

# Genetic diversity of symbiotic dinoflagellates associated with anthozoans from Korean waters

M. Rodriguez-Lanetty<sup>1,2</sup>, H. R. Cha<sup>1</sup> and J. I. Song<sup>1</sup>

## ABSTRACT

Anthozoans are important components of the intertidal marine fauna along the coastline of South Korea. Although the systematics of this group have been studied extensively in Korea, the diversity of the symbiotic dinoflagellates associated with these cnidarians is still unexplored. In this study, we examined the genetic diversity of symbiotic dinoflagellates associated with anthozoans from three locations within Korean waters. Partial nucleotide sequences of 28S ribosomal DNA genes were used to identify the symbionts extracted from three species of actiniarian (*Anthopleura japonica*, *A. kurogane* and *Parasicyonis* sp.), and two species of scleractinian (*Alveopora japonica* and *Dendrophyllia* sp.). At two temperate sites along the southeast coast of mainland Korea we found *Symbiodinium* in clade A in *Anthopleura japonica* and *A. kurogane*. In contrast, at an island under the influence of warm water currents from subtropical areas off the southernmost tip of mainland Korea, we found *Symbiodinium* in clade A in *Anthopleura kurogane* and *Dendrophyllia* sp., but *Symbiodinium* in clade C in *Parasicyonis* sp. and *Symbiodinium* in clade C or F in *Alveopora japonica*. This represents the first report of *Symbiodinium* in clade F in scleractinians, which to date has only been reported in association with foraminiferans.

**Keywords** *Symbiodinium*, Symbiotic dinoflagellates, Zooxanthellae, Diversity, Anthozoa, Scleractinia, Actinaria, Ribosomal DNA

## Introduction

Until the early 1980s, symbiotic dinoflagellates associated with many marine invertebrates were thought to belong to a single pandemic species called *Symbiodinium microadriaticum* (Freudenthal 1962). We now understand this species to be exceptionally diverse. Both morphological and molecular studies have provided evidence for high diversity of these symbionts (Schoenberg and Trench 1980, Rowan and Powers 1991a, 1991b, Baker et al. 1997, Loh et al. 1998). Moreover, recent studies have provided evidence of diverse ecological roles for symbiotic dinoflagellates that relate to differences in environmental factors such as light (Rowan and Knowlton 1995, Rowan et al. 1997) and temperature (LaJeunesse and Trench 2000).

Whilst most studies of symbiotic dinoflagellate diversity have concentrated on tropical reef environments (see Rowan 1998 for review), only a few have explored the degree and pattern of divergence of these symbionts in temperate waters (Bythell et al. 1997, Saunders and Muller-Parker 1997, Baker 1999, Bates 2000, LaJeunesse and Trench 2000, Rodriguez-Lanetty et al. 2001).

In contrast to the relatively stable tropical environment, mutualistic associations occurring at high latitudes may be influenced by highly fluctuating environmental conditions. Changes in temperature and light between seasons are significant, and may affect symbiont population composition, especially if certain species are better adapted physiologically to warmer/high light or colder/low light conditions. Baker (1999) and Rodriguez-Lanetty et al. (2001) found that the widespread coral, *Plesiastrea versipora* associates with at least two taxa of symbiotic dinoflagellates and that the presence of these genotypes

varies with latitude. They suggested that selection on a coral-dinoflagellate association might lead to one symbiont type being exchanged for another under a different set of environmental conditions.

In Korean waters, zooxanthellate anthozoans are important components of the intertidal and subtidal marine fauna. Their biology and distribution within Korean waters have been extensively studied (Song 1982, 1984, 1991). The assemblage of symbiotic invertebrates occurring along the South Korean coastline and Cheju Island provides an opportunity to explore patterns of distribution in symbiotic dinoflagellates occurring within different hosts from temperate latitudes.

In this study, the authors explored the diversity of symbiotic dinoflagellates in different species of anthozoans living in temperate latitudes at two sites along the South Korean coastline, and at an offshore island lying further south.

## Methods

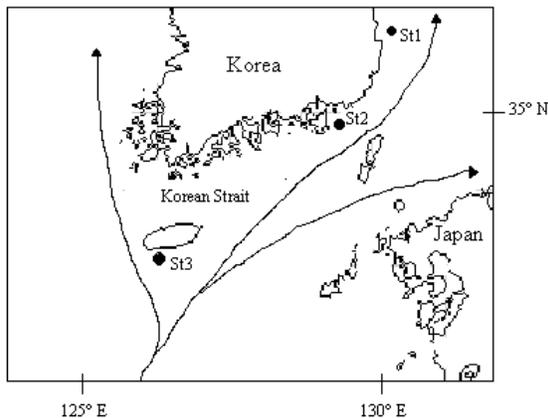
### Collection of anthozoans

After initial screening for the presence of symbiotic dinoflagellates in many anthozoans found within Korean waters, five species of anthozoans were selected for this study. These include three actinarians: *Anthopleura japonica* (n=2), *A. kurogane* (n=5) and *Parasicyonis* sp. (n=3); and two scleractinians: *Dendrophyllia* sp. (n=1) and *Alveopora japonica* (n=4). Samples were collected at three locations along the east and south coast of Korea during 1998 and 1999 (Table 1 and Fig. 1). In Korean waters, the distribution of *Alveopora japonica* and *Parasicyonis* sp. is restricted uniquely to Cheju Island reefs (Song 2000). In contrast, *Anthopleura japonica* and *A. kurogane* occur broadly along the Korean coastline. *Anthopleura japonica* and *A. kurogane* are intertidal, and the remaining species are subtidal, occurring at depths of

<sup>1</sup> Department of Biology, Ewha Womans University, Seoul 120-750, South Korea.

<sup>2</sup> Centre for Marine Studies, University of Queensland, St. Lucia, QLD 4072, Australia. Email: m.rodriguez@uq.edu.au

5-15 m. Samples were preserved in absolute alcohol immediately after collection.



**Fig. 1** Map showing patterns of surface currents within Korean waters. Site 1: Dangsa (East Sea); Site 2: Geo-je Island (Korean Straits); Site 3: Cheju Island (South of Korean Straits). Solid lines show warm water (Kuroshio) current from sub-tropical areas during summer (adapted from Song 1991).

#### DNA extraction

Preserved samples were washed in distilled water to remove all alcohol, then macerated in 2 mL of DNAB buffer (EDTA and Tris-base) to form a slurry. Tissue slurries were incubated in 1% SDS at 65°C for 1h followed by digestion with Proteinase K (Sigma) in a final concentration of 0.5 mg/mL at 39°C for 8h (Sambrook et al. 1989). DNA was extracted from the digestion in two steps using phenol-chloroform (25:24 v/v) and chloroform-isoamyl alcohol (24:1 v/v). DNA was precipitated at 0°C by the addition of 3M sodium acetate (pH 5.2) and cold isopropanol (1:10 v/v). The precipitate was washed with 70% ethanol, dried and resuspended in 50µL of sterile MQ water and stored at 70° C.

#### PCR amplification of 28S rDNA

The variable domains D1 and D2 of 28S rDNA were amplified using the *Symbiodinium*-specific primer set TohaF (5'- CCT CAG TAA TGG CGA ATG AAC A-3') and TohaR (5'- CCT TGG TCC GTG TTT CAA GA-3')(LOI 1998)

All PCR reactions contained 0.4 µg of template DNA, 10 µL of 10xPCR buffer (1 M Tris-HCl, pH 8.3), 6 µL of 25 mM MgCl<sub>2</sub>, 1.5 mM total dNTPs, 30 pmol of each primer and 0.5 µL of *Taq* polymerase (5 unit/µL) in a total volume of 100 µL. 28S rDNA was amplified using a DNA thermal cycler (PCR express, Hybaid) with the following profile: 94 °C for 1 min, 65 °C for 2 min and 72 °C for 3 min (30 cycles).

#### Sequencing

28S rDNA PCR products were sequenced directly using GFX™ PCR kits (Amersham Pharmacia Biotech Inc). The sequence was determined in both directions by the dye-primer technique using an ABI 377 automated

DNA sequencer (SUPEMAC, University of Sydney, Australia).

#### Sequence identity and phylogenetic analysis

28S rDNA sequences were aligned with one another and with representative rDNA sequences from *Symbiodinium* clade A (Genbank Accession No. U63480), B (U63484), C (U63485) [Baker and Rowan 1997], D (AF170148) [Baker 1999], F (AJ291521 and AJ291522) [Pawlowski et al. 2001], and *Symbiodinium* (= *Gymnodinium*) *varians* (AF060899) [Wilcox 1998]. *Gymnodinium beii* (AF060900) was used as the outgroup. Alignment of sequences was performed using CLUSTAL W (Thompson et al. 1994). Phylogenetic analyses based on the maximum parsimony and distance (neighbor-joining) methods were conducted using PAUP (Swofford 1998) with 1000 bootstrap replicates to estimate the statistical support for each major clade in the consensus tree. The nodes were considered significantly robust if bootstrap values were >95% (Felsenstein 1985).

#### Results and Discussion

Partial 28S rDNA sequences (D1/D2 domain) of symbiotic dinoflagellates from the anthozoans in this study were aligned with GenBank sequences of *Symbiodinium* and *Gymnodinium* species. A length of 391 bp per sequence was used for the phylogenetic analysis. Of these, 78 variable sites were informative under parsimony.

**Table 1** List of host species, sampling localities and symbiont identity. Shown are Site 1 (Dang-sa), Site 2 (Geo-je Island) and Site 3 (Cheju Island). Numbers of samples per locality are given within parenthesis.

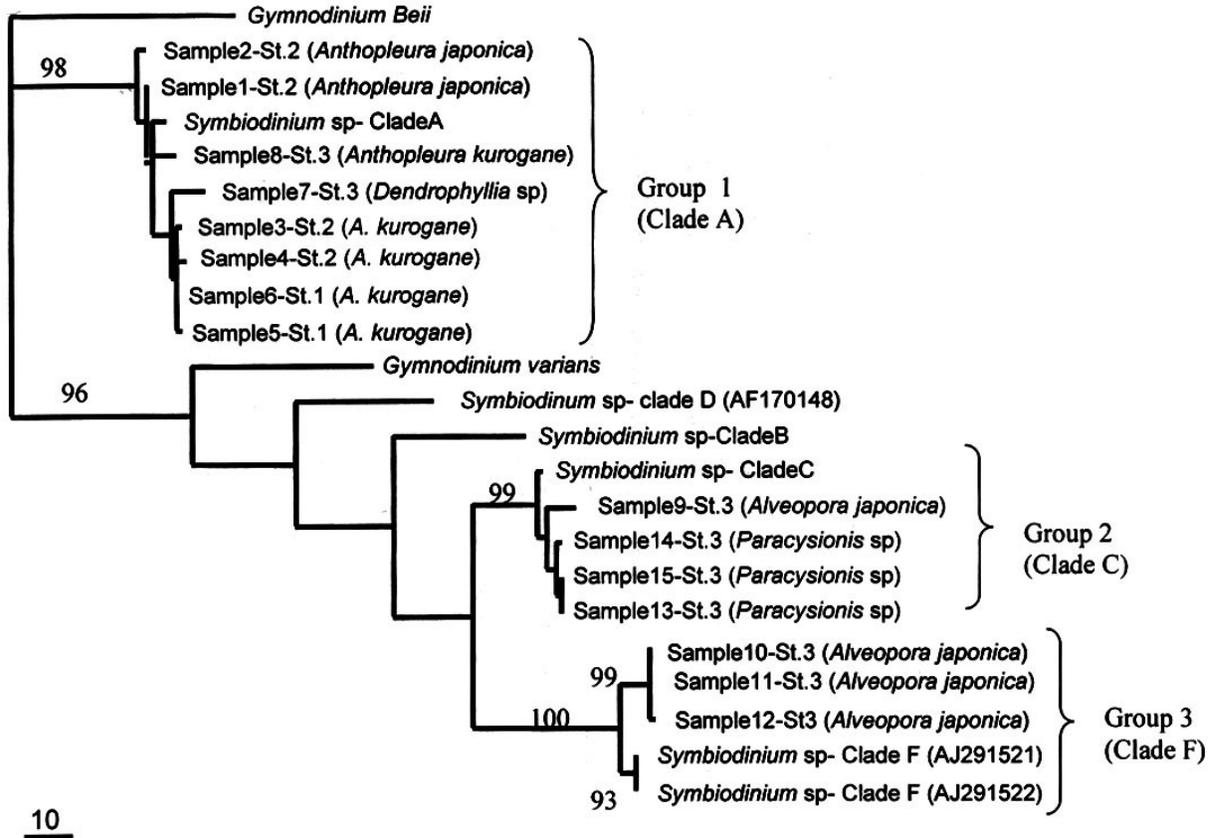
Host Species	<i>Symbiodinium</i> clade		
	Site 1	Site 2	Site 3
Order: Actiniaria			
<i>Anthopleura kurogane</i>	A (2)	A (2)	A (1)
<i>Anthopleura japonica</i>		A (2)	C (3)
<i>Parasicyonis</i> sp.			
Order: Scleractinia			
<i>Dendrophyllia</i> sp.			A (1)
<i>Alveopora japonica</i>			F (3), C (1)

The phylogenetic trees constructed using parsimony and distance criteria showed similar topologies. For simplicity, only the parsimony-based tree is shown in Fig. 2. This tree shows three well-defined groups supported by high bootstrap values. One group is represented by all the sequences from symbionts of *Anthopleura kurogane* (from all locations), *Anthopleura japonica* (from Geo-je Island) and *Dendrophyllia* sp. (from Cheju Island). As this group also includes a *Symbiodinium* classified as clade A by Baker and Rowan (1997), our symbionts

probably belong to this clade. The identification of this type of symbiont at each station indicates that it is widely distributed within Korean waters. *A. kurogane* appears to have a specific association with this type of symbiont.

The other two groups are phylogenetically more closely related to each other. An important observation is

that both groups appear restricted to Cheju Island (Site 3). One of these groups consists of symbionts from *Paracysionis* sp. and one sample of *Alveopora japonica*.



**Fig. 2** Maximum parsimony tree of 28 rDNA sequences of symbiotic dinoflagellates from Korean anthozoans. This tree is a strict consensus of 5 trees. The rescaled consistency index (RC) and homoplasy index (HI) were 0.66 and 0.30, respectively. Numerals above the branches indicate the percentage of 1000 bootstrap replications supporting each node. Host names are given within parentheses. Site 1: Dang-sa; Site 2: Geo-je Island; Site 3: Cheju Island.

This group includes a reference *Symbiodinium* classified as a member of clade C (Baker and Rowan 1997), suggesting that our symbionts are part of the same clade. The final group is composed entirely of sequences from symbionts of *Alveopora japonica* (from Cheju Island) and reference symbiont sequences from foraminiferan hosts belonging to *Symbiodinium* clade F (Pawlowski et al. 2001, LaJeunesse, Pochon and Baker, unpublished data). This is the first report of a symbiotic cnidarian hosting a symbiont belonging to this lineage. Sequence divergence between members of this clade and those of its most closely related clade (*Symbiodinium* C) is 13.8%, similar to the difference observed between *Symbiodinium* clades C and B (14.8%). As shown in Fig. 2, *Symbiodinium* clade F appears to be a sister clade of *Symbiodinium* clade C (LaJeunesse, Pochon and Baker, unpublished data). We

suggest further biogeographic studies should be carried out to achieve a better understanding of the distribution and specificity of this group of *Symbiodinium* within the Anthozoa.

Our results indicate that there is no apparent host-specific association between Korean anthozoans and the type of symbionts they host at an ordinal level of host taxonomy, i.e. actinarians and scleractinians do not host mutually exclusive types of symbionts. However, all specimens of *Anthopleura kurogane* (n=5) contained members of *Symbiodinium* clade A. We suggest that members of this clade may be phenotypically plastic, able to survive in all environmental conditions throughout the Korean Peninsula and Cheju Island.

The highest diversity of symbionts among all sites was observed at Cheju Island. This may be due to a higher

diversity of host species at this location compared to the Korean mainland coastline. For example, *Alveopora japonica* and *Parasicyonis* sp. are only found around Cheju Island within Korean waters.

Despite the fact that Cheju Island is located within a latitudinally-defined temperate zone, its marine ecosystems are composed of sub-tropical fauna because of the influence of the northern extension of the Kuroshio current, which bring warm waters from tropical areas around the Philippines (Fig. 1). We suggest that the higher diversity of symbionts associated with several anthozoans in Cheju Island, and especially the presence of *Symbiodinium* in clade C (normally observed in subtropical and tropical waters), could be linked to the particular oceanographic patterns around this island.

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