Coral Reefs (2006)
DOI 10.1007/s00338-006-0151-4

NOTE

James D. Reimer · Kiyotaka Takishita
Tadashi Maruyama

Molecular identification of symbiotic dinoflagellates (*Symbiodinium* spp.) from *Palythoa* spp. (Anthozoa: Hexacorallia) in Japan

Received: 25 May 2006 / Accepted: 11 August 2006

Abstract In Japan, zooxanthellate *Palythoa tuberculosa* Klunzinger and *Palythoa mutuki* Verrill (Anthozoa: Hexacorallia: Zoantharia) are found over a 1,000 + km latitudinal range, often in environments where most other zooxanthellate anthozoans are not found (i.e. tidal lagoon pools, around shallow water hydrothermal vents, subtropical rocky shorelines). Sequences of internal transcribed spacer of ribosomal DNA (ITS-rDNA) of the symbiotic dinoflagellate genus *Symbiodinium* (zooxanthellae) Freudenthal (Order Suessiales) from *P. tuberculosa* and *P. mutuki* from several locations in Japan (34°11′N–24°16′N) were analysed. Unexpectedly, despite the ability of the genus *Palythoa* to be flexible in association with different *Symbiodinium* subclades, most (35 of 36) *Palythoa* investigated here specifically associate with subclade C1 and closely related types. *Symbiodinium* subclade C1 has been characterized as a "generalist" in terms of the ability to associate with a range of hosts, but present results suggest that subclade C1 may also be a "generalist" in terms of being able to live in a variety of environments over a latitudinal range.

Communicated by Biology Editor M. van Oppen.

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00338-006-0151-4 and is accessible for authorized users.

J. D. Reimer (✉) · K. Takishita · T. Maruyama
Research Program for Marine Biology and Ecology,
Extremobiosphere Research Center,
Japan Agency for Marine-Earth Science and Technology
(JAMSTEC), 2-15 Natsushima, Yokosuka,
Kanagawa 237-0061, Japan
E-mail: jreimer@jamstec.go.jp
Tel.: +81-46-8679524
Fax: +81-46-8679525

Present Address: J. D. Reimer
Kuroshio Biological Research Foundation,
560 Nishidomari, Otsuki, Kochi 788-0333, Japan

Keywords ITS-rDNA · *Symbiodinium* · Symbiosis · *Palythoa* · Zooxanthellae · Zoanthid

Introduction

Many marine organisms contain symbiotic dinoflagellates of the genus *Symbiodinium* (Order Suessiales) and such algae-host symbiotic relationships are numerous in the coral reef ecosystems of the world's shallow sub-tropical and tropical oceans (Muscatine and Porter 1977). It has been established that there are at least eight major "clades" (of unknown taxonomic level) of this dinoflagellate genus (Pochon et al. 2006). Within each clade are numerous subclades; differences in physiology between some subclades have been demonstrated (Tchernov et al. 2004; LaJeunesse 2005). Various animal hosts contain different *Symbiodinium* populations; some show specificity to single *Symbiodinium* strains (LaJeunesse 2005).

Recent work has begun to characterize the genetic diversity of the symbiotic dinoflagellate genus *Symbiodinium* (or zooxanthellae) in zoanthids (Anthozoa: Hexacorallia). For the genus *Palythoa* (family Sphenopidae) in the Indo-Pacific however, only one detailed report exists, in which *Palythoa caesia* Dana from the Indian Ocean was shown to possess clade D around Madagascar, and clades C and D in the western Indian Ocean based on 18S ribosomal DNA (Burnett 2002). Other previous work (with a limited number of samples) has shown *Palythoa* spp. to harbor zoanthid-specific *Symbiodinium* subclade C24 (LaJeunesse et al. 2003), and generalists C1 and C3 on the Great Barrier Reef (LaJeunesse et al. 2004b), C1 and C3 in the Caribbean (LaJeunesse 2002), Hawaiian *Palythoa* harbors subclades C1 and C3 (LaJeunesse et al. 2004a), and *Palythoa* in Florida has subclades C1 and D1a (Kemp et al. 2006), demonstrating *Palythoa* species do have the ability to form symbioses with various different *Symbiodinium* types.

In Japan, the encrusting anemone zoanthid species *Palythoa tuberculosa* Klunzinger and *Palythoa mutuki*

Verrill (for photographic identification see Electronic Supplementary Material) are found in shallow waters (intertidal to 30 m depth) over a wide latitudinal range, from a reported northern limit of Miyakejima, Tokyo (34°11′N) to the southern boundaries of Japan in Okinawa (approx. 24°N) (Fig. 1) (see Reimer et al. 2006a). Additionally, both species are often found in conditions where few other zooxanthellate anthozoans exist in shallow tide pools, in areas high in the intertidal zone, and even (in the case of *P. tuberculosa*) in the immediate vicinity of shallow water hydrothermal vents (see Nakamura et al. 2006). Short-term (e.g. less than 6 hr duration) ocean water temperatures at these sites can range from maxima approaching 40°C (in shallow tide pools at Tomori, Amami in summer) to minima below 15.0°C in winter (at Miyakejima) (data not shown). *Palythoa* spp., like *Zoanthus* spp. (Ono et al. 2005), acquire *Symbiodinium* horizontally, presumably after larvae have settled on substrata (Ryland 1997). Thus, *Palythoa* spp. appear to be excellent candidates for possessing high levels of *Symbiodinium* genetic diversity.

Here, *Symbiodinium* has been characterized at the molecular level using internal transcribed spacer of ribosomal DNA (ITS-rDNA) from samples collected at 12 sites over a 1000 + km latitudinal range in order to answer the following question: Is there any genotypic difference among *Symbiodinium* in zooxanthellate *Palythoa* spp. over a latitudinal range in Japan?

Materials and methods

Sampling, DNA extraction and PCR-amplification

Clonal polyps of *Palythoa tuberculosa* ($n = 29$) and *Palythoa mutuki* ($n = 7$) were collected between August 2004 and May 2006 from 12 locations over a latitudinal range (34°11′N–24°16′N) in Japan (Fig. 1; Table 1). Previous research has indicated that *P. mutuki* may in fact be two very closely related groups (*P. mutuki* 1 [samples PmMiI1, PmBA1, PmYS1] and 2 [sample PmAT1]) (Reimer et al. 2006a), but for the purpose of simplicity both groups are referred to as *P. mutuki* in this study. Sample polyps were collected from the center of each colony, and immediately placed in 99.5% ethanol and stored at -30°C until further analyses. DNA extraction and PCR-amplification methods followed Reimer et al. (2006c).

Cloning and sequencing

PCR-amplified products from *Palythoa* colonies were obtained and processed as described in Reimer et al. (2006c). PCR-amplified products from *P. tuberculosa* samples from four selected locations (most northern = Miyakejima, mid-northern = Kushimoto, mid-southern = Tomori–Amami, and most southern = Kataguwa–Ishigaki) were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA, USA). Several clones (5–11) of ITS-rDNA from each sample were sequenced with an ABI PRISMTM 3700 DNA Analyzer (PE Applied Biosystems, Foster City, CA, USA) using a BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The sequences were analysed using DNASIS Mac v3.6 (Hitachi Software Engineering Company, Ltd., Tokyo, Japan). PCR-amplified products from *Palythoa* polyps from other locations were sequenced directly.

Phylogenetic analyses

New sequences obtained in the present study were deposited in GenBank (accession numbers DQ480593–DQ480644, DQ889726–DQ889746). Previously reported ITS-rDNA sequences of *Symbiodinium* clade C (for

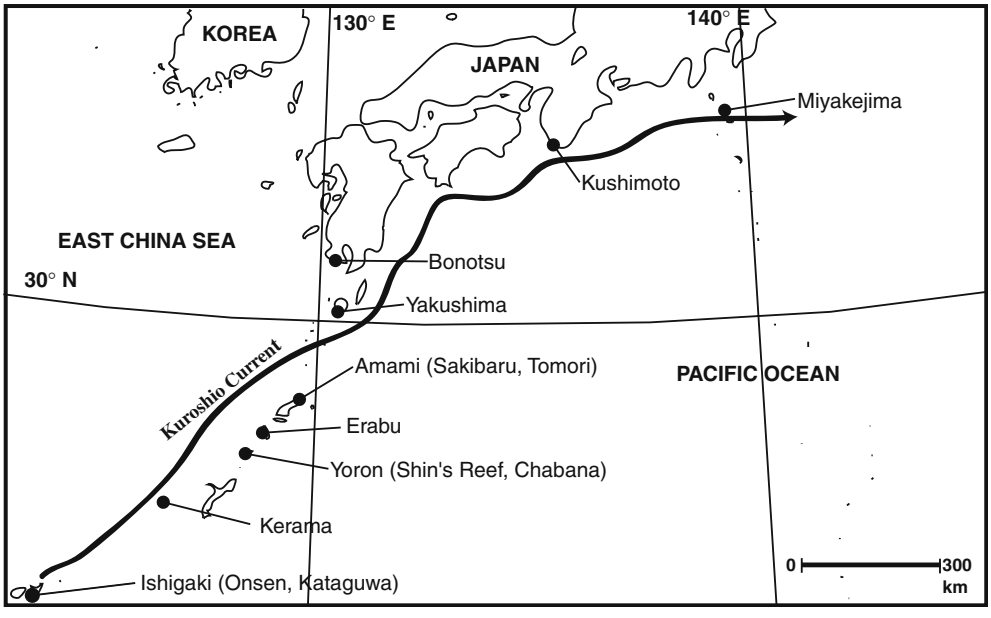

Fig. 1 Southern Japan sampling locations in this study. For location descriptions and information see Table 1

Table 1 *Palythoa* samples used in this study, sampling location description, and associated *Symbiodinium* types

Species	Location	Sample name(s)	Latitude (°N)	Depth(s) (m)	Maximum temperature (°C)[a]	Minimum temperature (°C)[a]	Average temperature (°C)[a]	Environment description	ZX clade
P. tuberculosa	Izushita, Miyakejima, Tokyo	PtMiI1[b]	34.11	−2.0	31.5	13.6	22.4	Non-reefal, algae-dominated, exposed rocky shoreline. Sub-tropical/temperate.	C1
	Marine Center, Kushimoto, Wakayama	PtWK1[b]	33.47	−2.0	30.8	10.1	22.0	Non-reefal, coral community, exposed rocky shoreline. Sub-tropical/temperate	C1
		PtWK2		−2.0					C1
	Akamizu, Bonotsu, Kagoshima	PtBA1	31.26	−1.0	30.9	10.2	21.8	Non-reefal, coral community, exposed rocky shoreline. Sub-tropical	C1
		PtBA2		−1.0					C1
	Sangohama, Yakushima, Kagoshima	PtYS1	30.30	−9.0	31.0	15.0	23.2	Coral reef mixed with granite rock. Tropical	C1
		PtYS2		−10.0					C1
		PtYS4		−1.5					
	Tomori, Amami, Kagoshima	PtAT1[b]	28.44	−2.0	31.4	17.6	24.6	Coral reef lagoon. Tropical	C1
		PtAT2		−1.5					C1
	Sakibaru, Amami, Kagoshima	PtAS1	28.48	−1.0	31.4	17.6	24.6	Rubble and rock shoreline, bleached coral. Tropical	C1
		PtAS2		−1.0					C1
	Okidomari, Erabu, Kagoshima	PtE1	27.42	0.0	30.1	18.4	24.4	Outer patch reef. Tropical	C1
		PtE2		−2.0					C1
		PtE3		−2.0					C1
	Shin's Reef, Yoron, Kagoshima	PtYoS1	27.04	−1.0	30.1	18.4	24.4	Reef lagoon. Tropical	C1
		PtYoS2		−1.5					C1
	Chabana, Yoron, Kagoshima	PtYoC1	27.03	−1.0	30.1	18.4	24.4	Coral reef lagoon. Tropical	C1
		PtYoC2		−1.0					C1
	Kuroshima-kita, Kerama, Okinawa	PtKK1	26.24	−6.0	31.7	19.7	25.9	Coral reef forefront. Tropical	C1
		PtKK2		−6.5					C1
		PtKK3		−2.0					C1
		PtKK4		NA					C1
	Onsen, Ishigaki, Okinawa	PtIsO1	24.20	−8.5	30.6 (JODC), approx. 35[c]	21.2	26.1	Directly next to shallow hydrothermal vent, dead coral and sand substrate. Tropical	C1
	Kataguwa, Ishigaki, Okinawa	PtIsK1[b]	24.16	−10.0	30.6	21.2	26.1	Outer patch reef. Tropical	C1, C3, C33
		PtIsK2		−9.5					C1
		PtIsK3		−10.0					C1
		PtIsK4		−10.0					C1
		PtIsK5		−10.0					C1
P. mutuki	Izushita, Miyakejima, Tokyo	PmMiI1	34.11	0.0	31.5	13.6	22.4	Non-reefal, algae-dominated, exposed rocky shoreline. Sub-tropical/temperate	C1
	Akamizu, Bonotsu, Kagoshima	PmBA1	31.26	0.0	30.9	10.2	21.8	Non-reefal, coral community, exposed rocky shoreline. Sub-tropical	C1
		PmBA2		0.0					C1
	Sangohama, Yakushima, Kagoshima	PmYS1	30.30	0.0	31.0	15.0	23.2	Coral reef mixed with granite rock. Tropical	C1
		PmYS2		0.0					C1
	Tomori, Amami, Kagoshima	PmAT1	28.44	+0.5	31.4	17.6	24.6	Coral reef lagoon. Tropical	C1
		PmAT2		0.0					C1

[a] Data from the Japan oceanographic data center (JODC) (1906–2003). Data are product of statistical analyses of temperature data from serial station data for 1° × 1° areas

[b] Samples were cloned (see Materials and methods for details)

[c] Maximum ocean temperature from the Onsen location is much higher than JODC data due to the presence of a hydrothermal vent. Estimated maximum ocean temperature to occasionally reach as high as 35°C (Yasuo Furushima, JAMSTEC, personal communication) for brief periods (e.g. < 5 min) of time

NA not available

Accession Numbers see Fig. 2 and electronic supplementary material) were retrieved from the DNA data bank of Japan (DDBJ) and were aligned with present data using Clustal W version 1.8 (Thompson et al. 1997).

Two sequence alignments were generated. In addition to a full ITS-rDNA alignment (see electronic supplementary material), an ITS-2 alignment was made (Fig. 2). ITS-2 has been frequently used to identify subclades of *Symbiodinium* (see LaJeunesse et al. 2003, 2004a, b; LaJeunesse 2005), and compared to the full ITS-rDNA region much more sequence data are available from GenBank. The alignments were inspected by eye and manually edited. Some sequences that were identical (and thus redundant) to other sequences were not included in the alignments (see Fig. 2 legend). The alignment datasets are available in the electronic supplementary material. The datasets (full ITS-rDNA region (72 taxa/636 sites; ITS-2 region (77 taxa/253 sites) were independently subjected to analyses with the maximum-likelihood (PhyML) (Guindon and Gascuel 2003) and neighbor-joining (NJ) (Saitou and Nei 1987) methods. PhyML was performed using input trees generated by BIONJ with the general time-reversible model (Rodriguez et al. 1990) of nucleotide substitution incorporating invariable sites and a discrete gamma distribution (eight categories) (GTR + I + Γ). The proportion of invariable sites, discrete gamma distributions, and base frequencies of the models were estimated from the dataset. PhyML bootstrap trees (500 replicates) were constructed using the same parameters as the individual ML trees. The

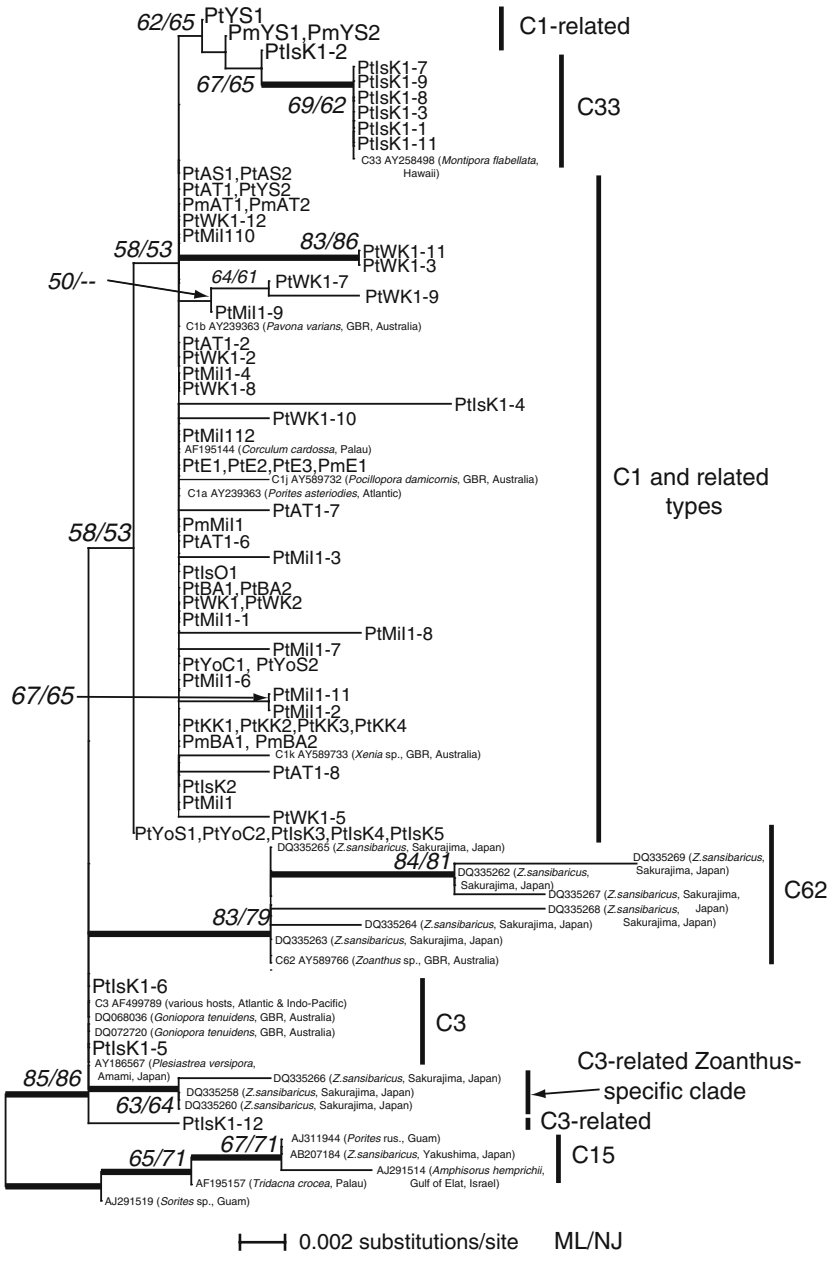

Fig. 2 Maximum-likelihood (PhyML) tree of *Symbiodinium* internal transcribed spacer of ribosomal DNA (ITS-2) sequences. Values at branch nodes represent ML and neighbour-joining (NJ) bootstrap probability, respectively (> 50%). Monophylies with more than 0.95 Bayesian posterior probability are shown by thick branches. For sample name information refer to Table 1. For sequences acquired from GenBank, the Accession Number is followed by the host species and sampling location. Note that some sequences from samples that had identical ITS-2 sequences to other sequences used in the alignment to generate this tree were not included in our analyses, and instead added to the tree after generation (sequences PmYS2, PtYS2, PtAS2, PmAT2, PtE2, PtE3, PmE1, PtBA2, PtWK2, PtYoS2, PtKK2, PtKK3, PtKK4, PmBA2, PtIsK3, PtIsK4, PtIsK5, PtAT2, PtYoC2)

distances were calculated using Kimura's 2-parameter model (Kimura 1980). Support for NJ branches was tested by bootstrap analyses (Felsenstein 1985) of 1,000 replicates. PAUP version 4 was used for phylogenetic analyses (Swofford 1998).

Bayesian trees were reconstructed by using the program MrBayes 3.0 (Ronquist and Huelsenbeck 2003). The proportion of invariable sites, discrete gamma distributions, and base frequencies of the models were estimated from the datasets. One cold and three heated Markov chain Monte Carlo (MCMC) chains with default-chain temperatures were run for 1,000,000 generations, sampling log-likelihoods (InLs), and trees at 100-generations intervals (10,000 InLs and trees were saved during MCMC) for both datasets. The likelihood plots for the dataset suggested that MCMC reached the stationary phase after the first 40,000 generations for both datasets. Thus, the remaining 960,000 trees for both datasets were used to obtain clade probabilities and branch-length estimates.

Temperature data

Temperature data at the sea surface (0 m) were obtained from the Japan oceanographic data center (JODC) for the period of 1906–2003. Data are product of statistical analyses of temperature data from serial station data for one-degree latitude by one-degree longitude areas. Temperature data (maximum observed temperature 1906–2003, minimum observed temperature 1906–2003, and annual average 1906–2003) for each sampling site were obtained for the relevant 1° × 1° areas (Table 1).

Results and discussion

ITS-rDNA sequence data and analyses

All PCR products could be sequenced directly with minimal ambiguity (i.e. observable double peaks at 0–11 of 636 sites [0–1.7%] for the full ITS-rDNA region) (except PtIsK1), indicating that samples at least possessed a majority of *Symbiodinium* of very similar genetic identity (i.e., to the subclade level, as in Rodriguez-Lanetty and Hoegh-Guldberg 2003). This was further confirmed as all cloned sequences except sequences from sample PtIsK1 were very similar. The number of variable sites over the full ITS-rDNA region was 10/636 bp (1.57%, $n = 40$) for all samples used in phylogenetic analyses excluding PtIsK1 sequences, and 17/636 bp (2.67%, $n = 52$) for all samples used in phylogenetic analyses including PtIsK1 sequences. A summary of samples and obtained ITS-rDNA sequences is shown in Table 1 and a phylogenetic tree of the ITS-2 region in Fig. 2. No major differences were seen between the ITS-rDNA full region and the ITS-2 topologies. All examined colonies of *Palythoa* were unambiguously shown (Fig. 2) to harbor *Symbiodinium* of clade C (*sensu* Rowan and Power 1992). Most obtained sequences were subclade C1 (99–100% similarity with DQ068044 from *Symbiodinium* in *Heliofungia actinoformes* on the Great Barrier Reef, Bui et al. unpublished), with two subclades of cloned sequences from sample PtIsK1 slightly different, one group forming a monophyly most closely related (bootstrap support [ITS-2 region] 85 and 86% for ML and NJ, respectively) to *Symbiodinium* subclade C3 (ITS-2 99–100% similarity with AF499789 from LaJeunesse 2002), and the other most closely related to C33 (ITS-2 100% similarity with AY258498 [LaJeunesse et al. 2004a], bootstrap support [ITS-2 region] 69% and 62% for ML and NJ respectively, Bayesian posterior probability = 0.98 [Fig. 2]). Obtained *Symbiodinium* sequences from *Palythoa* spp. showed less variation (different genotypes within and among subclades) than *Symbiodinium* sequences used in analyses here obtained from a single polyp of *Z. sansibaricus* at Sakurajima (Reimer et al. 2006b) despite examining a far smaller number of *Symbiodinium* sequences from *Z. sansibaricus* (number of variable sites = 21/637 bp = 3.30%, $n = 10$).

Why do *Palythoa* spp. have a strong association with *Symbiodinium* subclade C1?

The data demonstrate that the two *Palythoa* species investigated here (excepting PtIsK1), despite having the potential for flexibility in *Symbiodinium* association, are specifically selecting an association with subclade C1.

Symbiodinium subclade C1 is one of the most common *Symbiodinium* subclades throughout the East Pacific–Indian Ocean region (LaJeunesse 2005). The majority of the data here showing *Symbiodinium* subclade C1 in *Palythoa* spp. reflect previous findings from Zamami, Okinawa, Japan, which found *Symbiodinium* subclade C1 in a single sample of *Palythoa* sp. (LaJeunesse et al. 2004b); LaJeunesse (2005) states that subclade C1 is a "generalist" *Symbiodinium* found in a wide range of different hosts.

All of the *Palythoa* samples here except the Ishigaki samples (PtIsO1, PtIsK1–PtIsK5) were from environments where occasionally ocean temperatures drop below 20°C (Table 1). These other *P. tuberculosa* and *P. mutuki* samples were from more "marginal" habitats where the number of clonal cnidarians is much lower (i.e. high latitude non-reef communities [Miyakejima, Kushimoto, Bonotsu, Yakushima], intertidal/very shallow depths [both Amami samples, Erabu, both Yoron samples], reef lagoons [both Yoron samples, and Sakibaru at Amami], or hydrothermal vent area [Onsen, Ishigaki]). JODC ocean temperature data (1906–2003) showed all locations to have average temperatures within 5°C of each other (low (Akamizu = 21.8°C; high = both Ishigaki locations (Onsen, Kataguwa) = 26.1°C) (Table 1), as would be expected as all locations are located along the path of the warm Kuroshio Current (Fig. 1). All locations experienced maximum temperatures slightly over 30°C (30.1–31.7°C) (Table 1). However, only samples PtIsK1–PtIsK5 came from a

stable (i.e., ocean temperatures between 20 and 30°C) tropical coral reef environment where many other coral and colonial cnidarian species were present, and only PtIsK1 did not have a specific association with subclade C1. The observed *Palythoa*-C1 association may be similar to the specific and stable association between the coral *Oulastrea crispata* and *Symbiodinium* clade D observed by Chen et al. (2003) in East Asia who suggest association with *Symbiodinium* clade D allows *Oulastrea crispata* to live in subtropical nonreefal environments. These data suggest *Palythoa* may specifically associate with subclade C1 in order to live in a wide variety of environments, including "marginal" habitats where many other zooxanthellate cnidarians cannot. In other words, subclade C1 may not only be a "host generalist" but also may be a "generalist" in terms of environment. However, unknown factor(s) in *Palythoa* spp. may also be partially or entirely responsible for subclade C1 being present in a wide variety of environments, and further investigations into the physiology of subclade C1 *Symbiodinium* and *Palythoa* are needed.

It may be that sample PtIsK1, possessing subclades C1 (generalist), C33 (found thus far only in Hawaiian *Montipora flabellata* at 2 m depth, see LaJeunesse et al. 2004a) and C3 (hypothetically deeper-water adapted, see LaJeunesse 2002), has a different physiology than C1-specific *Palythoa*, and this difference may be linked to differences in the environment at Kataguwa (e.g. a more "stable" environment, or more, different *Symbiodinium* present) compared to the other environments sampled here. Alternatively, it may be possible that PtIsK1 sequence variation is the result of errors associated with PCR-amplification/cloning or intragenomic variation (e.g. LaJeunesse 2002), although it has been shown this may be unlikely (see Reimer et al. 2006b; Speksnijder et al. 2001).

In conclusion, most *P. tuberculosa* and *P. mutuki* in southern Japan specifically associate with "generalist" (in terms of hosts and perhaps environment) *Symbiodinium* subclade C1 despite the potential for flexible associations with other *Symbiodinium* types. *Palythoa* spp. investigated here often exist in marginal environments where most other zooxanthellae cnidarians do not, and this wide-ranging ability may be conferred in part by this specific "generalist" subclade C1 association. Further investigations into the physiology of *Symbiodinium* subclade C1 and the sexual and asexual reproductive traits of *Palythoa* spp. (and how they are related to horizontal and vertical *Symbiodinium* transmission–which may affect *Symbiodinium* genetic diversity within the host) may help shed light on possible causes for differing levels of symbiont specificity seen worldwide in *Palythoa*.

Acknowledgments The authors thank Masaru Kawato (JAMSTEC) for sequencing assistance, and Drs. Shusuke Ono and Junzo Tsukahara for their ideas on *Palythoa*. The following people helped with sampling: Dr. Yasuo Furushima (JAMSTEC), Tatsu Yarabu, Mamoru Uehara, Masanori Uehara, Osamu Tamashiro, Yuzo Tamashiro, (Ishigaki), Hideo Noda, Kei Sasahara (Miyakejima), Ryan Cox (Bonotsu), Mika Reimer, Denny Probizanski (Amami), Shin Hiasashi (Yoron and Erabu), and Dr. Hiroomi Uchida (Kushimoto). At JAMSTEC JDR was supported by a Japan Society for the Promotion of Science (JSPS) post-doctoral fellowship.

References

Burnett WJ (2002) Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. Mar Ecol Prog Ser 234:105–109

Chen AC, Lam KK, Nakano Y, Tsai WS (2003) A stable association of the stress-tolerant zooxanthellae, *Symbiodinium* Clade D, with the low temperature-tolerant coral, *Oulastrea crispata* (Scleractinia: Faviidae) in subtropical non-reefal coral communities. Zool Stud 42:540–550

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Kemp DW, Cook CB, LaJeunesse TC, Brookes WR (2006) A comparison of the thermal bleaching responses of the zoanthid *Palythoa caribaeorum* from three different geographically different regions in south Florida. J Exp Mar Biol 335 306:266–276

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141:387–400

LaJeunesse TC (2005) "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene–Pliocene transition. Mol Biol Evol 22:570–581

LaJeunesse TC, Loh WKW, Van Woesik R, Hoegh-Guldberg O, Schmidt GW, Fitt WK (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnol Oceanogr 48:2046–2054

LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG, Fitt WK, Schmidt GW (2004a) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. Coral Reefs 23:596–603

LaJeunesse TC, Bhagooli R, Hidaka M, deVantier L, Done T, Schmidt GW, Fitt WK, Hoegh-Guldberg O (2004b) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef communities across environmental, latitudinal and biogeographic gradients. Mar Ecol Prog Ser 284:147–161

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. BioScience 27:454–460

Nakamura T, Yamazaki SS, Sakai K, Yamasaki H, Furushima Y, Yamamoto H (2006) Acroporid corals growing over a methane-bubbling hydrothermal vent, Southern Ryukyu Archipelago. Coral Reefs 25:382

Ono S, Reimer JD, Tsukahara J (2005) Reproduction of *Zoanthus sansibaricus* in the infra-littoral zone at Taisho Lava Field, Sakurajima, Kagoshima, Japan. Zool Sci 22:247–255

Pochon X, Montoya-Burgos JI, Stadelmann B, Pawlowski J (2006) Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. Mol Phylogenet Evol 1:20–30

Reimer JD, Ono S, Takishita K, Tsukahara J, Maruyama T. (2006a) Molecular evidence suggesting species in the zoanthid genera *Palythoa* and *Protopalythoa* (Anthozoa: Hexacorallia) are congeneric. Zool Sci 23:87–94

Reimer JD, Ono S, Tsukahara J, Takishita K, Maruyama T. (2006b). Non-seasonal clade-specificity and subclade microvariation in symbiotic dinoflagellates (*Symbiodinium* spp.) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia) at Kagoshima Bay, Japan. Phycol Res (in press)

Reimer JD, Takishita K, Ono S, Tsukahara J, Maruyama T (2006c) Latitudinal and intracolony ITS-rDNA sequence variation in

the symbiotic dinoflagellate genus *Symbiodinium* (Dinophyceae) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia). Phycol Res 54:122–132

Rodriguez F, Oliver JL, Marin A, Medina JR (1990) The general stochiatic model of nucleotide substitution. J Theor Biol 142:485–501

Rodriguez-Lanetty M, Hoegh-Guldberg O (2003) Symbiont diversity within the scleractinian coral *Plesiastrea versipora*, across the northwestern Pacific. Mar Biol 143:501–509

Ronquist F, Huelsenbeck JP (2003) Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxf) 19:1572–1574

Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). Proc Natl Acad Sci USA 89:3639–3643

Ryland JS (1997) Reproduction in Zoanthidea (Anthozoa: Hexacorallia). Invertebr Reprod Dev 31:177–188

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Speksnijder AGCL, Kowalchuk GA, De Jong S, Kline E, Stephen JR, Laanbroeak HJ (2001) Microvariation artifacts introduced by PCR and cloning of closely related 16S rRNA gene sequences. Appl Environ Microbiol 67:469–472

Swofford DL (1998) PAUP*. V Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, MA, USA

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, Haggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc Natl Acad Sci USA 101:13531–13535