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Two anthozoans, *Entacmaea quadricolor* (order Actiniaria) and *Alveopora japonica* (order Scleractinia), host consistent genotypes of *Symbiodinium* spp. across geographic ranges in the northwestern Pacific Ocean

Soo-Jung Chang[a], Mauricio Rodriguez-Lanetty[b], Kensuke Yanagi[c], Satoshi Nojima[d] and Jun-Im Song[e]*

[a]*Fisheries Resources and Environment Division, Southwest Sea Fisheries Institute, Yeosu-si, Jeollanam-do 556-823, South Korea;* [b]*Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504, USA;* [c]*Coastal Branch of Natural History Museum and Institute, Chiba 299-5242, Japan;* [d]*Department of Biology, Kyushu University, Tomioka, Reihoku-Amakusa, 863-2507, Kumamoto, Japan;* [e]*Department of Life Sciences, Ewha Womans University, Seoul 120-750, South Korea*

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The actiniarian sea anemone, *Entacmaea quadricolor*, and the scleractinian coral, *Alveopora japonica*, host symbiotic dinoflagellates belonging to the genus *Symbiodinium* (Freudenthal). We studied the host–symbiont specificity of these two anthozoan hosts in the northwestern Pacific Ocean. Symbionts within the two hosts were identified using partial large subunit (LSU) ribosomal DNA (rDNA) and complete internal transcribed spacers (ITS) 1 rDNA regions. The host, *E. quadricolor*, was identified using the partial LSU rDNA molecular marker. Genetic analysis showed that *E. quadricolor* only harbors dinoflagellates belonging to subclade C1/3 of the genus *Symbiodinium*. Moreover, no genetic variation was detected among the symbionts of *E. quadricolor* within the study region (Korea and Japan), even though the two distant sites were separated by more than 1000 km, at collection depths of 1 m in shallow and 13–16 m in deep water. Whilst scleractinian corals host multiple *Symbiodinium* clades in tropical waters, *A. japonica*, sampled over a wide geographical range (800 km) within the study region, only hosts *Symbiodinium* sp. clade F3. The high specificity of endosymbionts in *E. quadricolor* and *A. japonica* within the northwestern Pacific Ocean could be accounted for because symbiotic dinoflagellates within the host anemones appear to be acquired maternally, and the Kuroshio Current might affect the marine biota of the northwestern Pacific. However, the consistency of the symbiotic relationships between these two anthozoan hosts and their endosymbionts could change after climate change, so this symbiotic specificity should be monitored.

Keywords: *Symbiodinium* spp.; symbiotic dinoflagellates; Anthozoa; symbiosis specificity; ribosomal DNA

Introduction

Symbiotic dinoflagellates belonging to the genus *Symbiodinium* are unicellular algae that occur as endosymbionts in many hundreds of marine invertebrate species (Taylor 1974; Trench and Blank 1987; Rowan and Powers 1991b). Symbioses between cnidarians and endosymbiotic dinoflagellates of the genus *Symbiodinium* are especially widely known in shallow subtidal and intertidal areas of tropical oceans. The endosymbionts translocate photosynthetically fixed carbon for the hosts' respiration, growth and reproduction, and help to make host skeleton with carbonate calcification fixation (Iglesias-Prieto and Trench 1994; Davy et al. 1997). On the other hand, the hosts provide a safe shelter to their symbiotic algae from direct sunlight and predators. They have emerged as a potent biogeochemical force, serving physically and biologically to structure and stabilize shallow-water marine reef ecosystems (Taylor 1983; Hoegh-Guldberg and Salvat 1995).

These endosymbiotic dinoflagellates are taxonomically described using molecular methods. DNA sequencing of the ribosomal small subunit (SSU) (Carlos et al. 1999), the partial large subunit (LSU) (Pawlowski et al. 2001; Pochon et al. 2001), and the internal transcribed spacers (ITS1 and 2) and 5.8S rDNA region (LaJeunesse 2001; van Oppen et al. 2001) revealed a diverse clade of *Symbiodinium* sp. The D1 and D2 region of LSU and ITS1 rDNA are well-resolved markers to identify *Symbiodinium* as clades A, B, C, D, E, F and G (Baker 2003; Rodriguez-Lanetty 2003).

The traditional view that symbiotic marine invertebrates host homogeneous algal populations is well known (Schoenberg and Trench 1980; Rowan and Powers 1991a, b). However, many researchers have shown that some species of symbiotic marine invertebrates contain more than one type of endosymbiotic dinoflagellate (Rowan 1998). Baker (1999) reported that 38 of 107 species (36%) of scleractinian corals contained multiple *Symbiodinium* clades in the Great Barrier

*Corresponding author. Email: jisong@ewha.ac.kr

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Reef (GBR). Hosting multiple symbiont species may guarantee symbioses under changeable and extreme conditions, and may supply an evolutionarily advantageous strategy (Saunders and Muller-Parker 1996).

Korean waters contain dinoflagellates of *Symbiodinium* sp. clade A within the actiniarians, *Anthopleura japonica*, *Anthopleura kurogane* and the scleractinian *Dendrophyllia* sp., dinoflagellates of *Symbiodinium* sp. clade C within the actiniarian *Heteractis* sp., and dinoflagellates of *Symbiodinium* sp. clade F within *Alveopora japonica* (Rodriguez-Lanetty et al. 2000). In this study, endosymbiotic dinoflagellates associated with the Korean and Japan anthozoan hosts – *Alveopora japonica* and *Entacmaea quadricolor* – were studied for their degree of specificity.

The 'giant' sea anemone, *E. quadricolor*, is one of the most widely distributed Actiniidae within the Indo-Pacific Ocean (Dunn 1981) and is a very popular sea anemone species in the aquarium trade. Its distribution extends from the warm waters of the Red Sea to the Indonesian Archipelago, and extends across latitudes to the subtropical waters of both the Ryukyu Archipelago (Japan) in the northern hemisphere and the eastern/western Australian seaboards in the southern hemisphere. *Entacmaea quadricolor* plays a variety of important ecological roles. At a macro level, this sea anemone hosts a number of marine animals (ectosymbionts), including the obligate symbiotic anemonefishes and at least seven species of caridean shrimps (Allen 1975; Dunn 1981). Moreover, at a micro level, this sea anemone hosts photosynthetic unicellular dinoflagellates, no bigger than 13 μm in diameter, but living in high concentrations in the gastroderm cells of the host. The eggs of host sea anemones contain an abundance of endosymbiotic dinoflagellates at spawning (Scott and Harrison 2007). Host anemones absorb most of the ammonia produced by the resident fish, and endosymbiotic dinoflagellate photosynthesis drives ammonia uptake (Roopin et al. 2008).

Alveopora japonica is a small poritid scleractinian coral occurring in a subtropical zone from Japan to the southern part of Korea (Song 1991; Veron 1992) in the northwestern Pacific Ocean. Endosymbionts within *A. japonica* help to build its skeleton with their symbiotic products. The species is a hermaphroditic brooder that releases planulae containing symbiotic dinoflagellates from September to October (Harii et al. 2001).

The purpose of this study is to identify host–symbiont specificity within the northwestern Pacific Ocean. We studied the genetic diversity and specificity of symbiotic dinoflagellates associated with the two common anthozoan hosts, the scleractinian *A. japonica* and the actiniarian *E. quadricolor*, in Korea and Japan.

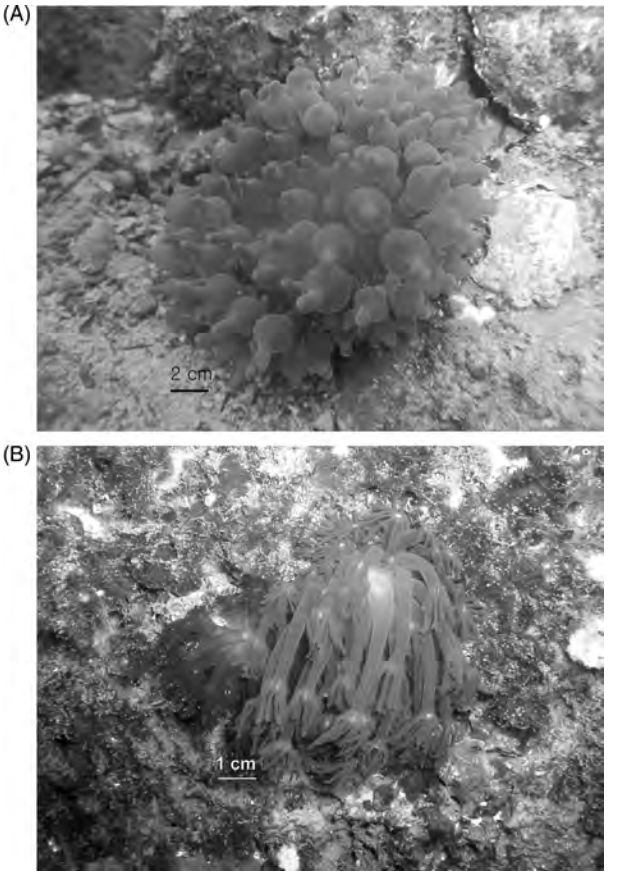

Figure 1. Anthozoan hosts associated with symbiotic dinoflagellates. A. *Entacmaea quadricolor.* B. *Alveopora japonica.*

Materials and Methods

Study sites and sample collection

We collected 10–15-cm-diameter samples of *E. quadricolor* (Figure 1A) from three locations in the northwestern Pacific Ocean (Figure 2). Five specimens were collected from Natto-ura (34°38′35″N, 137°47′14″E) and from Kogane-zaki (34°50′15″N, 138°45′26″E) on the central eastern coast of Honshu Island, Japan, and six specimens were collected from Moonsum (126°38′56″N, 33°13′43″E) on the south coast of Jeju Island, South Korea. At Natto-ura and Kogazane-zaki, the anemones were found at a depth of 1 m, whereas at Moonsum anemones were collected at depths between 13 and 16 m. We collected specimens more than 3 m apart. The annual range in seawater temperature was 14.1–30.1°C at Natto-ura and Kogane-zaki and 15.8–22.7°C at Moonsum. *A.*

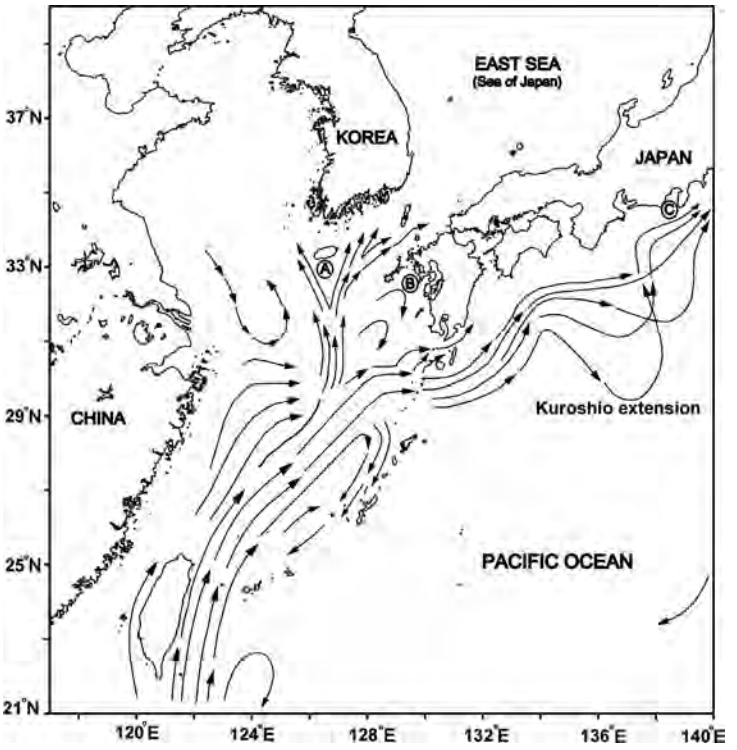

Figure 2. The sampling sites and current system. A, Jeju Island, Korea; B, Aitsu, Akaiwa, Satsuki, Japan; C, Natto-ura, Koganezaki, Japan.

japonica coral samples 5–10 cm in diameter (Figure 1B) were collected from four locations in the northwestern Pacific Ocean. Specimens of *A. japonica* were collected from Aitsu ($n = 7$) (32°18′01″N, 130°10′08″E), Akaiwa ($n = 6$) (32°18′07″N, 129°58′01″E) and Satsuki ($n = 5$) (34°10′50″N, 130°00′30″E) on the western coast of Honshu Island, Japan, and Moonsum ($n = 5$) on the south coast of Jeju Island (Korea). The annual range in seawater temperature was 6.1–24.4°C at Aitsu, Akaiwa and Satsuki and 15.8–22.7°C at Moonsum. All specimens were collected and preserved in 99% ethanol.

DNA extraction

Preserved samples were washed in distilled water to remove all ethanol and then macerated in 2 mL DNAB buffer (EDTA and Tris-base) to form a slurry. We randomly excised 1 mg of tissue from the bodies of the anemones and five or six polyps from the coral colonies for DNA extraction. Tissue slurries were incubated in 1% SDS at 65°C for 1 h followed by digestion with proteinase K (Sigma) in a final concentration of 0.5 mg/mL at 39°C for 8 h. 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 3 M sodium acetate (pH 5.2) and cold isopropanol (1:10 v/v). The precipitate was washed with 70% ethanol, dried and resuspended in 50 μL of sterile MQ water and stored at 70°C.

Amplification of LSU and ITS1 rDNA from symbiotic dinoflagellates

The symbiotic dinoflagellates were genetically identified using the ribosomal DNA regions, LSU and ITS1. The variable domains D1 and D2 of LSU 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). The ITS1 rDNA region was amplified using another 'zooxanthella-specific' primer designed by Bui et al. (2000): forward (its-dino) 5′–GTG TAT TAT TCG GAC TGA CG–3′ and the universal reverse (ITS4): 5′–TCC TCC GCT TAT TGA TAT GC–3′.

All PCR reactions contained 0.4 μg of template DNA, 10 μL of 10 × PCR buffer (1 M Tris-HCL, pH 8.3), 6 μL of 25 mM $MgCl_2$, 1.5 mM total dNTP, 30 pmol of each primer and 0.5 μL of Taq polymerase (5 unit/μL) in a total volume of 100 μL. Both rDNA regions were amplified using a DNA thermal cycler (PCR express, Hybaid) with the following profile: 94°C for 1 min, 65°C (28S rDNA) and 55°C (ITS1 rDNA) for 2 min, and 72°C for 3 min (30 cycles).

Amplification of LSU rDNA from E. quadricolor *hosts*

The partial LSU rDNA region from the hosts was amplified using the following anthozoan-specific primers designed by Chen et al. (1995): forward primer, No. 1: 5′–GGC GAC CCG CTG AAT TCA AGC ATA T–3′, and reverse primer: 5′–GCT TTG GGC TGC AGT CCC AAG CAA CCC ACT C–3′.

All PCR reactions contained 0.4 μg of template DNA, 10 μL of 10 × PCR buffer (1 M Tris-HCL, pH 8.3), 6 μL of 25 mM $MgCl_2$, 1.5 mM total dNTP, 30 pmol of each primer and 0.5 μL of Taq polymerase (5 unit/μL) in a total volume of 100 μL. Both rDNA regions were amplified using a DNA thermal cycler (PCR express, Hybaid) with the following profile: one cycle at 95°C for 3 min, four cycles at 94°C (30 s), 50°C (1 min), and 72°C (2 min), and 25 cycles at 94°C (30 s), 57°C (1 min), and 72°C (2 min).

Sequence identification and phylogenetic analysis

The PCR products were sequenced directly using GFXTM 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. The symbiont genotype sequences (28S and ITS1 rDNA), one in *A. japonica*, and two in *E. quadricolor*, were lodged in the GenBank database under the accession numbers HQ668059-72 and HQ668079-82. The *E. quadricolor* genotype sequences (LSU rDNA), one each from Jeju Island in Korean waters and Natto-ura and Kogane-zaki in Japanes waters, were lodged in the GenBank database under the accession numbers HQ668073-8.

Symbiont LSU rDNA sequences were also aligned with one another and with other representative rDNA sequences found in GenBank. These sequences were from *Symbiodinium* sp. clade A (GenBank accession no. AF170140), B (AF170152) (Baker 1999), subclade C1 (FJ529523), C3 (FJ529524) (Sampayo et al. 2009), F3 (AJ291521) (Pawlowski et al. 2001), F3 (AJ830911) (Pochon et al. 2006), the symbionts of *Heteractis* sp. (AY186623) (Rodriguez-Lanetty et al. 2003), and *Gymnodinium beii* (accession no. AF060900) (Wilcox 1998). *Symbiodinium* sp. clade A, B and *Gymnodinium beii* were used as an outgroup.

ITS1 sequences were aligned with other related sequences obtained in GenBank (through Fasta Search) from the following endosymbionts: *Symbiodinium* sp. clade A (accession no. AF427467) (Santos et al. 2002), B (AF360555) (Santos et al. 2001), subclade C1 (AB259647) (Ono et al. 2010), C1 (EU074892) (Thornhill et al. 2007), C2 (AF3805570) (van Oppen et al. 2001), C3 (Sampayo et al. 2009) and *Symbiodinium* sp. subclade F3 (AJ291521 and AJ291522) (Pawlowski et al. 2001), and *Symbiodinium goreauii* (accession no. AF333515) (LaJeunesse 2001). *Symbiodinium* sp. clade A and B were used as an outgroup.

Host LSU rDNA sequences were aligned to determine genetic distances between the samples and for comparison with other rDNA sequences from two related species of the family Actiniidae, order Actiniaria, in the GenBank database (*Anemonia viridis*, accession no. U69685, *Anthopleura dixoniana*, no. U69686 and *Stichodactyla tapetum*, no. U69687) (Chen et al. 1995). We also included a sequence of *E. quadricolor* from a specimen collected on the eastern coast of Australia (accession no. U69687) (Chen et al. 1995). *Anemonia viridis*, *Anthopleura dixoniana*, and *Stichodactyla tapetum* were used as an outgroup.

Sequences were aligned using CLUSTAL X (Thompson et al. 1997). Modeltest v3.7 (Posada and Crandall 1988) was used to identify the best model of DNA evolution for each of our data sets using maximum likelihood (ML) analysis. Modeltest v3.7 was used to find the optimal model of DNA substitution for ML construction and suggested the GTR + G + I model as the best-fit model for the 28S and ITS rDNA dataset. The ML method was then performed with a heuristic search and random addition of sequences as implemented in PAUP 4.0b10 (Swofford 2002), with a starting tree obtained via stepwise addition of taxa, and then swapped using the tree bisection reconnection (TBR) algorithm. One thousand bootstrap replicates were used to estimate the statistical support for each major clade in the consensus tree. An ML tree, based on the 28S rDNA of *Symbiodinium* sp., was developed with the selected GTR + G + I model in PAUP 4.0b10 (Swofford 2002), using the following likelihood settings determined from the above Modeltest: base frequencies A = 0.24390, C = 0.19630, G = 0.29490, T = 0.26490; base substitution rates AC = 0.64251, AG = 2.32874, AT = 0.45733, CG = 0.32290, CT = 4.79480, GT = 1.00000; assumed proportion of invariable sites = 0.0995760; and gamma distribution shape parameter = 0.656640. An ML tree, based on ITS rDNA of *Symbiodinium* sp., was developed with the selected GTR + G + I model in PAUP 4.0b10 (Swofford 2002), using the following likelihood settings determined from the above Modeltest: base frequencies A = 0.19800, C = 0.21880, G = 0.27770, T = 0.30550; base substitution rates AC = 1.06412, AG = 2.83484, AT = 1.07551, CG = 0.53787, CT = 3.94220, GT = 1.00000; assumed proportion of invariable sites = 0.000000; and gamma distribution shape parameter = 1.768303. The nodes were considered significantly robust if the bootstrap values were > 95% (Felsenstein 1985). Maximum parsimony (MP) trees were constructed by using 100 repetitions of random

sequence additions of taxa. Starting trees were obtained by stepwise addition and branches were swapped using the TBR option. Support for branches in the MP trees was tested by bootstrap analysis with 1000 replicates.

Results

No diversity of endosymbiotic Symbiodinium *sp. within* E. quadricolor *and* A. japonica

The genetic diversity of endosymbionts in the anthozoans *E. quadricolor* and *A. japonica* based on the partial LSU (455 bp) and ITS1 (214 bp) rDNA region is shown in Figure 3. All the endosymbiont sequences associated with *E. quadricolor* grouped in a monophyletic group showed no genetic variation at all regardless of different collection sites (Korea and Japan) and depth (1 and 13–16 m). This symbiont clade was phylogenetically related to the symbiotic dinoflagellate *Symbiodinium* sp. subclade C1/3. The other reference sequences from *Symbiodinium* sp. in clades A, B and F grouped in separate clusters.

All the endosymbionts associated with *A. japonica* grouped in a single clade showed no genetic difference based on ITS rDNA regardless of where they were collected at Jeju Island, Korea, and in Japan. This symbiont clade was phylogenetically related to the symbiotic dinoflagellate *Symbiodinium* sp. subclade F3 (GenBank Acc. Num. AJ291521 and AJ830911) (Figure 3A, B). Three *Symbiodinium* genotypes (differing by one and three pair bases) were detected in association with the coral *A. japonica* using LSU rDNA regions. Genotype AJ-Jeju was found in the samples from locations in the southern Korean Sea, genotype AJ-Sat and AJ-Ait-Aka were found in the samples from western Japanese waters. These three

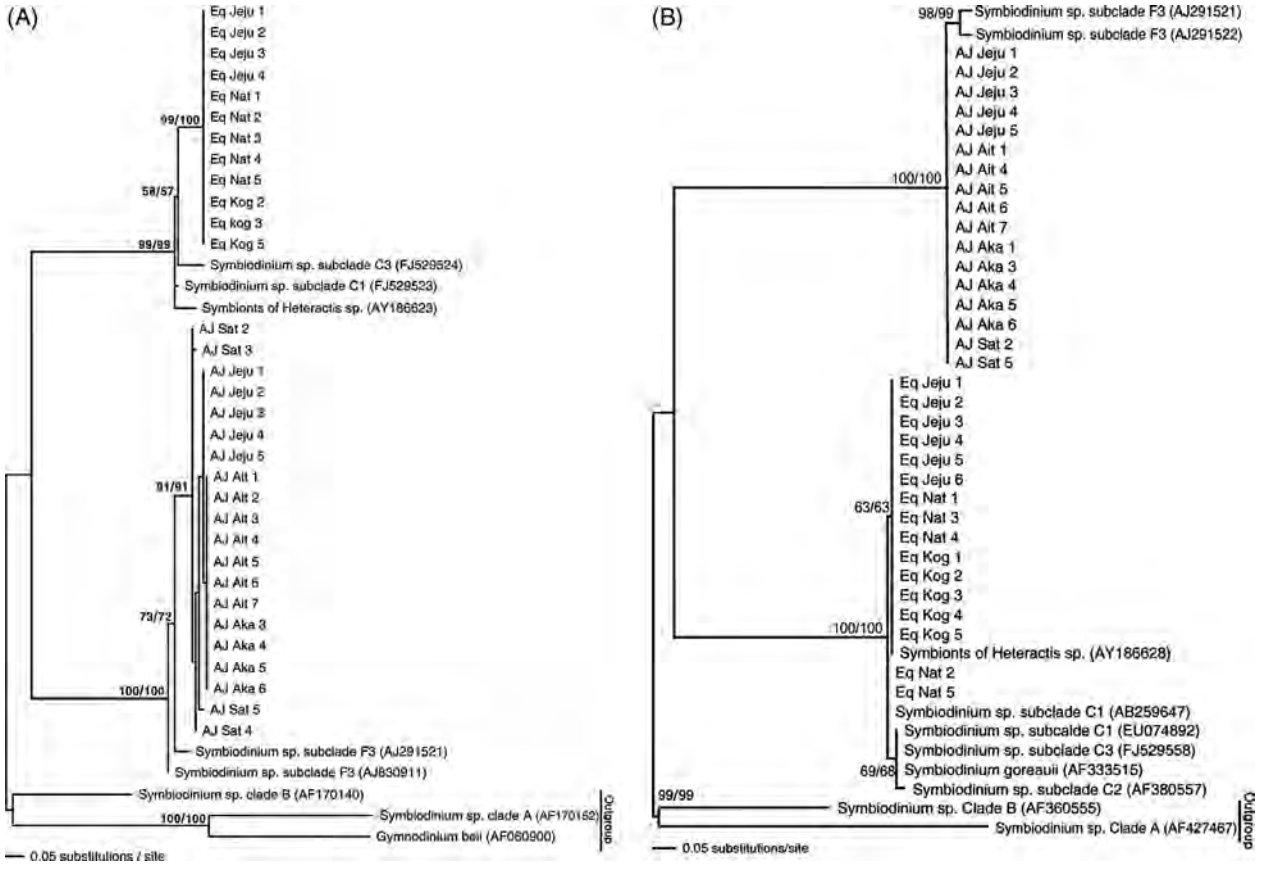

Figure 3. Maximum likelihood and maximum parsimony trees of LSU rDNA (A) and ITS1 rDNA (B) sequences of symbiotic dinoflagellates living within two anthozoans. Sample names starting with Eq and AJ represent symbiont sequences from the host *Entacmaea quadricolor* and *Alveopora japonica*. Within samples, 'Ait' refers to location Aitsu, 'Aka' to location Akaiwa, 'Sat' to location Satsuki, 'Jeju' to location Jeju Island, 'Nat' to location Natto-ura, and 'Kog' to location Kogane-zaki. Numbers (1–7) represent the individual number. Sequences from *Symbiodinium* sp. clades A, B, subclades C1, C2, C3 and F3, and two symbionts associated with the actiniidae *Heteractis* sp. were used as reference sequences within the Phylogram. Numerals above the branches mean the percentage of 1000 bootstrap replications supporting each node. Bootstrap indices under maximum likelihood and parsimony are shown at each node (ML/MP).

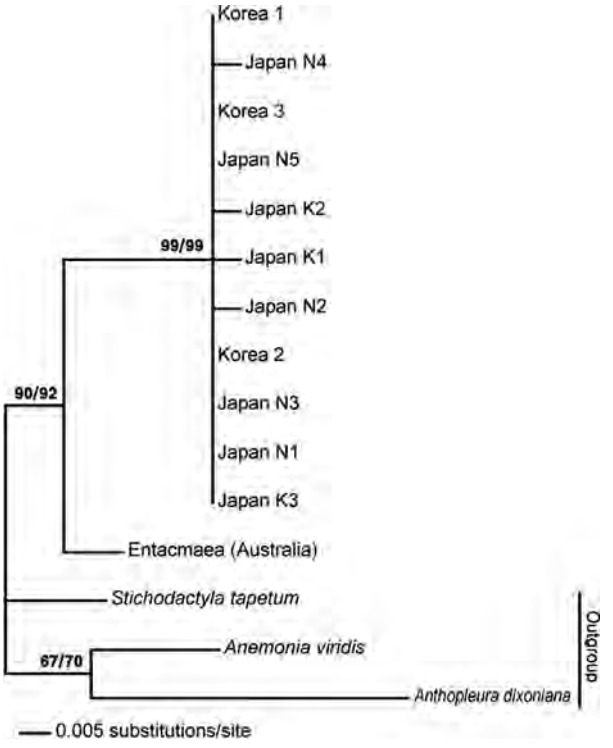

Figure 4. Maximum likelihood and parsimony tree of LSU rDNA sequences from *Entacmaea quadricolor* hosts. Sequences from three other actiniidae were used as outgroups. Samples are from Korea and Japan (see Figure 2). Within Japanese samples, 'N' refers to location Natto-ura, and 'K' to location Kogane-zaki. Bootstrap indices under maximum likelihood and parsimony are shown at each node (ML/MP).

Symbiodinium genotypes were phylogenetically related to the symbiotic dinoflagellates in subclade F3.

Host LSU rDNA

The maximum likelihood tree of the LSU rDNA sequences of *E. quadricolor* is shown in Figure 4. The LSU rDNA sequence of the host, *E. quadricolor*, from Korean and Japanese samples showed very little genetic difference (less than 0.6%) within and between them, which suggests that all these samples belong to the same sea anemone species, *E. quadricolor* (Table 1). The reference sequences from the same species obtained from a sample collected in the Great Barrier Reef, Australia (GBR) showed it to be phylogenetically related to the Japanese and Korean sequences (Figure 4). However, comparisons with another LSU rDNA sequence from *E. quadricolor* collected in the GBR (Chen et al. 1995) showed a higher genetic difference of between 3.15% and 3.45% (Table 1). The other reference sequences of LSU rDNA sequences from the other anemone species (*Anemone viridis* and *Anthopleura dixionia*) grouped in separate clusters within the phylogenetic tree and showed pairwise differences over 4.5% to those of Japan/Korea *E. quadricolor* sequences.

Discussion

Using the ITS1 rDNA marker, which can classify eight subclades of *Symbiodinium* sp. clade C (Sampayo et al. 2009), we found that the *Symbiodinium* endosymbionts of *E. quadricolor* in the northwestern Pacific (Korean and Japanese waters) are of subclade C1/3. These symbionts were strongly related phylogenetically to the *Symbiodinium* sp. of clade C1/3 that was observed in *Heteractis* sp. They belong to the same order of Actiniaria and are distributed at the same habitat at Jeju Island, Korea (Rodriguez Lanetty et al. 2003) and in *E. quadricolor* at Kyushu, Japan (Ono et al. 2010). Even though *Symbiodinium* species of subclade C1/3 were known to be host 'generalists' (LaJeunesse 2005), these symbionts were only found in *Heteractis* sp. and *E. quadricolor* in Korean waters.

No genetic variation was detected among the samples from Honshu Island, Japan, and Jeju Island, Korea, even though these two distinct geographical areas were separated by more than 1000 km, and the collection depths were different. Sea anemone hosts from both sites in Japan were sampled at a depth of 1 m, whereas the samples from the Korean site came from depths between 13 and 16 m. The annual range in seawater temperature at the Japanese sampling sites (14.1–30.1°C) was greater than that at the Korean site

Table 1. Pairwise sequence difference of 28S rDNA among samples of *Entacmaea quadricolor* from Japan, Korea, and Australia, and several other actiniidae species used as references.

	Korea	Japan-N	Japan-K	Australia	*Anemonia viridis*	*Anthopleura dixoniana*
Korea	**0**	0.30	0.18	3.15	5.41	7.66
Japan-N		**0.60**	0.48	3.45	5.71	7.96
Japan-K			**0.36**	3.33	5.58	7.84
Australia				**0**	4.50	7.21
A. viridis					**0**	5.86
A. dixoniana						**0**

(15.8–22.7°C). Even though *Symbiodinium* lineages have been shown to exhibit distinct local depth zonation patterns within the same host species (Rowan et al. 1997; LaJeunesse 2002), our *E. quadricolor* samples, obtained at different depths and from waters with different temperature ranges, were only associated with *Symbiodinium* spp. subclade C1.

The scleractinian coral, *A. japonica*, harbors symbionts belonging to *Symbiodinium* in clade F. More specifically, the coral endosymbionts resolved in subclade F3 were originally represented by endosymbionts associated with foraminiferans in the Red Sea (Rodriguez-Lanetty 2003). The affinity of *A. japonica* for this type of endosymbiont seems to span a wide geographical range (800 km) exposed to the Kuroshio Current within temperate environments in the northwestern Pacific Ocean. This evidence confirms the high affinity that *A. japonica* has for subclade F3 symbionts over a wide geographical range within temperate environments of the northwestern Pacific Ocean.

In other studies carried out within smaller geographical areas, symbiont differentiation within the same host species has been reported (Baker 1999; Loh et al. 2001). However, most of these studies were carried out in tropical waters where many coral species seem to establish a nonstable (or more flexible) association with symbiotic dinoflagellates (Baker 2001), and where horizontal transmission of symbionts (i.e. acquired from surrounding water) is a common mechanism of zooxanthellae acquisition (Stat et al. 2008). Coral species may change symbionts or may host multiple symbionts at different depths or at different geographic locations on the reef. Caribbean scleractinian corals, *Montastraea annularis* and *M. faveolata*, are associated with *Symbiodinium* clades A, B, C and D depending on the depth – clades A, B and D shallow water (0–6 m) and clade C deep water (3–14 m) (Rowan and Knowlton 1995; Rowan et al. 1997; Toller et al. 2001). *Anthopleura elegantissima* hosts two species of *Symbiodinium* that vary in their distribution along the Pacific coast of North America, with northern populations containing *Symbiodinium* clade B and southern populations hosting mixtures of *Symbiodinium* clades B and E (LaJeunesse and Trench 2000). Within tropical waters, many of these symbiotic relationships are known to be unstable when exposed to environmental change (Baker 2001; Toller et al. 2001). Cnidarians may expel the symbionts in the presence of various environmental stimuli, including changes in water temperature, decreased salinity and high levels of sunlight (Steen and Muscatine 1987; Muscatine et al. 1991). The phenomenon of bleaching, or loss of symbiotic dinoflagellates, is of global concern in coral reefs because it indicates a high degree of stress in the ecosystem (Jokiel and Coles 1990). Under extreme environmental changes, some host species lose their endosymbionts and recover by the acquisition of new endosymbiont partners (Toller et al. 2001).

On the other hand, in temperate waters, anthozoan–symbiotic dinoflagellate symbioses seem to be more stable, and the mechanism of maternal symbiont transmission appears to be predominant (Muller-Parker and Davy 2001; Davy and Turner 2003). Davy et al. (1997) pointed out that a predominance of vertical transmission (maternally) of zooxanthellae at high latitudes could relate to a scarcity of potential donors and selection against hosts with horizontal (indirect) transmission mechanisms. *E. quadricolor* and *A. japonica* already contain endosymbiotic dinoflagellates at the larval stage (Harii et al. 2001; Scott and Harrison 2007). In our study, the high conspecificity of endosymbionts in *E. quadricolor* and *A. japonica* within the geographical area studied (temperate waters) could be because the symbionts within the host anemones appear to be acquired maternally (vertical transmission). Sprung and Delbeek (1997) have documented the presence of zooxanthellae in embryos immediately after being brooded by female anemones of *E. quadricolor*. Likewise, during asexual reproduction, new anemone clones are always provided, before division, with a full set of zooxanthellae from the mother colony (Sprung and Delbeek 1997). Corals that transmit their symbionts maternally (vertical transmission) are associated with specific symbionts, but corals that obtain their symbionts from the environment (horizontal transmission) may host various types of symbiont (Barneah et al. 2004). Host *E. quadricolor* LSU rDNA sequences from Korean and Japanese samples showed very little genetic difference (less than 0.6%) within and between them, which suggests that all these samples belong to the same sea anemone species. Comparison with another LSU rDNA sequence from *E. quadricolor* collected in the GBR (Chen et al. 1995), however, showed a genetic difference of 3.25%. Populations of *E. quadricolor* in the GBR and nearby localities in the southwestern and Indo-Pacific Ocean might have been isolated and genetically unconnected with populations in the northwestern Pacific Ocean, such as those from the Ryukyu Archipelago, Korea Strait and Honshu Island. This isolation might have caused a remarkable genetic differentiation in evolutionary time between the northern and southern populations within the Pacific Ocean.

Although it seems, based on the current oceanographic features of the northwestern Pacific Ocean, that the anthozoan hosts and symbionts from the Japanese sites are not directly connected to populations found in Korean waters, both geographical areas are connected to the southern source population, in the north of the Philippines, by the main ocean current, the

Kuroshio (Xu and Su 1997). This current brings water from the Philippines through the Ryukyu Islands and then splits into two northern extensions: one that flows through the Korea Strait, and the other that flows along the east coast of Japan (Figure 4). The Tsushima warm current flows through the Korea Strait and reaches Jeju Island and Aitsu, Akaiwa and Satsuski, at the eastern part of Honshu Island, Japan. Moreover, the main Kuroshio Current reaches Natto-ura, Kogane-zaki at Honshu Island, Japan. We hypothesize that those identical symbionts found in *E. quadricolor* and *A. japonica* at Japanese and Korean sites have passed maternally from generation to generation, from populations that originally came from southern subtropical areas in the north of the Philippines. In other words, the endosymbionts residing in most of the *E. quadricolor* and *A. japonica* within our study area in the northwestern Pacific may represent a mega clone from a single symbiont genotype.

Coral *Pocillopora* sp., containing *Symbiodinium* clade D, are abundant in Panama reefs after ENSO (El Niño-Southern Oscillation) events; however, coral colonies that contained clade C bleached severely after thermal change (Baker et al. 2004). After many years, colonies containing clade D had become dominant on these reefs in Panama. Clade C *Symbiodinium* sp. are predominant in the western Indian Ocean, but clade D *Symbiodinium* sp. are more successful in the turbid, high-temperature conditions of the northeastern Indian Ocean (LaJeunesse et al. 2010). However, the relationship between the two hosts, *E. quadricolor* and *A. japonica*, and endosymbiotic dinoflagellates within the hosts is highly specific in this study.

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