, it can be suggested that *Symbiodinium* Clade C may be the dominant zooxanthellae clade in the South China Sea. This is in accordance with results of previous studies conducted in the Indo-Pacific oceans (Table 2, Rodriguez-Lanetty et al., 2002; LaJeunesse et al., 2003, 2004a, b; Chen et al., 2005; Visram & Douglas, 2006).

Symbiodinium Clade D appears to be uncommon. Various studies have suggested that *Symbiodinium* Clade D may be a relatively more stress tolerant zooxanthellae than other clades of the genus *Symbiodinium* (Chen et al., 2003; Rowan, 2004; Berkelmans & van Oppen, 2006). However, *Symbiodinium* Clade D does not appear to be the dominant symbiont of scleractinian corals in most of the oceans surveyed so far, except in the Persian Gulf (Lien et al., 2007). In the present survey, the scleractinian corals *Montipora aequituberculata*, *Galaxea fascicularis*, and *Plerogyra sinuosa* were found to host *Symbiodinium* Clade D. A previous study in the Zhubi Coral Reef of the Nansha Islands showed that *Goniastrea aspera* and *Acropora formosa* harbored *Symbiodinium* Clade D (Huang et al., 2006). *Symbiodinium* Clade D was the dominant zooxanthellae in *Pocillpora damicornis*, *Favia fava*, *Galaxea fascicularis*, and *Acropora horrida* in the Luhuitou fringing reef of Sanya, Hainan Island (Dong et al., 2008a). These results suggest that *Symbiodinium* Clade D is not specific to any particular host species and appears to have a somewhat haphazard distribution (LaJeunesse, 2002; Baker, 2003).

3.2 Symbiosis polymorphism

Symbiosis polymorphism describes coral species that can host multiple zooxanthellae clades not only within the same coral colonies, but also within the various coral colonies over various depths on the same reef, in different geographical areas, or sampled over different times (Goulet, 2006). Previously, it was widely accepted that each scleractinian coral could harbor only one algal symbiont (Rowan & Powers, 1991a; Trench, 1993). However, more recent research suggests that some scleractinian coral species can associate with multiple clades of zooxanthellae in single coral colonies (Rowan & Knowlton, 1995; Baker, 2001; Chen et al., 2005).

Whether each coral species can host multiple species of zooxanthellae remains unknown. Goulet (2006) analyzed data on 442 coral species from 43 studies and revealed that only 23% of coral species had the ability to host multiple zooxanthellae clades. Scleractinian corals that can host multiple clades may demonstrate a shift or switch in clade composition with environmental changes. However, the phenomena of clade composition shifts or switches do not occur in most scleractinian corals. It was concluded by Goulet (2006) that if global climate warming continues, coral reefs may undergo a change in biodiversity with the exception of one subset of symbiotic corals. However, another study (Baker & Romanski, 2007) suggested that scleractinian corals commonly host multiple symbiotic partnerships based on a re-evaluation of the data analyzed by Goulet (2006). One of the most powerful reasons for this is the fact that the coral species investigated to date have been severely undersampled (sample size <5), whereas 75% of coral species sampled frequently (sample size >10) harbored multiple *Symbiodinium* clades or multiple *Symbiodinium* subclades within the same clade (Baker & Romanski, 2007).

During the investigation of the diversity of symbiotic algae of the genus *Symbiodinium* in

scleractinian corals of the South China Sea, it was found that only a few coral species examined could harbor multiple zooxanthellae clades simultaneously or sequentially in the Xisha Islands, in the Zhubi coral reef of the Nansha Islands, in the Luhuitou fringing reef of Sanya, and in the Dongshan bay of Fujian province (Huang et al., 2006; Dong et al., 2008a, b; present study). In the present study, we found that the corals *Montipora aequituberculata*, *Galaxea fascicularis*, and *Plerogyra sinuosa* harbored two clades of the genus *Symbiodinium*. In addition, four colonies of *Pocillpora damicornis* were found to harbor *Symbiodinium* Clade C in the Xisha Islands, whereas one colony of *Pocillpora damicornis* was found to harbor *Symbiodinium* Clade D in the Luhuitou Fringing reef of Sanya (Dong et al., 2008a). In comparison, many more coral species have been found to be able to host multiple zooxanthellae clades worldwide. For example, *Pocillpora damicornis* collected in the Xisha Islands harbored only *Symbiodinium* Clade C (present study), but *Pocillpora damicornis* sampled in the Society Archipelago of French Polynesia and Okinawa, Japan, was found to harbor *Symbiodinium* Clades D and A (Magalon et al., 2007). These results imply that geographically distinct varieties of zooxanthellae clades within the same scleractinian coral are likely to be associated with physiological differences in the zooxanthellae (Rodriguez-Lanetty et al., 2001).

More recently real-time PCR assays have demonstrated the presence of background or cryptic *Symbiodinium* clades (Mieog et al., 2007). Therefore, further investigations are required to identify symbiosis polymorphism using these techniques.

3.3 Implications for the survival of the South China Sea coral reefs

Symbiodinium Clade C has been found in scleractinian corals globally, and is particularly abundant in the Indo-Pacific oceans (Baker, 2003). However, *Symbiodinium* Clade C seems to be susceptible to coral bleaching events, being more vulnerable than other *Symbiodinium* clades (Pochon et al., 2006). Although it has been proposed that scleractinian coral may obtain symbiotic algae that are better suited to the changing environment by shuffling or switching of partners, there is little real indication of this occurring in field surveys (Buddemeier & Fautin, 1993; Goulet, 2006). If it does occur, the predominance of *Symbiodinium* Clade C in the most common coral species of the coral reefs in the South China Sea may not survive future global warming events that lead to coral bleaching.

4 Conclusions

The present study has demonstrated that *Symbiodinium* Clade C is the dominant zooxanthellae in the South China Sea. *Symbiodinium* Clade D appears to be uncommon and not specific to any particular host species. Symbiosis polymorphism seems to be uncommon for scleractinian corals in the South China Sea compared with those worldwide.

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