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Temporal stability of *Symbiodinium* phylotype in scleractinian coral *Galaxea fascicularis* from a tropical fringing reef in the South China Sea*

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Abstract *Symbiodinium* sp. occurs in a symbiotic association with various marine invertebrates, including the scleractinian corals. Understanding the flexibility and specificity in coral-algal symbiosis can have important implications for predicting the future of coral reefs in the era of global climate change. In the present study, we conducted *Symbiodinium* phylotype analysis, based on polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP), in the scleractinian coral, *Galaxea fascicularis*, from a tropical fringing reef in Hainan Island, over a 1-yr period. Our results showed that *Galaxea fascicularis* could associate with *Symbiodinium* clade C and D either individually or simultaneously. However, during the sampling period, the *Symbiodinium* phylotype did not change significantly in the scleractinian coral *Galaxea fascicularis*, although the seawater temperature decreased sharply in the winter season. This study further suggests that the shift in *Symbiodinium* communities in response to seasonally fluctuating environments might not be a universal feature of coral-algal associations.

Keyword: coral; *Symbiodinium*; symbiosis; South China Sea

1 INTRODUCTION

Symbiotic algae of the genus *Symbiodinium* supply up to 95% of the host coral's energy requirements, and play an important role in the coral reef ecosystem (Muscatine, 1990). Because of the limited morphological variation, early studies suggested that symbiotic algae of the scleractinian coral belonged to a single dinoflagellate species, *Symbiodinium microadriaticum* (Freudenthal, 1962). However, recent studies revealed that the genus *Symbiodinium* consists of at least nine major clades (A-I), based on the analysis of nuclear ribosomal DNA and chloroplast large subunit (cp23S) rDNA genes (Rowan and Powers, 1991; van Oppen et al., 2001; Santos et al., 2002; Pochon and Gates, 2010). Among these, six clades (A–D, F and G) are currently known to associate with the scleractinian corals (van Oppen et al., 2001, 2005; Baker, 2003), and clades A, C and D are predominant (Baker, 2003; Goulet, 2006).

The holobionts (coral and *Symbiodinium*) are sensitive to environmental factors such as elevated sea surface temperature, altered irradiance and the presence of pollutants and other environmental changes (Glynn, 1996; Brown et al., 2000; Douglas, 2003). The loss of *Symbiodinium* or photosynthetic pigments is known as "coral bleaching" and may cause coral death and reef degradation if they are not recovered in a short time (Hoegh-Guldberg, 1999; Glynn et al., 2001). Bleaching susceptibilities are variable, not only among coral species, but also among conspecific populations in different areas (Hoegh-Guldberg and Salvat, 1995; Berkelmans and Oliver, 1999; Loya et al., 2001). Several studies

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have proved that the different clades of *Symbiodinium* in the same coral species have distinctly physiological and ecological responses to environmental changes (Baker, 2001; Diekmann et al., 2002; Rowan, 2004; Berkelmans and van Oppen, 2006). Therefore, the symbiotic algae of the genus *Symbiodinium* are thought to play an important role in the adaption or acclimatization of the host corals to environmental changes (Baker, 2003). For example, environmental changes may induce a shift in *Symbiodinium* communities by providing an opportunity for a previously rare phylotype of *Symbiodinium* to become dominant in the scleractinian coral, or by the coral attaining a new phylotype of *Symbiodinium* from the environment (Baker, 2003). The composition of *Symbiodinium* phylotypes in a single colony may also fluctuate with a particular perturbation (Chen et al., 2005; Thornhill et al., 2006a), but stability in coral and *Symbiodinium* associations has also been showed in previous studies (Thornhill et al., 2006b; Goulet et al., 2008; Sampayo et al., 2008; Stat et al., 2009). Therefore, it remains controversial whether *Symbiodinium* and their host corals are flexible.

Galaxea fascicularis is a spawning scleractinian coral species common in Indo-Pacific regions. Previous studies have shown that *G. fascicularis* harbors two clades of *Symbiodinium* (Dong et al., 2009; Huang et al., 2006, 2011). It is unknown whether environmental disturbance can induce a shift in the scleractinian coral *G. fascicularis* in a fringing reef region in the South China Sea. In the present study, we investigated the composition of *Symbiodinium* phylotypes in *G. fascicularis* in a fringing reef of Hainan Island, from July 2007 to October 2008, based on PCR-RFLP (restriction fragment length polymorphism) analysis. This research may contribute to understanding the relationship between coral and *Symbiodinium* under natural environments.

2 MATERIAL AND METHOD

2.1 Study site and collection of corals

The study area was located at Xiaodonghai Bay (XDH) on the south coast of Hainan Island, situated in the north of the South China Sea (Fig.1), where there is a typical fringing reef. From July 2007 to October 2008, fragments of *G. fascicularis* were haphazardly sampled, by scuba diving, from a reef flat at XDH, on a bimonthly basis. Nearly 20 adult colonies at two different depths (3 m and 9 m) were randomly sampled along a fixed transect line laid on the reef flat. Due to the fixed nature of the transect line, most colonies could have been re-sampled during our study period. All samples were placed in pre-labeled plastic bags and filled with local sea water, and immediately transported to the laboratory of Tropical Marine Biological Research Station in Hainan, near the sampling area.

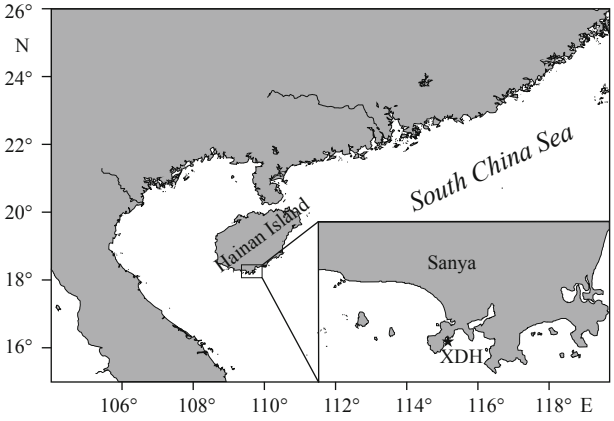

Fig.1 Location of research site in Sanya, Hainan Island
The star mark indicates sampling area. XDH means Xiaodonghai Bay.

2.2 Molecular phylotyping

DNA was extracted from the stored coral samples as described by Chen and Yu (2000) and Zhang and Lin (2005) with slightly modifications. Briefly, the fragments were preserved in DNA isolation buffer (containing 0.1 mol/L EDTA, 1% (w/v) sodium dodecyl sulfate, 10% (w/v) cetyltrimethylammonium bromide). Proteinase K (Promega) was then added, to a final concentration of 0.5 mg/mL, prior to incubation at 55°C for at least 10 h. DNA was then isolated by adding 17 μL of 5 mol/L NaCl and incubating at 55°C for 10 min, followed by one chloroform extraction and one phenol-chloroform extraction. The DNA was then purified by being passed twice through DNA Clean and Concentrator columns (Zymo Research, Orange, CA). The DNA was resuspended in 50 μL aliquots of TE buffer and stored at -20°C until PCR was performed. The 5′ end of the 28S rDNA region of 520 bp in length was amplified using a host-excluding primer pair (5S: 5′-GCCGACCCGCTGAATTCAAGCATAT-3′, and D23zoox: 5′-TGTGGCAYGTGACGCGCAAGC TAAG-3′) (Chen et al., 2005). The PCR was performed in a PTC-200 thermal sequencer (MJ Research, USA), using the following thermal cycles: 1 cycle at 95°C (3 min), 50°C (1 min) and 72°C (2 min); 4 cycles at 94°C (30 sec), 50°C (1 min) and 72°C (2 min); 25 cycles at 94°C (30 s), 57°C (1 min) and 72°C (2 min). The amplification reaction was

conducted with 50 to 200 ng of a DNA template and *Taq* polymerase (Fermentas, Germany), in a 50 μL reaction volume, using a buffer supplied with the enzyme, under conditions recommended by the manufacturer. The PCR products were electrophoresed in a 1.0% agarose (BioWest, Spain) gel, using 1×TAE buffer to assess the yield. PCR products were then characterized using the restriction enzyme, *Rsa* I (Fermentas, Germany). Restriction fragments were examined by electrophoresis on 2.0% low melting temperature agarose (BioWest, Spain).The profiles were photographed and analyzed using the Syngene Gene Genius imaging system (Syngene, USA).

2.3 Seawater temperature measurements

Seawater temperature was recorded every 15 min at the sampling station in XDH at depths of 3 m and 9 m, using data loggers (HOBO Water Temp Pro, Onset. Accuracy ± 0.1°C).

2.4 Statistical analyses

Statistical tests were performed using SPSS v 11.0 (SPSS Inc). Non-parametric statistics were used since data were not distributed normally. The significance of temporal fluctuations of *Symbiodinium* compositions in the bimonthly population survey were examined using the Chi-Square test.

3 RESULT AND DISCUSSION

Genomic DNA was successfully extracted from all 206 *G. fascicularis* colonies. A 520 bp fragment of 28S rDNA in *Symbiodinium* was amplified. As described by Chen et al. (2005), the restriction fragment length polymorphisms (RFLPs) patterns of 28S rDNA digested with the restriction enzymes *Rsa* I indicated the existence of two phylotypes of *Symbiodinium* in *G. fascicularis*: clade D consisting of three fragments of 220, 200 and 100 bp; clade C consisting of two fragments of 320 and 200 bp. There was also a mixture of clades D and C consisting of all four of these fragments (Fig.2).

PCR-RFLP analysis of 28S rDNA indicated that *G. fascicularis* associated with clade C and/or D zooxanthellae in spite of the changes of sampling time and depth (Fig.2). The phylotype frequencies of the populations did not change significantly over 1-year at either the 3 m depth ($\chi^2=1.540$, $P=1.00>0.05$) or the 9 m depth ($\chi^2=0.753$, $P=1.00>0.05$). Therefore, no temporal changes in the composition of *Symbiodinium* phylotypes were observed in the scleractinian coral *G. fascicularis*.

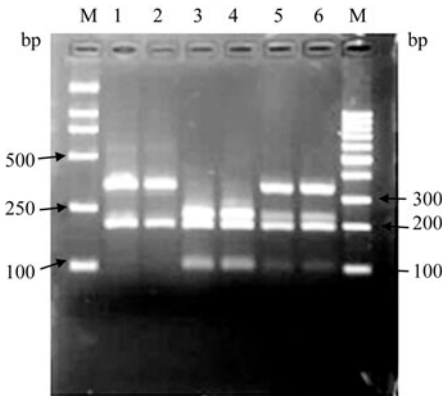

Fig.2 The typical profile of RFLP band patterns of *Symbiodinium* in *Galaxea fascicularis* obtained in this study

Lanes 1 and 2 were RFLP band pattern of *Symbiodinium* clade C; lanes 3 and 4 were RFLP band pattern of *Symbiodinium* clade D; lanes 5 and 6 were the mixture of *Symbiodinium* clades C and D; lanes at both ends of the gels labeled with M were molecular weight standards of a 100-bp DNA ladder and DL 2000 marker. RFLP means restriction fragment length polymorphism.

No coral bleaching events or other mortality were observed during the period of sample collection.

During the study period, the seawater temperature of the sampling station ranged from 19°C (January to February, winter) to 30°C (September to October, summer) (Fig.4). At the depth of 3 m, the highest temperature reached was 31.7°C, on Jun. 25th, 2007, and the lowest temperature reached was 19.9°C, on Feb. 2nd, 2008. At the depth of 9 m, the highest temperature reached was 30.8°C, on Jun. 25th, 2007, and the lowest temperature reached was 19.7°C, on Feb. 13rd, 2008. The average daily temperatures showed little difference between the different depths most of the time, except in the rainy season (May to October).

Our present results suggest that the dominant strain of *Symbiodinium* in the scleractinian coral *G. fascicularis* did not significantly change with the fluctuations of seawater temperature. It may indicate a high degree of stability in *Symbiodinium* communities within the scleractinian coral. These results are consistent with some previous studies showing a lack of significant change over time in symbiont populations within anthozoans, including scleractinian corals, soft corals and sea anemones (LaJeunesse et al., 2004; Thornhill et al., 2006a, b; Goulet et al., 2008; Sampayo et al., 2008; Stat et al., 2009). Despite the fact that many of these studies encompassed serious environmental disturbances such as bleaching events (Goulet et al., 2008; Stat et al., 2009) or coral diseases (Kirk et al., 2005),

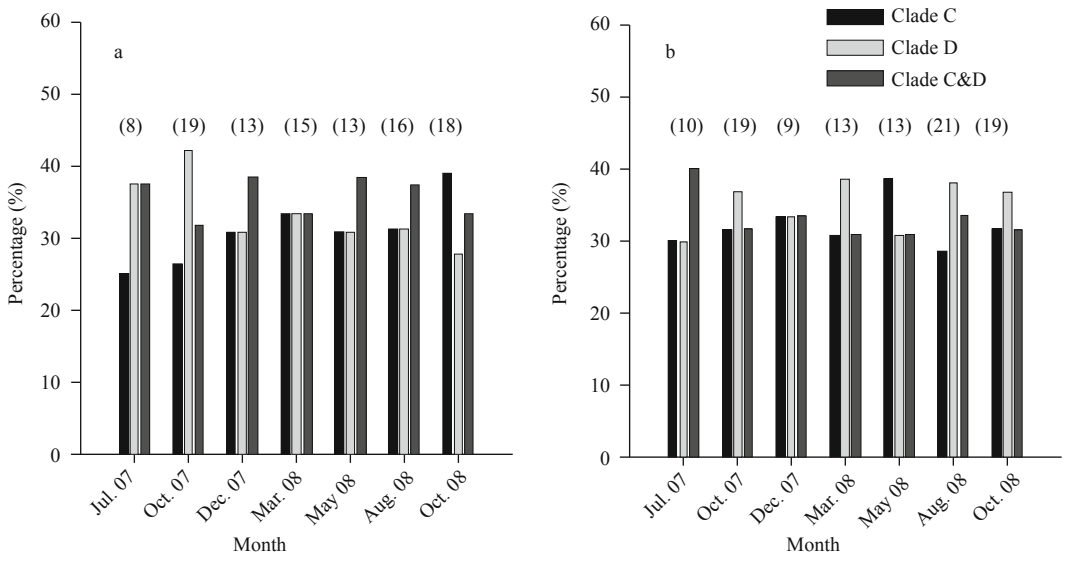

Fig.3 Proportions of *Galaxea fascicularis* colonies possessing each symbiont type in samples from reef building corals in Xiaodonghai Bay

a. Symbiont type composition at 3 m depth; b. Symbiont type composition at 9 m depth; The numbers of samples collected each time are indicated in parentheses above the bars.

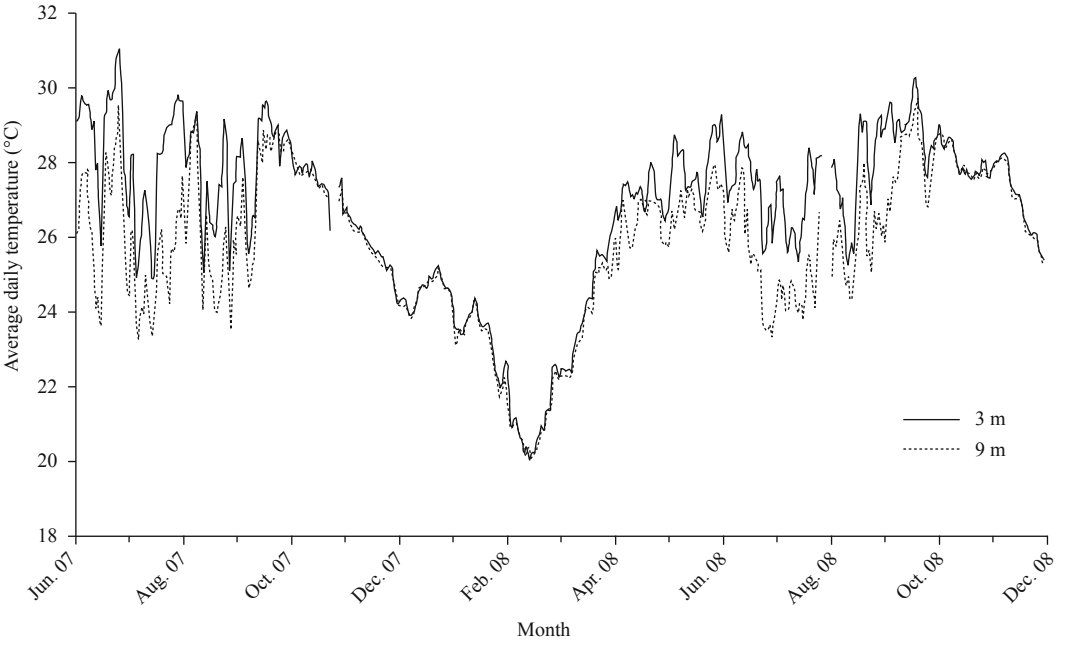

Fig.4 Average daily seawater temperature at depths of 3 m and 9 m in Xiaodonghai Bay from June 2007 to December 2008

the overriding trend is the stability in symbiont composition. The host coral and its symbiont could have formed a stable symbiotic relationship in the long period of co-evolution (Stat et al., 2009). Goulet (2006) reported that most corals do not change their symbiotic algae over time.

However, Buddemeier and Fautin (1993) proposed the "Adaptive Bleaching Hypothesis" (ABH), which hypothesized that this symbiont flexibility enables the corals to respond to thermal stress events due to a relative increase in abundance of heat-resistant symbionts at the cost of heat-sensitive ones (symbiont shuffling), resulting in a rapid increase in thermo-tolerance (Baker, 2003; Berkelmans and van Oppen, 2006). In the present study, the lack of fluctuation in symbiont type during the winter and summer may suggest that these holobionts in fringing reefs maintain their association over the range of

environmental fluctuations. In contrast, spatial and temporal variability in *Symbiodinium* phylotypes have been found to occur in some species of scleractinian corals (Chen et al., 2005; Thornhill et al., 2006a; Jones et al., 2008). However, it must be noted the above studies tracked individual colonies over the study period and monitored the changes of the phylotypes in each colony, whereas the present study sampled along the fixed transect line and might overlook the variation of symbiont among individual coral colonies. Although the method of PCR-RFLP is commonly used for assessing the cladal diversity of *Symbiodinium*, it is thought that it underestimates the potential symbiont diversity partly due to some phylotypes having low densities in corals (Baker, 2003; Goulet, 2006). More research in the future is needed to ascertain the actual diversity of *Symbiodinium* using more effective methods like SSCP (single-strand conformation polymorphism), DGGE (denaturing gradient gel electrophoresis) and DNA. Collectively, our results suggest that the shift in *Symbiodinium* communities in response to the seasonally fluctuating environments might not be a universal feature of coral-algal associations, and there may be coral species-specific differences in response to environmental change (van Oppen et al., 2001; Goulet et al., 2008).

The present study contributes to an increasing number of investigations that suggest many coral species may not respond to the seasonally fluctuating environments by significantly changing symbionts at the clade level and points to the need for more similar studies (Thornhill et al., 2006b; Jones et al., 2008; Sampayo et al., 2008; Stat et al., 2009) that track the longer term trends of coral-algal symbiosis. The flexibility and specificity in corals and *Symbiodinium* across temporal and spatial scales need to be researched more to understand the future of coral reefs under rapid change of global climate.

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