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## SHORT REPORT

# Latitudinal variation in algal symbionts within the scleractinian coral *Galaxea fascicularis* in the South China Sea

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### Abstract

The diversity of symbiotic algae of the genus *Symbiodinium* (*Symbiodinium* sp.) within a widespread ranging scleractinian coral *Galaxea fascicularis* along latitudinal gradients in the South China Sea was examined using restriction fragment length polymorphism (RFLP) of the large subunit rDNA and sequencing. It revealed that *G. fascicularis* was associated with two distinct zooxanthellae clades. *Galaxea fascicularis* sampled in Daya bay in Guangdong province harboured *Symbiodinium* Clade C, while *G. fascicularis* sampled in Sanya Bay of Hainan Island, Yongxing Island of Xisha Islands, and Zhubi Reef of Nansha Islands harboured *Symbiodinium* Clade C and D either simultaneously or sequentially, indicating symbiosis flexibility. A distinct latitudinal distribution of *Symbiodinium* sp. in *G. fascicularis* was also revealed. *Galaxea fascicularis* hosting two clades of *Symbiodinium* sp., especially *Symbiodinium* clade D, may provide hosts with a flexible mechanism for adaptation to environmental change and can more easily survive mass coral bleaching events.

**Key words:** *Galaxea fascicularis*, restriction fragment length polymorphism, *Symbiodinium*, symbiotic algae

### Introduction

It is a general supposition that all scleractinian corals harbour symbiotic algae (*Symbiodinium* sp.) in their endodermal cells (Rowan & Powers 1991). The lack of observed sexual reproduction and difficulties in culturing the microalgae for morphological description limited the use of a biological species concept to define species in the genus of *Symbiodinium* (Baker 2003). Currently, molecular DNA techniques are widely used by most researchers to study diversity in the genus *Symbiodinium*. These different DNA markers have included ribosomal small subunit (18S), large subunit (28S), internal transcribed spacers (ITS) and chloroplast rDNA (cp23S) (Baker 2003; Pochon et al. 2006; Sampayo et al. 2009). Restriction fragment length polymorphisms (RFLP) analysis of small or large subunit ribosomal DNA

genes is considered to be appropriate for cladal assessment of *Symbiodinium* diversity (Chen et al. 2005).

*Galaxea fascicularis* (Linnaeus, 1767) is an ecologically important and widespread scleractinian coral species in the Indo-Pacific Ocean and may be a dominant species on inshore fringing reefs (Veron 2000). It survives in cold waters but also in warm waters from the northern Japanese Archipelago to the waters off southern Australia. In the South China Sea, *G. fascicularis* is also widely distributed from the Daya Bay in Guangdong Province to the Nansha Islands, and is especially dominant in Sanya Bay of Hainan Island. Previous field investigation suggested that the scleractinian coral *G. fascicularis* were relatively less affected by coral bleaching than other coral taxa (Yamazato 1999; Marshall & Baird 2000). The symbiosis between host coral *G. fascicularis* and their

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symbiotic algae may play a key role in understanding how *G. fascicularis* achieves such a physiological flexibility.

In this study, the diversity of *Symbiodinium* sp. was investigated in the wide-ranging scleractinian coral *G. fascicularis*, by collecting widely from two inshore and two offshore sites in the South China Sea, between Daya Bay in Guangdong province, Sanya fringing reef in Hainan Island, Yongxing Island of Xisha Islands and Zhubi Reef of Nansha Islands. The 28S rDNA was used as the studied DNA region to investigate the cladal diversity of symbiotic algae in order to address the issue of environmental adaptation.

## Materials and methods

Samples of *G. fascicularis* were collected from a depth of 0–5 m from four different sites in the South China Sea (Figure 1). Three opportunistic samples were obtained in the Daya Bay in Guangdong province (DYB), in Sanya Bay in Hainan Island (SYB), in Yongxing Islands of Xisha Islands (YXI) and in Zhubi Reef of Nansha Islands (ZBR). In total, 12 individual *G. fascicularis* colonies were sampled. Fragments of coral tissue were placed in labelled bags, and preserved in 95% (v/w) ethanol immediately for DNA analysis in laboratory.

DNA extraction was modified according to methods described by Chen et al. (2005) and Huang et al. (2006). An approximate 520-bp fragment corresponding to

the region 5'-end of the 28S rDNA gene of symbiotic algae was amplified using PCR. Amplifications were performed with 0.05 mmol/L primers (28Szoof: 5'-CCTCAGTAATGGCGAATGAAC-3'; 28Szoor: 5'-CCTTGGTCCGTGTTTCAAGA-3') and 20 ng total DNA in 50 µl PCR buffer (1 × PCR buffer, dimethylsulphoxide (DMSO), 3 mmol/l MgCl<sub>2</sub>, 0.1 mmol/l dNTPs, and 0.5 U *Taq* DNA polymerase). The PCR amplification conditions included an initial 3 min denaturization 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, and a final 10 min extension at 72°C. The PCR products were electrophoresed in a 1% agarose gel to assess the yield. The amplified PCR products were stored at –20°C.

For RFLP analysis, the PCR products were digested using *Rsa*I for approximately 12 h overnight. Restriction fragments were visualized by electrophoresis on 2% low melting temperature agarose mixed with 1% agarose. PCR products were cleaned using a DNA purification kit (BBI). All PCR fragments of 28S rDNA gene were sequenced in one direction on an ABI 3730XL DNA sequencer (Guangzhou Top Genomics, Ltd). In addition, one sample collected from YXI which harbours two clades according to the RFLP pattern was cloned and sequenced. All sequences were initially aligned using CLUSTALX W version 1.8 followed by manual editing. The alignment data sets are available on request from the corresponding author. Phylogenetic analysis based on the maximum parsimony method was conducted using Mega 3.1. A total of 1000 bootstrap replicates were performed to estimate the statistical support for each major clade in the consensus tree.

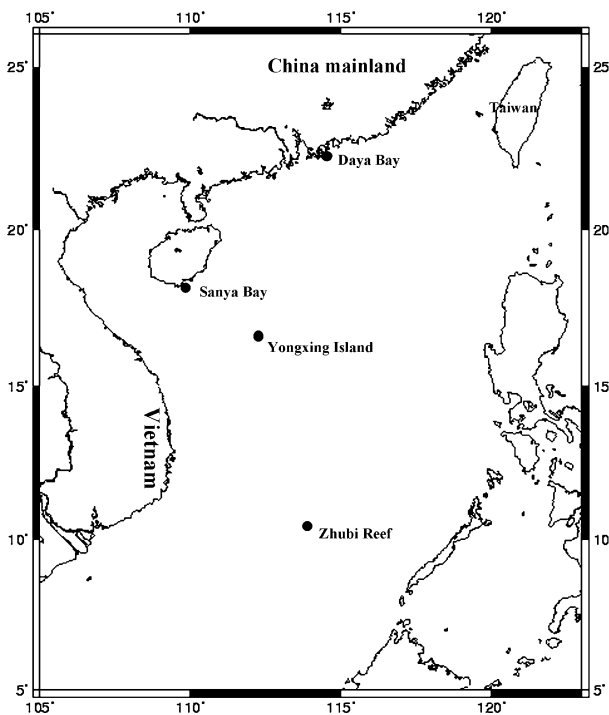


Figure 1. Location of sampling sites of *Galaxea fascicularis* in the South China Sea.

## Results and discussions

PCR amplification of 28S rDNA genes from symbiotic algae of all *Galaxea fascicularis* colonies produced a single amplicon of approximate 520 base pair (Figure 2A). *Rsa*I digestion of the amplified *G. fascicularis* symbionts' DNA produced three distinct RFLP patterns (Figure 2B). *Symbiodinium* sp. from three DYB (Figure 2B, lanes 4, 5, 6), one ZBR (Figure 2B, lane 7) and two SYB (Figure 2B, lanes 2, 3) *G. fascicularis* colonies produced an RFLP pattern typical for *Symbiodinium* Clade C. However, amplicons of symbionts produced a second RFLP pattern for *Symbiodinium* Clade D from the remaining one SYB (Figure 2B, lane 1), two ZBR (Figure 2B, lanes 8, 9) and two YXI *G. fascicularis* colonies (Figure 2B, lanes 10, 12). The remaining one YXI *G. fascicularis* colony (Figure 2B, lane 11) harboured both *Symbiodinium* Clade C and D according to the

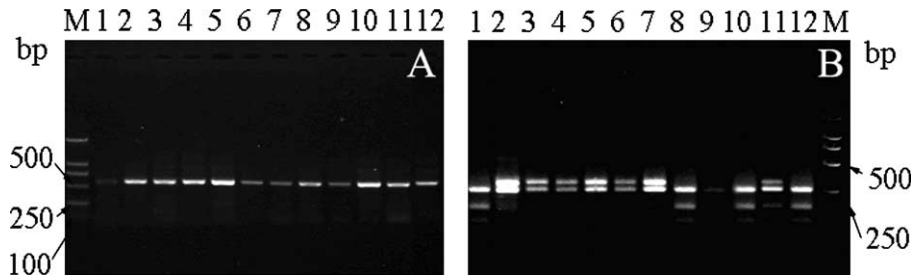


Figure 2. A: PCR products of 28S rDNA gene; B: restriction fragment length polymorphism patterns derived from *RsaI* digestion of the amplified symbiotic algae 28S rDNA gene. Lanes 1–3 are *Symbiodinium* from SYB; lanes 4–6 are *Symbiodinium* from DYB; lanes 7–9 are *Symbiodinium* from ZBR; lanes 10–12 are *Symbiodinium* from YXI. M, DNA ladder.

RFLP pattern observed in previous studies (Chen et al. 2005; Huang et al. 2006).

Approximately 520 bp nucleotides of the 28S rDNA from the 12 *G. fascicularis* colonies were sequenced in this study, representing each clade and RFLP pattern. Newly obtained sequences were deposited in GenBank (accession numbers: EU560882–EU560894). Meanwhile, a FastA search in GenBank using these sequences verified the clade identity suggested by the RFLP patterns. These sequences were aligned with each other and the variable sites between each clade were identified (data not shown). There were four variable sites among clade C symbionts, two variable sites among clade D symbionts and 92 variable sites between clade C and clade D symbionts. Maximum-parsimony analysis of aligned 28S rDNA sequences produced trees that were congruent topology. Clade C branch is composed of symbionts from three DYB, one YXI, one ZBR and two SYB *G. fascicularis* colonies, while the remaining symbionts formed clade D branch. Meanwhile, three DYB sequences clustered with one ZBR sequence, while one YXI sequence formed another group with two SYB sequences (Figure 3). It indicates that there are different species of clade C *Symbiodinium* that can associate with *G. fascicularis*, whose distributions may also depend on latitude.

In the current study, the symbiotic algae in *G. fascicularis* sampled in DYB are closely related to *Symbiodinium* clade C, while those corals collected in lower latitude locations harboured both *Symbiodinium* clades C and D either simultaneously or sequentially. Investigation for the symbionts diversity of scleractinian corals in Zamami Island, Okinawa of Japan showed *G. fascicularis* harboured *Symbiodinium* Clade C21a (LaJeunesse et al. 2004; Table I). Chen et al. (2005; Table I) examined the genetic affinity of symbiotic algae from *G. fascicularis* in Taiwan seawaters and the result showed that all of the five coral samples associated with *Symbiodinium* clade C. Visram & Douglas (2006; Table I) also investigated the genetic diversity of *G. fascicularis* in Kenya coral reefs in tropical waters near the equator.

All five coral colonies harboured *Symbiodinium* clade D. In west Pacific waters, *G. fascicularis* can harbour *Symbiodinium* clades C1 and D1a in shallow waters, and *Symbiodinium* clade D1a in deep waters (LaJeunesse et al. 2004; Table I). *Galaxea fascicularis* from the island of Mauritius in the southwest Indian Ocean harboured *Symbiodinium* clade D or C (McClanahan et al. 2005; Table I). LaJeunesse et al. (2003; Table I) also examined a *G. fascicularis* colony from Heron Island in the southern GBR and found it associating with *Symbiodinium* clade C1. Therefore, a latitudinal gradient of *Symbiodinium* sp. in *G. fascicularis* may be revealed here, and it appears that *Symbiodinium* clade D is limited to lower latitude locations.

Examination of the oceanographic characteristics of the sampling sites suggests a possible explanation for symbiont zonation along latitudinal gradients. It showed a distinct decreasing temperature continuum from the equator to the pole. Meanwhile, light was also believed to have the greatest influence

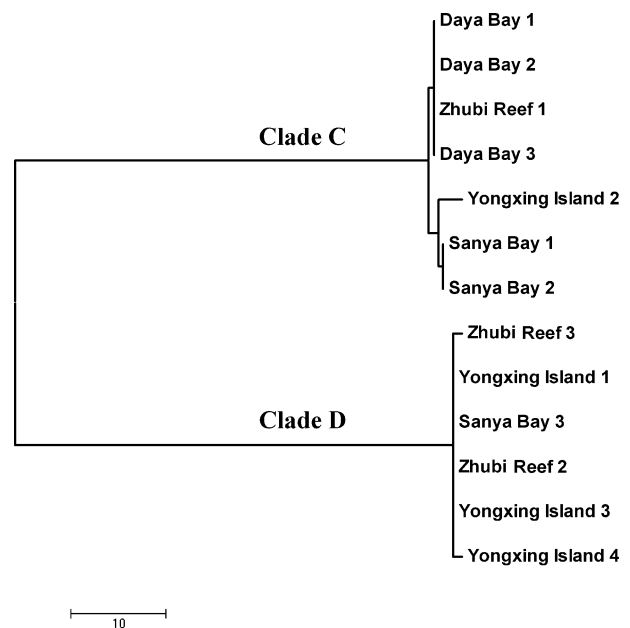


Figure 3. Maximum parsimony phylogeny based on 488 unambiguously aligned nucleotide sites.

Table I. Currently reported *Symbiodinium* diversity from *Galaxea fascicularis*. Symbiont identity is listed as *Symbiodinium* type followed by sample size.

Country	Latitude	Symbiont identity	Reference
Zamami Island, Okinawa, Japan	26°N	C21a	LaJeunesse et al. 2004
Daya Bay, Guangdong, China	23°N	C (3)	This study
Kenting, Taiwan, China	22°N	C (5)	Chen et al. 2005
Sanya Bay, Hainan, China	18°N	C (2)/D (1)	This study
Yongxing Island, China	17°N	C+D (1)/D (2)	This study
Zhubi Reef, China	11°N	C (1)/D (2)	This study
Kenya	2°S–5°S	D (5)	Visram & Douglas 2006
Feather Reef, GBR, Australia	17°S	C1(1)/D1a (2)	LaJeunesse et al. 2004
Mauritius	20°S	C (3)/D (1)	McClanahan et al. 2005
Heron Island, GBR, Australia	23°S	C1	LaJeunesse et al. 2003

on zooxanthellae clades within a particular coral host (Rowan et al. 1997). Therefore, temperature and irradiance associated with decreased latitude may explain the *Symbiodinium* variation in *G. fascicularis* and other scleractinian corals.

Previous studies also suggested that scleractinian corals associating with *Symbiodinium* clade D appeared more tolerant to mass coral bleaching than those hosting *Symbiodinium* clade C (Baker 2003). Marshall et al. (2000) analysed the bleaching responses for 4160 coral colonies after the breakout of large-scale bleaching in 1998 in the Great Barrier Reef. Their results showed that the genus *Galaxea* was relatively unaffected by bleaching, while most *Acropora* were highly susceptible. The present study suggests that the symbiotic association of *Galaxea fascicularis* and their symbiotic algae is flexible, and such a flexible holobiont may lead to a broad adaptation to environmental conditions. *Galaxea fascicularis* hosting two zooxanthellae taxa, especially *Symbiodinium* clade D, may provide hosts with a flexible mechanism for adaptation to environmental changes (temperature, irradiance, sedimentation, etc.) and can more easily survive from the mass coral bleaching events.

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