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Research Article

Marine invertebrate diversity in the oceanic Ogasawara Islands: a molecular examination of zoanthids (Anthozoa: Hexacorallia) and their *Symbiodinium* (Dinophyceae)

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The Ogasawara (Bonin) Islands are oceanic islands approximately 1000 km south of mainland Japan noted for their high levels of both terrestrial and marine endemicity. Despite their unique location, the marine fauna of many taxa remains relatively under-examined. In this study, we specifically investigated the diversity of shallow water zoanthids (Cnidaria: Anthozoa: Hexacorallia) and their symbiotic zooxanthellae (*Symbiodinium* spp.) of the Ogasawara Islands for the first time. Using *in situ* field examinations combined with DNA sequencing and phylogenetic analyses, our results show the presence of five zoanthid species in the Ogasawara Islands; *Zoanthus sansibaricus*, *Z. kuroshio*, *Palythoa tuberculosa*, *P. mutuki*, and one potentially undescribed species of *Palythoa*. While most collected specimens were in symbiosis with clade C *Symbiodinium* as seen in southern Japan and other Indo-Pacific locations, one specimen of *Z. kuroshio* was unexpectedly in symbiosis with clade A, and no *Z. sansibaricus* colonies contained specific C1z type zooxanthellae previously observed at other locations. Overall, the Ogasawara Islands showed a lower zooxanthellate zoanthid species diversity (n = 5) than the non-oceanic Okinawa Islands (n > 10), which are at roughly the same latitude. Instead, the islands' relatively depauperate zoanthid/symbiont fauna more closely resembles that of the northern Izu Islands.

Key words: Kuroshio recirculation, Ogasawara (Bonin) Islands, *Palythoa*, *Symbiodinium*, zoanthid, *Zoanthus*, zooxanthellae

Introduction

Zoanthids are an order of anthozoans found in many marine ecosystems and are particularly common in coral reef ecosystems worldwide. Similar to many other benthic cnidarians of coral reefs, zoanthids are often in symbioses with endosymbiotic dinoflagellates of the genus *Symbiodinium* (Reimer, 1971; LaJeunesse, 2002; LaJeunesse *et al.*, 2004; Reimer *et al.*, 2006*b*, 2006*c*), and obtain a substantial portion of their nutritional requirements from their symbionts (Trench, 1974). Zoanthids of the genera *Palythoa* (family Sphenopidae) and *Zoanthus* (Zoanthidae) are commonly seen in the intertidal and shallow sublittoral zones in tropical and subtropical waters, and can be the dominant benthic invertebrate in these areas (Sebens, 1982; Karlson, 1983; Oigman-Pszczol *et al.*, 2004; Irei *et al.*, pers. comm.). Thus, an understanding of the distribution and diversity of *Palythoa*, *Zoanthus*, and their *Symbiodinium* is critical to understanding the ecosystems in which they are found.

However, zoanthids, particularly of the genera *Palythoa* and *Zoanthus*, are notoriously difficult to identify to the level of species. The reasons for this are multiple, and include high levels of intraspecific morphological variation (Burnett *et al.*, 1994, 1997; Reimer *et al.*,

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2004), colony and polyp morphology being influenced by microenvironment (Burnett *et al.*, 1997), and sand encrustation in the mesoglea of most zoanthids making histology very difficult (Reimer *et al.*, 2010*c*). As well, the taxonomic status of species within *Palythoa* and *Zoanthus* is very confused, with over 150 species described for each genus (Fautin, 2009), although many are likely inadvertent redescriptions (Burnett *et al.*, 1994, 1995, 1997; Reimer *et al.*, 2004).

Over the past two decades, molecular techniques including allozyme analyses (Burnett *et al.*, 1994, 1995, 1997) and phylogenetics of DNA markers (Reimer *et al.*, 2004; Sinniger *et al.*, 2005) have resulted in a re-examination of zoanthid species diversity. Research has demonstrated the utility of molecular examination of zoanthids (Reimer *et al.*, 2006*a*; Sinniger *et al.*, 2008). Currently, a large-scale revision of the higher-level classifications (i.e. > genera) is now underway (Reimer *et al.*, 2008*a*; Reimer & Fujii, 2010; Sinniger *et al.*, 2010). With basic DNA molecular phylogenetic protocol now established, examination and comparison of relative zoanthid diversity from different regions is now possible.

The Ogasawara Islands, formerly known as the Bonin Islands, are located approximately 1000 kilometres south of Tokyo and north of the Marianas Islands in the northwestern Pacific (Fig. 1). These islands are oceanic, and are noted for their unique terrestrial floral and fauna (e.g. Ono & Masuda, 1981). As the Ogasawara Islands are isolated from both the East Asia landmasses by geography and by currents, the coral reef ecosystems are much different than found in the Ryukyu Islands and the southern Pacific coast of Japan. However, marine fauna of the Ogasawara Islands is not yet well inventoried, although diversity of hard corals in this region is known to be much lower than the Ryukyu Islands (Inaba, 2004), which are further east at the same latitude and influenced by the Kuroshio Current. The few studies examining marine biodiversity have confirmed the presence of endemic invertebrate species in the Ogasawara Islands (e.g. Carlgren, 1943; Okutani, 1986; Asakura, 1991; Asakura *et al.*, 1993). However, zoanthid diversity in these islands remains generally unexamined.

In this study, we examined the diversity of shallow water zooxanthellae zoanthid (primarily *Palythoa* and *Zoanthus*) species and their endosymbiotic *Symbiodinium* in the Ogasawara Islands to answer the following questions:

1. Is there endemic zoanthid and/or *Symbiodinium* diversity in the Ogasawara Islands?
2. How does the diversity of Ogasawara zoanthids and their symbionts compare with results from previous recent studies in the Ryukyu Islands and along the Pacific coast of Japan (Reimer *et al.*, 2006*b*, 2007*b*; Reimer, 2007, 2010; Ono *et al.*, 2008)?

Materials and methods

Specimen collection and initial identification

Twenty-nine zoanthid specimens (specimens = colonies) representing all observed zoanthid morphotypes were collected in May 2009 (as in Reimer *et al.*, 2010*b*) by snorkelling or scuba diving from six locations in the Ogasawara Islands; Miyanohama (27.1044°N, 142.1942°E), Nishi-kaigan (27.0443°N, 142.2245°E) in Tatsumi Bay, Higashi-kaigan (27.0527°N, 142.2302°E) in Tatsumi Bay, Byobudani in Futami Bay (27.0937°N, 142.2029°E), all on Chichijima Island, and on Anijima Island, Takinoura (27.1185°N, 142.2013°E) and Takinoura Higashi (27.1137°N, 142.2011°E; a small beach south of Takinoura), both in Takinoura Bay (Fig. 2, Table 1). No

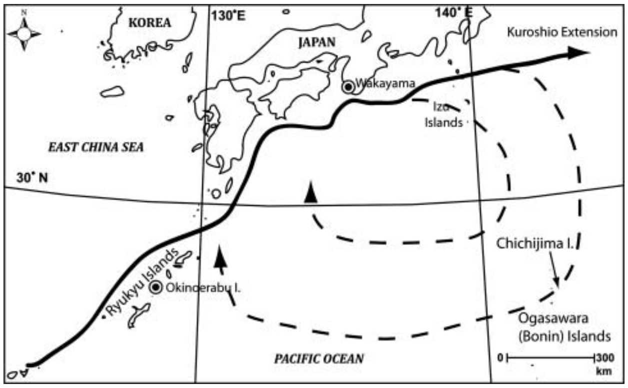


Fig. 1. Map showing the position of Ogasawara Islands and the sampling location for the current study with the approximate location of the Kuroshio Current (solid line), and the Kuroshio Recirculation (dotted line). Other locations mentioned in the text marked as closed circles.

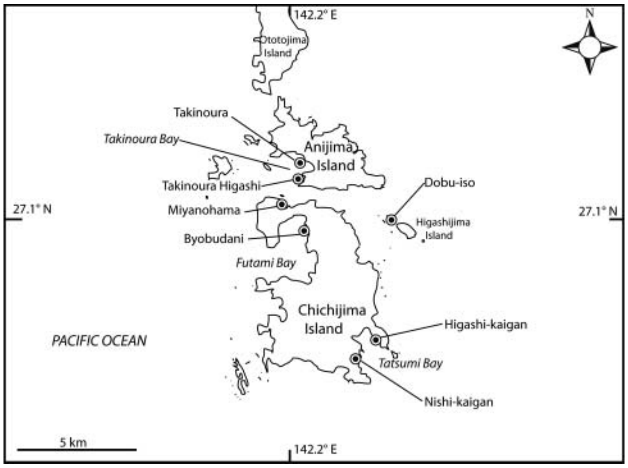


Fig. 2. Sampling locations around Chichijima Island and Anijima Island, designated by closed circles.

Table 1. Zooxanthellate zoanthid specimens collected in May 2009 from the Ogasawara Islands examined in this study.

MISE Specimen number	Genus	Species	Locality	Depth (m)	Collector(s)	mt 16S rDNA Acc. No.	COI Acc. No.	*Symbiodinium* type (ITS-rDNA Acc. No.)
1134	*Palythoa*	*tuberculosa*	Miyanohama, Chichijima Island	1	JD Reimer	JF419751	JF419793	NA
1135	*Palythoa*	*tuberculosa*	Miyanohama, Chichijima Island	1	JD Reimer	JF419748	JF419795	C1 (JF419765)
1137	*Palythoa*	*tuberculosa*	Miyanohama, Chichijima Island	1	JD Reimer	JF419744	JF419789	C1 related (JF419766)
1139	*Zoanthus*	*sansibaricus*	Miyanohama, Chichijima Island	1	JD Reimer	JF419762	JF419801	C3 (JF419772)
1141	*Palythoa*	unknown	Takinoura, Takinoura Bay, Anijima Island	3	JD Reimer	NA	NA	NA
1142	*Palythoa*	unknown	Takinoura, Takinoura Bay, Anijima Island	2	JD Reimer	JF419755	JF419797	C1 (JF419773)
1143	*Zoanthus*	*kuroshio*	Takinoura Higashi, Takinoura Bay, Anijima	Inter-tidal	JD Reimer	JF419760	Short but identical to *Z. kuroshio*	C15/C91-derived (JF419767)
1144	*Palythoa*	*tuberculosa*	Takinoura Higashi, Takinoura Bay, Anijima	2	JD Reimer	JF419753	JF419792	C1 (JF419770)
1145	*Zoanthus*	*kuroshio*	Takinoura Higashi, Takinoura Bay, Anijima	Inter-tidal	JD Reimer	JF419757	JF419802	C15/C91-derived (JF419768)
1146	*Zoanthus*	*sansibaricus*	Takinoura Higashi, Takinoura Bay, Anijima	Inter-tidal	JD Reimer	JF419761	JF419800	Short, but C1 or C1 related
1147	*Zoanthus*	*kuroshio*	Takinoura Higashi, Takinoura Bay, Anijima	Inter-tidal	JD Reimer	NA	NA	NA
1148	*Zoanthus*	*kuroshio*	Takinoura Higashi, Takinoura Bay, Anijima	Inter-tidal	JD Reimer	JF419758	JF419803	A1 (JF419782)
1149	*Zoanthus*	*kuroshio*	Takinoura Higashi, Takinoura Bay, Anijima	Inter-tidal	JD Reimer	JF419759	JF419804	C15/C91-derived (JF419769)
1151	*Palythoa*	*tuberculosa*	Higashi-kaigan, Tatsumi Bay, Chichijima	2	JD Reimer	JF419745	JF419787	C1 (JF419778)
1152	*Palythoa*	*tuberculosa*	Higashi-kaigan, Tatsumi Bay, Chichijima	3	JD Reimer	JF419752	JF419791	C1 related (JF419777)
1153	*Palythoa*	*tuberculosa*	Higashi-kaigan, Tatsumi Bay, Chichijima	2	JD Reimer	JF419742	JF419783	C1 related (JF419771)
1155	*Palythoa*	*tuberculosa*	Nishi-kaigan, Tatsumi Bay, Chichijima	2	JD Reimer	JF419749	JF419786	C1 (JF419775)
1156	*Palythoa*	*tuberculosa*	Takinoura Higashi, Takinoura Bay, Anijima	NA	K Yanagi	NA	Short but identical to *P. tuberculosa*	NA
1157	*Palythoa*	*tuberculosa*	Byobudani, Futami Bay, Chichijima	1	JD Reimer	JF419743	JF419790	C1 (JF419781)
1158	*Palythoa*	*tuberculosa*	Byobudani, Futami Bay, Chichijima	1	JD Reimer	JF419750	JF419784	C1 related (JF419780)
1159	*Palythoa*	*tuberculosa*	Byobudani, Futami Bay, Chichijima	1	JD Reimer	NA	NA	NA
1160	*Zoanthus*	*kuroshio*	Byobudani, Futami Bay, Chichijima	Inter-tidal	JD Reimer	NA	NA	NA
1161	*Palythoa*	*tuberculosa*	Byobudani, Futami Bay, Chichijima	1	JD Reimer	JF419747	JF419794	C1 (JF419774)
1162	*Palythoa*	*mutuki*	Byobudani, Futami Bay, Chichijima	1	JD Reimer	JF419754	JF419796	C1 (JF419779)
1163	*Palythoa*	*mutuki*	Byobudani, Futami Bay, Chichijima	1	K Yanagi	JF419746	JF419788	C1 related (JF419776)
1164	*Zoanthus*	*sansibaricus*	Miyanohama, Chichijima Island	Inter-tidal	JD Reimer	JF419764	JF419798	Short, but C1 or C1 related
1165	*Zoanthus*	*sansibaricus*	Miyanohama, Chichijima Island	Inter-tidal	JD Reimer	NA	JF419799	NA
1166	*Zoanthus*	*sansibaricus*	Miyanohama, Chichijima Island	Inter-tidal	JD Reimer	JF419763	Short but identical to *Z. sansibaricus*	NA
1167	*Palythoa*	unknown	Miyanohama, Chichijima Island	1	K Yanagi	JF419756	JF419785	Short, but C1 or C1 related

NA = not available.

zooxanthellate zoanthids were observed at one additional location, Dobu-iso (27.0983°N, 142.2368°E), northwest off Higashijima Island (Fig. 2).

Specimens were preliminarily identified using morphological characteristics used in previous literature (e.g. sand-encrusted or not, oral disk colour, polyp form, tentacle number, etc.) based on *in situ* photographs (Canon G11 digital camera with underwater housing) and *ex situ* physical examination. Specimens were subsequently stored in 99.5% ethanol at ambient temperature. All samples were finally deposited in the first author's collection at the University of the Ryukyus (Nishihara, Okinawa, Japan).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from portions of specimen weighing 5–20 mg using spin-column Dneasy Animal Extraction protocol (Qiagen, Santa Clarita, CA, USA). PCR amplification using the genomic DNA as a template was performed using HotStarTaq DNA polymerase (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. Mitochondrial (mt) 16S rDNA was amplified following procedures outlined in Sinniger *et al*. (2005). COI was amplified using zoanthid-specific primer COIZoanF (Reimer *et al*., 2007*a*) and general COI primer HCO2198 (Folmer *et al*., 1994). PCR amplification was performed on the samples under the following conditions: an initial denaturing step at 95 °C for 15 minute, followed by 35 cycles of 1 minute denature at 94 °C, 1 minute annealing at 40 °C, and 90 s extension at 72 °C, followed by 7 minutes' extension at 72 °C.

ITS-rDNA, and particularly ITS-2, has been shown to have good utility in identifying *Symbiodinium* types (e.g. LaJeunesse, 2002). The internal transcribed spacer region of ribosomal DNA (ITS-rDNA) of endosymbiotic *Symbiodinium* was amplified following procedures outlined in Reimer *et al*. (2006*c*). The amplified products were visualized by 1.0% agarose gel electrophoresis. PCR products were treated with Exonuclease I and Alkaline Phosphatate (Shrimp) (Takara) prior to sequencing reactions using DTCS Quick Start Master Mix (Beckman Coulter). The products were analysed using a CEQ8800 (Beckman Coulter) automated DNA sequencing system.

Phylogenetic analyses

New sequences obtained from this study were deposited in GenBank (accession numbers JF419472-JF419804). Nucleotide sequences of mt 16S rDNA and COI from specimens were aligned manually with publically available mt 16S rDNA and COI sequences from zoanthid specimens representing species in the genera *Palythoa* (family Sphenopidae), *Zoanthus*, *Neozoanthus* and *Isaurus* (families Zoanthidae+Neozoanthidae). Originally, outgroup sequences for mt 16S rDNA and COI trees were from families Epizoanthidae, Parazoanthidae and Hydrozoanthidae (in suborder Macrocnemina), and once the monophylies of Sphenopidae and Zoanthidae+Neozoanthidae (suborder Brachycnemina) were confirmed, final phylogenetic trees were made with only hydrozoanthid outgroups to improve resolution.

For *Symbiodinium* ITS-rDNA sequences, different *Symbiodinium* clade sequences are highly divergent from each other, and two alignments of ITS-rDNA were constructed, one with clade A and a clade E outgroup, and another with clade C with a clade F outgroup. GenBank Accession Numbers of *Symbiodinium* sequences used are given in the resulting phylogenetic trees.

All alignments were inspected by eye and all ambiguous sites of the alignments were removed from the dataset for phylogenetic analyses. Consequently, four alignment datasets were generated: (1) 833 sites of 34 sequences (mt 16S rDNA); (2) 461 sites of 35 sequences (COI); (3) 336 sites of 15 sequences (clade A *Symbiodinium* ITS-2), and (4) 281 sites of 33 sequences (clade C *Symbiodinium* ITS-2). The alignment data are available on request from the corresponding author and at the homepage: http://web.me.com/miseryukyu/.

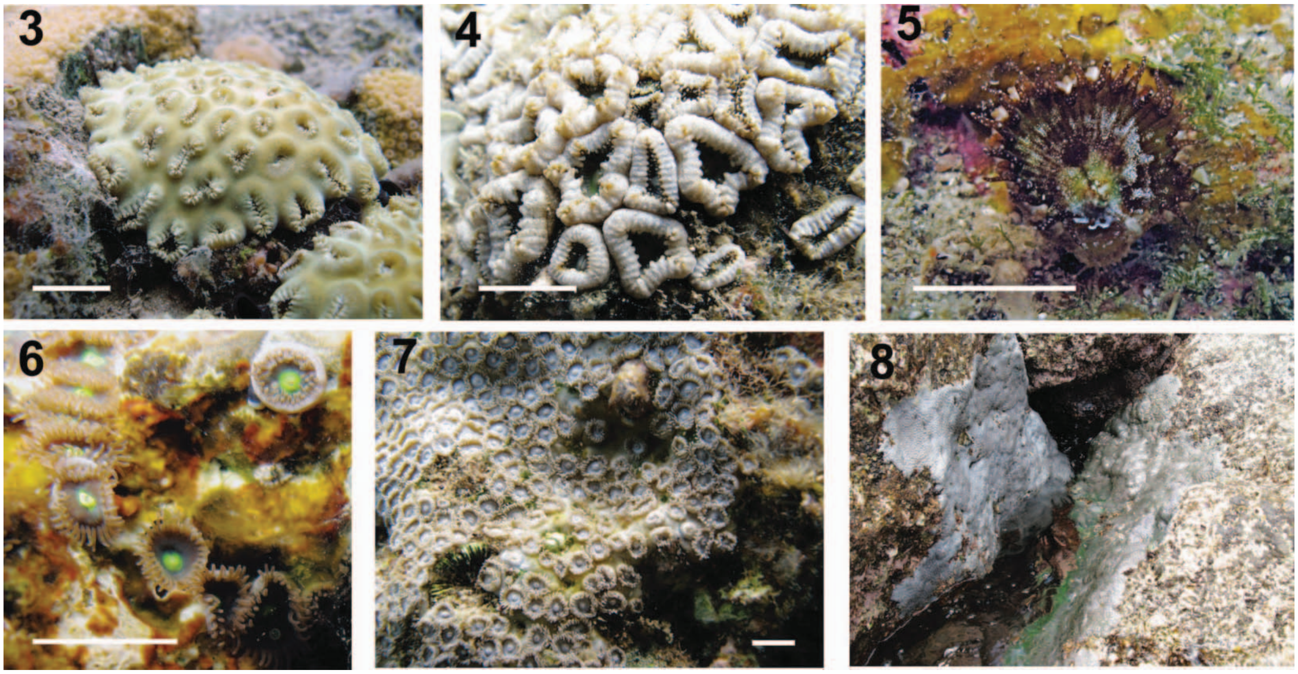
For the phylogenetic analyses of the four alignments, the same methods were applied independently. Alignments were subjected to analyses with the maximum-likelihood (ML) with PhyML (Guindon & Gascuel, 2003) and neighbour-joining (NJ) methods. PhyML was performed using an input tree generated by BIONJ with the general time-reversible model (Rodriguez *et al*., 1990) of nucleotide substitution incorporating a discrete gamma distribution (eight categories) (GTR + Γ). The discrete gamma distribution and base frequencies of the model were estimated from the dataset. PhyML bootstrap trees (500 replicates) were constructed using the same parameters as the individual ML tree. The distances were calculated using a Kimura's 2-parameter model (Kimura, 1980). Support for NJ branches was tested by bootstrap analysis (Felsenstein, 1985) of 1000 replicates. CLC Free Workbench 3.0 (Aarhus, Denmark) was used for NJ phylogenetic analyses (1000 replicates).

Results

In situ morphological identification

The 29 collected zoanthid specimens belonged to five morphological groups (= species groups).

Three species groups were identified as belonging to the genus *Palythoa* due to their heavy sand/particle encrustation and external brown or tan colouration. The most commonly observed zoanthid in the Ogasawara Islands was *Palythoa tuberculosa* Klunzinger, 1877 (n = 13), easily distinguishable by its embedded, 'immersae' (Pax, 1910) polyps (Fig. 3). This species was observed at Miyanohama, Takinoura Higashi, Higashi-kaigan, Nishi-kaigan and Byobudani sites (Table 1). *Palythoa mutuki* Haddon and Shackleton, 1891 (Fig. 4) was observed only rarely (n = 2) at two sites, Takinoura and Byobudani (Table 1). Three specimens (1141, 1142, 1167; Table 1, Fig. 5) did not closely match previously formally described *Palythoa* species from the northwestern Pacific. These three specimens were characterized by their elongated polyps with underdeveloped coenenchyme (= 'liberae'; Pax, 1910) and small colony size (= 1–3 polyps). Additionally, oral disk patterns did not match with *P. mutuki* or *P. heliodiscus* Ryland and



Figs 3–8. *In situ* images of zoanthid species observed in this study. Scale bars all represent 1 cm. (3) *Palythoa tuberculosa* (specimen 1157) at Byobudani. (4) *Palythoa mutuki* (1162) at Byobudani. (5) unknown *Palythoa* species (1142) at Takinoura. (6) *Zoanthus sansibaricus* (1139) at Miyanohama. (7) *Zoanthus kuroshio* (1160) at Byobudani. (8) Two *Zoanthus* colonies (not sampled) in the intertidal zone at Takinoura Higashi.

Lancaster, 2003 as shown in Reimer *et al*. (2006*a*, 2007*b*), with noticeable white markings or patterns present.

The remaining two observed species groups were clearly from the genus *Zoanthus*, as they had smooth, non-encrusted polyps attached directly to rocks or dead coral. While some specimens (n = 4) from Miyanohama and Byobudani sites matched with *Z. sansibaricus* Carlgren, 1900 (Fig. 6), and one other specimen from Byobudani matched morphologically with *Z. kuroshio* Reimer & Ono, 2006 (in Reimer *et al*., 2006*a*) (Fig. 7), several specimens (n = 6) from Takinoura Higashi site (Fig. 8) attached to large boulders in the intertidal zone were difficult to identify using only morphological characteristics such as oral disk colour and polyp form, as all were relatively 'immersae' similar to *Z. kuroshio* yet had oral disk colours similar to *Z. sansibaricus*.

Overall, aside from *P. tuberculosa*, and *Zoanthus* spp. at only the Takinoura Higashi site, zoanthids were seen to be generally rare in abundance at the sites examined when compared to previous zoanthid studies in the Ryukyu Islands or locations along the Pacific Coast of Japan. As well, observed zoanthid colonies were also generally small, often < 50 polyps, and no large encrusting *P. tuberculosa* colonies were observed. All observed zooxanthellate zoanthids were found in < 5 metres of water despite searching at greater depths at all sites.

DNA sequence and phylogenetic identification

mt 16S rDNA. The mitochondrial 16S ribosomal DNA (mt 16S rDNA) sequences from specimens in this study fell into two large genus-level clades, corresponding to *Zoanthus* and *Palythoa* (Fig. 9). Specimens 1143, 1145, 1148 and 1149 were identical to AB219191 from *Z. kuroshio*, and this subclade was highly supported (ML = 99%, NJ = 99%). This subclade was sister to a highly supported *Z. sansibaricus* monophyly (ML = 98%, NJ = 99%), which contained sequences from 1139, 1146, 1166 (identical to previously reported *Z. sansibaricus* sequences), and 1164 (one base pair difference).

The genus *Palythoa* was moderately supported (ML = 77%, NJ = 89%). The sequence from specimen 1167 was identical to two sequences from *Palythoa* sp. sakurajimensis *sensu* Reimer *et al*. (2007*b*), and these sequences were seen to be basal to the remaining *Palythoa* sequences to the exclusion of *P. heliodiscus*. 1142 was derived from and had one base pair difference from 1167 and *P*. sp. sakurajimensis. The remaining sequences only showed two base pair differences from each other, and showed poor resolution. Most sequences (1134, 1135, 1144, 1151, 1152, 1153, 1155, 1157, 1158, 1161, 1163) were identical to *P. tuberculosa* (AB219219), while one sequence (1162) was identical to *P. mutuki* (DQ997875), and these two sequences differed from

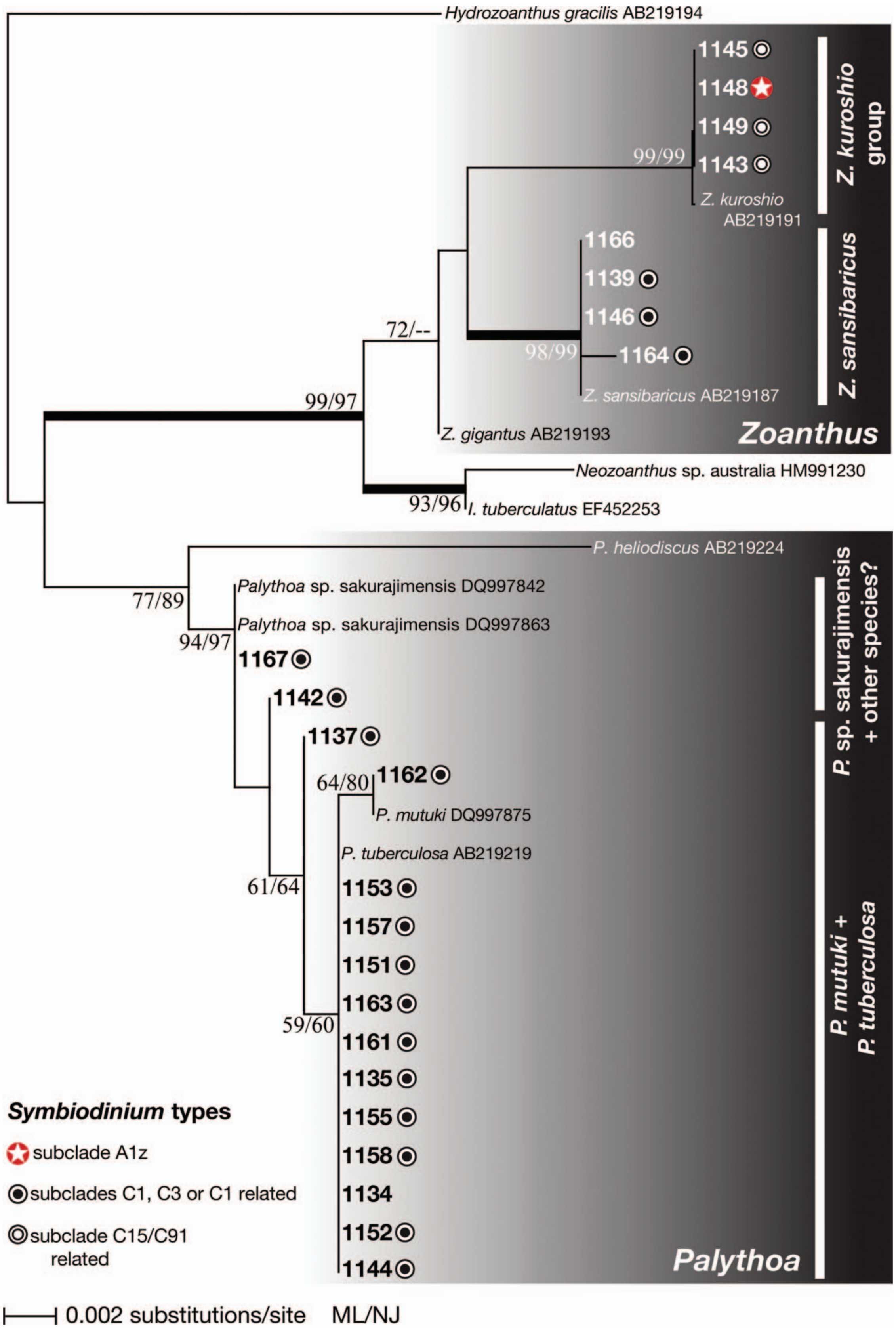


Fig. 9. Maximum likelihood tree of mitochondrial 16S ribosomal DNA sequences including novel sequences from specimens in this study. Values at branches represent maximum likelihood (ML) and neighbour-joining (NJ) bootstrap probabilities, respectively. Thick branches indicate monophylies supported by > 95% ML bootstrap in the corresponding cytochrome oxidase subunit I tree (Fig. S1, available via the Supplementary Content tab of the article's online page at http://doi.dx.org/10.1080/14772000.2011.569034). New sequences from this study in bold, with corresponding *Symbiodinium* zooxanthellae types represented by symbols as given in the legend.

the larger grouping by a single base pair. Together these two groups formed a poorly supported subclade (ML = 59%, NJ = 60%). One sequence from a specimen morphologically identified as *P. tuberculosa* (1137) was basal to this group by a single base pair change, and in turn derived from the sequence from 1142 by a single base pair.

COI. The resulting tree from the COI alignment showed similarities to the mt 16S rDNA tree, although the resolution was not as detailed (Fig. S1, see supplementary material, which is available via the Supplementary Content on the article's online page at http://dx.doi.org/10.1080/14772000.2011.569034.). Sequences from Ogasawara specimens again fell into either *Palythoa* or *Zoanthus* clades. In the very well supported *Zoanthus/Isaurus/Neozoanthus* clade (ML = 98%, NJ = 99%), specimens 1139, 1146, 1164 and 1165 were identical to and formed a well-supported monophyly with previously acquired *Z. sansibaricus* sequences (ML = 94%, NJ = 95%). The sequence from specimen 1166 was also identical over its length to *Z. sansibaricus* sequences AB214166 and AB194031, but was too short (approximately 270 bp) to be included in the alignment. Sequences from specimens 1145, 1148 and 1149 formed a poorly supported (ML = 62%, NJ = 65%) clade with sequence AB219191 from *Z. kuroshio*, although they were identical to this sequence. The sequence from specimen 1143 was also identical over its length to *Z. kuroshio* sequences, but was too short (approximately 335 bp) to be included in the alignment.

The genus *Palythoa* formed a polytomy, although sequences were generally identical to previously reported sequences. 1134, 1135, 1137, 1144, 1151, 1152, 1157, 1161, and 1163 were identical to *P. tuberculosa* sequences AB219199 and EU333661. Together with three sequences with 1–2 base pair changes (1153, 1155, 1158), these formed a poorly supported clade (ML = 64%, NJ = 63%). Additionally, the sequence from specimen 1156 was also identical to *P. tuberculosa* sequences over its length, but was too short (approximately 385 bp) to be included in the alignment. Two additional sequences from this study (1142, 1162) were seen to be identical to previously acquired *P. mutuki* sequences DQ997847 and DQ997875, while a third novel sequence (1167) differed by a single base pair change. These '*P. mutuki*-related' sequences formed a polytomy basal to the *P. tuberculosa* clade.

Symbiodinium ITS-rDNA. No specimens were seen to have 'mixed' unclear ITS-rDNA sequences (e.g. multiple peak signals), and while we cannot discount the presence of minor amounts of non-dominant *Symbiodinium* types in the zoanthid specimens from this study ($<\sim$5–10% as in Mieog *et al*., 2007), ITS-rDNA results were therefore interpreted as corresponding to the dominant *Symbiodinium* type within each zoanthid specimen.

Clade C. The phylogenetic tree of *Symbiodinium* clade C ITS-2 sequences is shown in Fig. S2 (see supplementary material, which is available online). Most sequences (1135, 1142, 1144, 1151, 1155, 1157, 1161, 1162) were identical to previously reported subclade C1 sequences from *P. tuberculosa* (DQ480613), *Galaxea fascicularis* Linnaeus, 1767 (EU940369), *Isaurus tuberculatus* Gray, 1828 (EF452288), cultured C1 (EU074885), and were subsequently identified as subclade C1 *sensu* LaJeunesse (2002). Several other sequences were closely related to subclade C1 but differed by 1–2 base pairs from previously reported sequences (1137, 1152, 1153, 1158), and along with one additional sequence (1153; identical to EU074878, identified as subclade C1 in Thornhill *et al.*, 2007) were classified as 'subclade C1 related'. One additional sequence (1139) was identical to subclade C3 (FN298467 from Pochon *et al*., 2010, and numerous other sequences). All of the above sequences were different by at least two base pairs from previously observed *Z. sansibaricus*-specific 'C1z' types from southern Japan (Reimer *et al*., 2006*c*) and Singapore (Reimer & Todd, 2009). Additionally, sequences from specimens 1146, 1164 and 1167, although too short to be placed in the alignment, were identical over their readable portions to subclade C1, and were also classified as such. Sequences from specimens 1143, 1145 and 1149 were seen to be identical to sequences previously seen in *Z. kuroshio* and *Z*. aff. *vietnamensis* Pax and Muller, 1957, and formed a well-supported subclade (ML = 98%, NJ = 98%). No Ogasawara sequences were within a subclade of C1z sequences isolated from *Z. sansibaricus* in southern Japan and Singapore.

Clade A. The sequence from specimen 1148 was seen to be part of a subclade along with *Symbiodinium* from *Z. sansibaricus* from Amami Oshima, southern Japan (AB190280) and from *Cassiopea xamachana* Bigelow, 1892 (AF427466), identified as *Symbiodinium* subclade A1 (Fig. S3, see supplementary material, which is available online). This sequence, derived from subclade A1, has been designated A1z. These three sequences together with previously reported sequences, including some sequences from zoanthids in the Atlantic (Reimer *et al*., 2010*a*) and *Aiptasia pallida* Verrill, 1864 (AF427465), formed a well-supported monophyly (ML = 96%, NJ = 100%) within the *Symbiodinium* clade A radiation.

Discussion

The results of this study point to the presence of five shallow water zooxanthellate species in the Ogasawara Islands; *Palythoa tuberculosa*, *P. mutuki*, *Zoanthus sansibaricus*, *Z. kuroshio* and an additional undescribed *Palythoa* species. Of these five species, four are previously described species that are distributed widely in the western Pacific (e.g. Reimer, 2007, 2010), while the remaining one species does not match with descriptions of previously described *Palythoa* species from around Japan. This species may be either a previously unknown endemic species, or *P*. sp. sakurajimensis previously reported from Kagoshima and Wakayama, Japan.

Although only seven locations were investigated in the Ogasawara Islands during the course of this study, it appears that shallow water zoanthid diversity (= five species, four common) in the islands is lower than the Ryukyu Islands, which are found at the same latitude and have six species (four present in Ogasawara + *Zoanthus gigantus* Reimer & Tsukahara, 2006 in Reimer *et al*., 2006*a*, and *Palythoa heliodiscus*) that can be commonly found at almost all examined coral reef sites. Furthermore, it is believed there is a total of at least ten zooxanthellate zoanthid species in the Ryukyus (Reimer, 2010), far more than the total of five species in the Ogasawara Islands seen in this study. Other zoanthid species not observed in the Ogasawara Islands are generally locally abundant (*P*. sp. yoron *sensu* Reimer *et al*., 2007*b*; *P*. sp. tokashiki *sensu* Reimer, 2010; *Neozoanthus* sp. okinawa *sensu* Reimer, 2010) in the Ryukyus, with only one species apparently quite rare (*Isaurus tuberculatus*). It may be with further examinations some of these species will be found in the Ogasawara Islands. However, we feel it unlikely that the total number of shallow zoanthid species in the Ogasawara Islands will reach the total observed in the Ryukyus.

The most obvious explanation for this relatively low zoanthid diversity in the Ogasawara Islands is their isolated location. The Ogasawara Islands are oceanic, and have not been connected by low seas or land bridges to surrounding areas, unlike the Ryukyus, which have been connected to the Asian mainland and to the Japanese mainland at various times in recent geological history (Ota, 1998). Furthermore, the Ogasawara Islands are not in the area of direct influence of the Kuroshio Current, which brings warm tropical water up the Ryukyu Island chain and along the Pacific coast of Japan. This current also contributes to the high diversity of Ryukyu coral reefs, with species from the 'Coral Triangle' of the Philippines and Indonesia coming into this region (e.g. Chen & Shashank, 2009). Similar to the Mariana Islands to the south (Veron, 2000), the Ogasawara Islands also have lower hard coral diversity than the Ryukyus (Veron, 1992, 2000), and this appears to be true for zoanthid diversity as well.

If lower zoanthid diversity is due to the isolated location of the Ogasawara Islands, then the question as to how the zoanthid species present reached the islands in the first place must be asked. The four species previously noted from other locations (*P. tuberculosa*, *P. mutuki*, *Z. sansibaricus*, *Z. kuroshio*) seem to be very widespread in the Indo-Pacific, and have even been found in the Galapagos (Reimer & Hickman, 2009), a location much more isolated than the Ogasawara Islands; suggesting long larval stages (e.g. several weeks). Recently, it has been demonstrated that *P. tuberculosa* has a larval stage of over two weeks (Hirose *et al*., 2011), and it may be that these species have longer larval stages than other zoanthid species not observed in the Ogasawara Islands (*Z. gigantus*, *P. heliodiscus*). However, there are few data on the larval stages for zoanthid species in this study aside from *P. tuberculosa*, and this idea for now is only speculation. It is noteworthy that the four commonly observed species in the Ogasawara Islands are the only zooxanthellate zoanthids observed in the northern Izu Islands. The other two species of zooxanthellate zoanthids often seen in Japanese waters, *Zoanthus gigantus* and *Palythoa heliodiscus*, appear to have northern range limits of Wakayama and Okinoerabu Island, respectively (see Fig. 1). It has previously been speculated that these two species cannot withstand the colder winter waters of more northerly areas and are therefore limited to distribution to southern Japan.

Furthermore, most zoanthid specimens (excluding 1148) in this study possessed previously observed types of *Symbiodinium* as from other Indo-Pacific locations including the Ryukyus, and this strongly suggests that these specimens are not isolated, cryptic endemic species. Research has shown that sea surface waters of the Ogasawara Islands are often influenced by the Kuroshio Extension (Fig. 1, Felis *et al*., 2009), and it may be that zoanthid larvae and *Symbiodinium* from the northern Pacific coast of Japan reach(ed) the Ogasawara Islands via this 'one-way' route (also see Otsuka & Ishino, 1988). This would also adequately explain the absence of *Z. gigantus* and *P. heliodiscus* despite the waters of the Ogasawara Islands being much warmer than the northern Izu Islands. Only few data exist on whether larvae for the zoanthid species in this study are zooxanthellate or azooxanthallate; the eggs of *Z. sansibaricus* are known to be azooxanthellate (Ono *et al*., 2005) and larvae of *P. tuberculosa* are also apparently azooxanthellate (Hirose *et al*., 2011). However, egg bundles of *Z. sansibaricus* apparently contain numerous zooxanthellae on their surfaces (T. Mezaki, pers. comm.). Clearly more research is needed to clarify potential dispersal mechanisms for *Symbiodinium* associated with zoanthids.

Additionally, as part of the Izu-Mariana Arc of islands, the zoanthid/symbiont fauna of the Ogasawara Islands may have affinity with Guam and other islands to the south. However, zooxanthellate zoanthid data from the Mariana Islands is sparse, with only a single report listing four zooxanthellae zoanthid species; *P. caesia* Dana, 1848 (likely conspecific with *P. tuberculosa*), *Protopalythoa* sp. (possibly *P. mutuki* or *P. heliodiscus*), *I. tuberculatus* and *Z. vietnamensis* (possibly *Z. kuroshio*) (Paulay *et al*., 2003). Thus, the Marianas' zoanthid/symbiont diversity needs to be further examined in the near future, and compared to the results here.

At the same time, although the major component of the Ogasawara Islands' zoanthid diversity is the same as the northern Izu Islands, it does appear that the zoanthid/symbiont fauna of the islands also has an endemic component. Our results confirmed the presence of *Symbiodinium* clade A in *Z. kuroshio*, which has not been previously reported. Furthermore, it should be noted that *Z. sansibaricus* specimens from Ogasawara did not have previously observed specific types of *Symbiodinium* as seen in southern Japan (Reimer *et al*., 2006*c*) and Singapore (Reimer & Todd, 2009), but instead 'generalist' C1 and C3 types seen in *Palythoa* (Reimer *et al*., 2006*b*) and *Isaurus* (Reimer *et al*., 2008*b*) from Japan.

As well, specimens 1141, 1142 and 1167, while morphologically similar to *Palythoa mutuki*, were shown to have significant phylogenetic differences to *P. mutuki* and other described *Palythoa* species, and were phylogenetically similar to *P*. sp. sakurajimensis. An examination of *in situ* images of specimens 1141 and 1142 (no images available of specimen 1167) showed some small morphological differences in oral disk colouration and patterns with not only *P. mutuki*, but also with *P*. sp. sakurajimensis, indicating these specimens could belong to an undescribed species. Regardless of whether these specimens' identity is either *P*. sp. sakurajimensis or a different, unknown species, it appears there may be one or several species of cryptic and sporadically distributed *Palythoa* species in Japan. Further specimen collection and a detailed examination should help confirm this hypothesis. As well, examinations of zoanthid hosts and their *Symbiodinium* from the Ogasawara Islands, the Ryukyus and the Marianas with microsatellites would help researchers confirm if any fine-level differences are present between the different regions.

In conclusion, the zooxanthellate zoanthid/symbiont fauna of the Ogasawara Islands can be described as relatively depauperate compared with southern Japan and more similar to that of the northern Izu Islands, with a small unique, potentially endemic component. Furthermore, the results of this study demonstrate the potential influence of the Kuroshio Recirculation on the marine fauna of the Ogasawara Islands. It is hoped this study provides a basis for future biogeographical studies on zooxanthellate zoanthids in the Ogasawara Islands.

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