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Review article

Implications for different diversity levels of *Symbiodinium* spp. (Dinophyceae, Suessiales) within closely related hosts: zoanthids (Cnidaria: Hexacorallia: Anthozoa) as a case study

James D. REIMER[1,2,*]

[1] Department of Marine Science, Biology and Chemistry, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 901–0213, Japan

[2] 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

* Corresponding author: J.D. Reimer
E-mail: jreimer@sci.u-ryukyu.ac.jp

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Abstract In recent years, the diversity of symbiotic dinoflagellates of the genus *Symbiodinium* has become intensively investigated. Found in a variety of marine invertebrate hosts, *Symbiodinium* are a key element in the observed high diversity within many sub-tropical and tropical shallow marine ecosystems. One group of marine invertebrates for which *Symbiodinium* diversity has been examined is zooxanthellate zoanthids (Cnidaria: Anthozoa: Zoantharia) of three genera; *Zoanthus, Isaurus* and *Palythoa.* Focusing on specimens in southern Japan, zoanthid species from *Zoanthus* and *Palythoa* show varying patterns of both levels of *Symbiodinium* variation within individual colonies, and levels of *Symbiodinium* variation (=“flexibility”) over geographic ranges (*i.e.,* latitudinal/longitudinal variation, etc.). Here I examine and compare the results of previous studies based primarily on sequences of internal transcribed spacer of ribosomal DNA (ITS-rDNA), and focus on two zoanthid species with apparently very different *Symbiodinium* symbiotic system characters; *Zoanthus sansibaricus* and *Palythoa tuberculosa. Symbiodinium* (subclade C1z) within Z. sansibaricus are characterized by extremely high levels of ITS-rDNA sequence microvariation, while *Symbiodinium* within *P. tuberculosa* (subclade C1/C3) do not have such microvariation. I suggest that three factors may potentially contribute to apparently higher flexibility and diversity of *Symbiodinium* in *Z. sansibaricus*; 1) multiple copies of ITS-rDNA sequences, 2) strong reliance on sexual reproduction in *Z. sansibaricus* vs. asexual reproduction in *P. tuberculosa,* and 3) niche specialization by *Z. sansibaricus* vs. “generalist” *P. tuberculosa,*. Through an examination of past literature and results, it appears that both multiple intragenomic copies of ITS-rDNA and multiple types of *Symbiodinium* within *Z. sansibaricus* due to niche specialization are the most likely reasons behind such unusually high microvariation. It is hoped that in the near future more intensive investigations of *Symbiodinium* in a variety of host species will help researchers more fully understand the mechanisms driving varying symbiont flexibility and diversity.

Keywords ITS-rDNA, *Palythoa, Symbiodinium,* symbiosis, *Zoanthus,* zooxanthellae

Introduction

Symbiotic dinoflagellates of the genus *Symbiodinium* are found in symbioses with a wide variety of marine invertebrates from sub-tropical and tropical marine environments (e.g., foraminifers [Pochon et al. 2001], cnidarians [Rowan and Powers 1991a], mollusks [Carlos et al. 1999]). These symbioses are partly responsible for the high biodiversity of organisms in coral reef environments. While it was originally thought that *Symbiodinium* was a single species of dinoflagellate based on morphology, molecular studies over the past three decades have shown a high level of "hidden" diversity within this group, with eight "clades" (designated A-H) each containing numerous "subclades" or "types" (Pochon et al. 2004, 2005). Different *Symbiodinium* have been shown to potentially have different physiologies and adaptations to different environments (Tchernov et al. 2004). Thus, an understanding of the diversity patterns of *Symbiodinium* is a critical and basic step in comprehension of the ecology of subtropical and tropical marine invertebrates hosting these dinoflagellates.

One group of organisms known to be in symbioses with *Symbiodinium* are zoanthids (Cnidaria: Anthozoa: Hexacorallia: Zoantharia). The zooxanthellate genus *Zoanthus* belongs to the family Zoanthidae, while zooxanthellate *Palythoa,* distinguishable from *Zoanthus* by taking up sand and particles in its endoderm, belongs to the family Sphenopidae (phylogeny in Fig. 1). It is believed that *Zoanthus* spp. (Trench 1974) and most *Palythoa* spp. (Ryland and Lancaster 2003) rely to a large degree on *Symbiodinium* for their energy requirements. Much recent work has focused on understanding the diversity of zoanthids using molecular techniques (e.g., Reimer et al. 2004; Sinniger et al. 2005; Reimer et al. 2007a). *Zoanthus* and *Palythoa* are commonly seen in the sub-tropical waters of southern Japan (Reimer et al. 2006a, 2007c).

Other recent work has also investigated *Symbiodinium* diversity within these zooxanthellate zoanthid genera, using specimens collected primarily from southern Japan

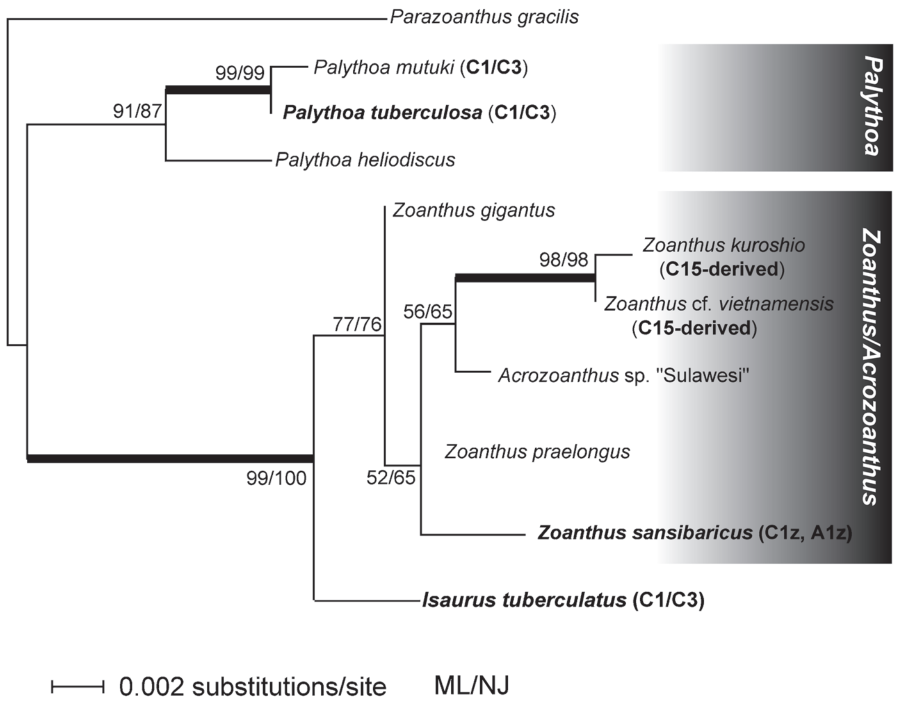

Fig. 1 General molecular phylogeny of the zoanthid genera *Palythoa, Zoanthus, Acrozoanthus* and *Isaurus* based on maximum likelihood tree of mitochondrial 16S ribosomal DNA sequences, adapted from Reimer et al. (2008). Values at branches represent ML and NJ bootstrap probabilities, respectively (>50%). Species names **in bold** are focused on in this study. Large bold letters and numbers after these species (e.g., **C1/C3**) are *Symbiodinium* types commonly associated with these species in southern Japan

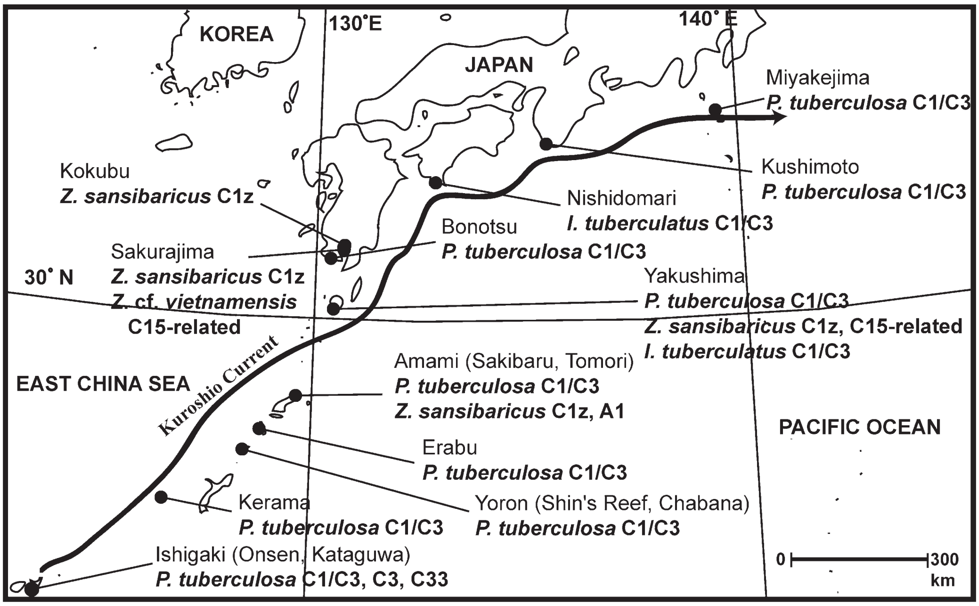

Fig. 2 Map showing sampling locations in Japan of zooxanthellate zoanthids and associated *Symbiodinium* types from previous published studies. Plain text next to locations shows location name, while bold italicized text designates zoanthid species investigated. Bold text after zoanthid species' names shows *Symbiodinium* types (sensu LaJeunesse) found in each zoanthid species. Abbreviations: *P. tuberculosa*=*Palythoa tuberculosa, Z. sansibaricus*=*Zoanthus sansibaricus, Z.* cf. *vietnamensis*=*Zoanthus* cf. *vietnamensis, I. tuberculatus*=*Isaurus tuberculatus*

(Fig. 2; see Reimer et al. 2006b, 2006c, 2007b). In both zoanthids and other organisms, *Symbiodinium* diversity has been most effectively investigated using molecular markers. While identifying *Symbiodinium* to the "clade" level has often used 18S and 28S ribosomal DNA sequence data (Rowan and Powers 1991a, 1991b), for identification with more resolution (i.e., the "subclade" and/or "type" level) faster evolving ITS-rDNA is the primary choice (see van Oppen et al. 2001; LaJeunesse 2001). The results of phylogenetic studies of *Symbiodinium* in different zoanthid species show differing levels of both intracolony ITS-rDNA sequence microvariation as well as flexibility in association with different *Symbiodinium* spp. (summarized in Fig. 3; see Reimer et al. 2006b, 2006c, 2007b). Here, I review these previous results, and attempt to make sense how such differing characteristics of these symbiotic systems may have arisen and what purpose they may serve by comparing *Symbiodinium* diversity with the ecology of investigated host species.

Ecological characters of host zoanthid species

Ecological characteristic data (primary reproduction method, habitat depth [minimum, maximum, average], larval ocean temperature [minimum]) of zoanthid species investigated were obtained from previously published studies (Table 1) with additional new distributional data from Okinawa observed by JDR (collected April 2007–January 2008).

The most noticeable differences are the presumed primary reproduction method between *Z. sansibaricus* (sexual) and *Palythoa tuberculosa* Klunzinger 1877 (asexual), and the ability of *Z. sansibaricus* to be present higher in the intertidal zone at sites in southern Japan (from Yakushima Island southwards) (Fig. 4). Both host species are known to have horizontal transmission of *Symbiodinium*; in other words, larvae are azooxanthellate and must somehow acquire symbionts from the environment. One other difference is it appears that larvae from *Zoanthus* can better withstand colder water temperatures (approx. 15℃) than larvae from *Palythoa,* which have not been found in ocean temperatures below 18℃ (Ryland et al. 2000).

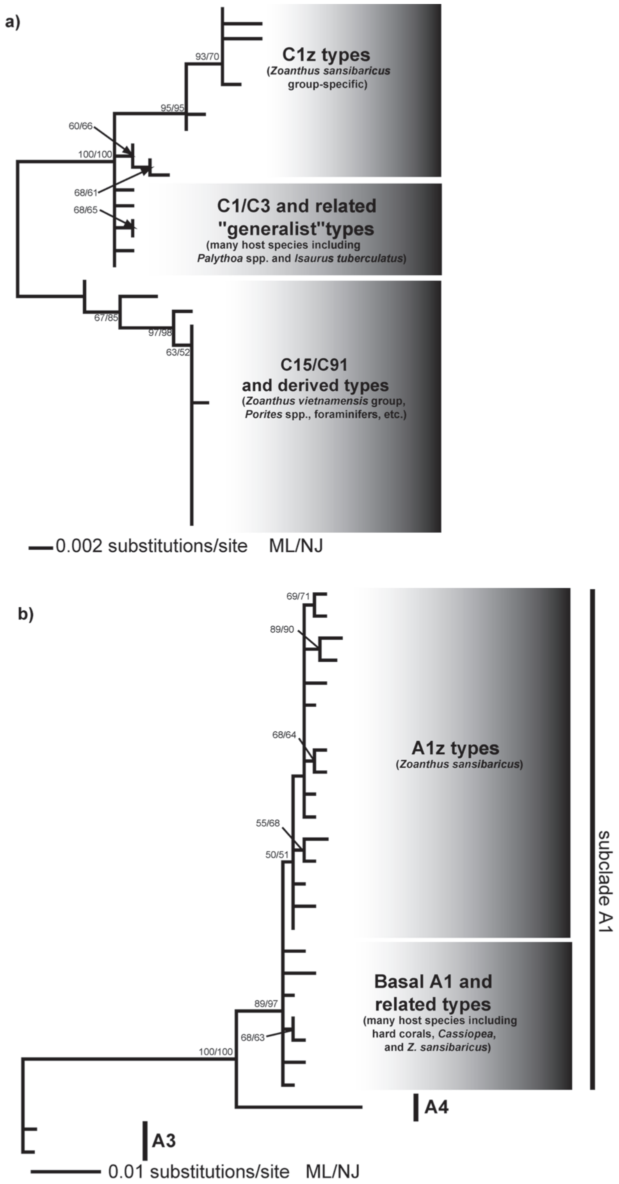

Fig. 3 Molecular phylogenies of *Symbiodinium* associated with zooxanthellate zoanthids in southern Japan based on maximum likelihood trees of internal transcribed spacer ribosomal DNA (ITS-rDNA) sequences. a) clade C *Symbiodinium,* b) clade A *Symbiodinium.* Values at branches represent ML and NJ bootstrap probabilities, respectively (>50%). a) and b) adapted from Reimer and Todd (unpublished data) and Reimer et al. (2006c), respectively

Symbiodinium diversity in zoanthid species

Sequences of the internal transcribed spacer region of ribosomal DNA (ITS-rDNA) *Symbiodinium* spp. from various zoanthid species in the genera *Zoanthus* (Reimer et al. 2006c, 2007b) and *Palythoa* (Reimer et al. 2006b) were previously obtained from zoanthid specimens over geographical (Reimer et al. 2006b, 2006c, 2008) and temporal (Reimer et al. 2007b) ranges (Fig. 2), for sampling details refer to the original publications. Additionally, for DNA analyses' details and GenBank Accession Numbers please refer to the original publications (listed in Table 1).

Results of previous studies of *Symbiodinium* spp. within zoanthids in southern Japan plus additional new data are summarized in Tables 1 and 2, and Figs. 1 and 3. Of special interest, the overall diversity of *Symbiodinium* species was much higher within *Zoanthus sansibaricus* Carlgren 1900 (Table 2; i.e., ～96% unique ITS-rDNA sequences [192/201 sequences], and higher percentage [6/22 total colonies ＝28%] of colonies with different *Symbiodinium* types), while *P. tuberculosa* had much lower levels of diversity (Table 2; only ～33% unique ITS-rDNA sequences [20/61 sequences], and lower percentage [1/28 colonies ＝4%] of colonies with different *Symbiodinium* types). For all zoanthid species examined (n＝50), the large majority (49, ＝98%) of individual colonies apparently associated with *Symbiodinium* from only one "subclade" (even if multiple "types" of this subclade were present within the colony) (Table 1). Note that the "*Zoanthus*-specific" C1 (also called C1/C3) *Symbiodinium* clade mentioned in Reimer et al. (2006b, 2006c, 2007b) is mentioned throughout this text as subclade C1z.

Validity of observed *Symbiodinium* ITS-rDNA sequence diversity in zoanthids

As shown in Table 2, the levels of ITS-rDNA sequence diversity in *Symbiodinium* in *Zoanthus sansibaricus* are much higher (～96% unique ITS-rDNA sequences, n＝201) than sequence diversity from *Symbiodinium* in *Palythoa tuberculosa* (～33% unique ITS-rDNA sequences, n＝61). As discussed in Reimer et al. (2006c, 2007b), it was initially feared that such unusual results were caused by experimental artifact(s) (i.e., sequencing error), but transition/transversion ratios of *Z. sansibaricus's Symbiodinium* ITS-rDNA sequences (71～74%

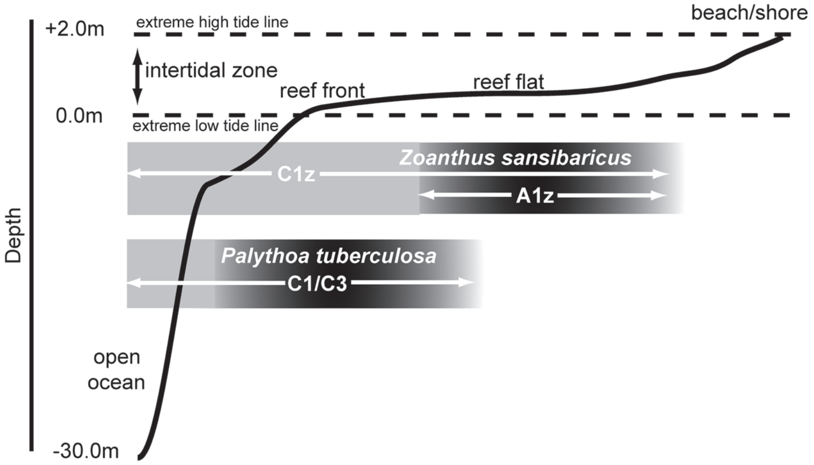

Fig. 4. Generalized representation of the different environments inhabited by the zooxanthellate zoanthids *Palythoa tuberculosa* and *Zoanthus sansibaricus* in Okinawa, and their major associated *Symbiodinium* types. The darker the color of the "habitat" boxes (with zoanthid species names in white), the more common the zoanthid is at that particular location. *P. tuberculosa* is commonly found from subtidal, deeper waters (depth= ∼−30.0 m) to the intertidal reef flat (0.0 m), and is the dominant colonial anthozoan on many reef fronts, containing C1/C3 *Symbiodinium*. *Z. sansibaricus* is found almost exclusively intertidally, and although present on the reef front and deeper in low numbers, is the dominant (and often only) colonial anthozoan on the upper part (average +0.3 m, up to +1.0 m) of the reef flat. While C1z *Symbiodinium* is found throughout the vertical distribution of *Z. sansibaricus,* A1z is found only intertidally in non-shaded colonies. The higher in the intertidal zone, the less stable temperatures become (i.e., higher and lower due to exposure to air). For detailed depth ranges and other data, see Table 1

transitions) were very close to the expected ratio (75%), and cloning of other *Symbiodinium* clades using the same methods produced no such ITS-rDNA sequence microvariation (both detailed in Reimer et al. 2006c). Additionally, the overall lack of *Symbiodinium* ITS-rDNA diversity from *P. tuberculosa* and *Isaurus tuberculatus* despite using the exact same PCR and cloning techniques (see Reimer et al. 2006c, 2007b, 2008) demonstrates that experimental error is likely not the cause of ITS-rDNA sequence microvariation observed in *Symbiodinium* from *Z. sansibaricus.* Similar results were reported by Thornhill et al. (2007), who have shown that PCR error is unlikely to account for any unexpectedly high rate of error/mutation when utilizing *Symbiodinium* ITS-rDNA.

Possible reasons for unusually high ITS-rDNA sequence microvariation in *Z. sansibaricus* compared to *P. tuberculosa* (and other host species)

The two zoanthid species for which *Symbiodinium* ITS-rDNA sequence diversity have been most intensively studied are *Z. sansibaricus* (Reimer et al. 2006c, 2007b) and *P. tuberculosa* (Reimer et al. 2006b). As mentioned above and in Table 1, *Z. sansibaricus* has significantly higher levels of ITS-rDNA sequence diversity than *P. tuberculosa* (∼96% unique genotypes vs. ∼33% unique genotypes). What could be the reasons behind such a stark difference between these two zoanthid species that have similar biogeographic distributions in southern Japan? I speculate there are three potential factors (listed below) that may potentially contribute to this unusual contrast of apparent *Symbiodinium* diversity levels.

a) Multiple copies of ITS-rDNA in the *Symbiodinium* genome

One explanation that cannot be excluded is multiple copies of the ITS-rDNA region in individual *Symbiodinium* genomes. Multiple copies of the ITS-rDNA region are known from the dinoflagellate *Alexandrium* (Scholin et al. 1993; Yeung et al. 1996), and it is generally believed that ITS-rDNA in *Symbiodinium* is also paralogous (Santos

Table 1 Some zooxanthellate zoanthid species of southern Japan, their associated *Symbiodinium spp.,* and reproductive and distributional ecological characteristics

Zoanthid species[a]	*Symbiodinium* subclades/types present (**major**, minor)	% unique *Symbiodinium* ITS-rDNA sequences (see Table 2)	Geographic flexibility in *Symbiodinium* association (see Table 2)?	*Symbiodinium* transmission method in sexual reproduction[b]	Proposed host species primary reproduction method[b]	Vertical distribution (**average**, and range [m]) in Okinawa[c]	Theorized larvae minimum ocean temperature (°C)[d]	*Symbiodinium* references
Zoanthus sansibaricus	**C1z and related types,** A1z, C15	~96%	Yes	Horizontal	Sexual spawner	**+0.3**, (+1.0 to >-30.0)	15.0	Reimer et al. 2006c, 2007b
Palythoa tuberculosa	**C1**, C3, C33	~33%	Limited	Horizontal	Asexual budding	**+0.0**, (+0.3 to >-30.0)	18.0	Reimer et al. 2006b

[a] Species names in bold are focus of this study.

[b] Reproduction and transmission data from Ono et al. (2005) for *Z. sansibaricus*, Tanner (2002), Ryland et al. (2003), and Acosta et al. (2005) for *Palythoa* spp., plus additional observations by J.D. Reimer and S. Ono.

[c] Data obtained in this study; positive depths above extreme low tide line (intertidal zone), negative depths below (subtidal), with 0.0m = extreme low tide line.

[d] From Ryland et al. (2000) discussing Family Zoanthidae (*Zoanthus* spp.) and Sphenopidae (*Palythoa* spp.) larval distributions.

Table 2 Variation in *Symbiodinium* ITS-rDNA sequences in colonies of *Zoanthus sansibaricus* and *Palythoa tuberculosa* from southern Japan based on previous studies[A]

Species	*Symbiodinium* identity	# colonies	% of total colonies	# ITS-rDNA sequences obtained	# unique ITS-rDNA sequences	% unique ITS-rDNA sequences
Zoanthus sansibaricus	C1z and related types	16	73	178	170	96
	A1z and related types	5	23	20	20	100
	C15 and related types	1	5	3	2	67
total	**NA**	**22**	**100**	**201**	**192**	**96**
Palythoa tuberculosa	C1 and related types	28	100	51	16	31
	C3 and related types	1	4	3	2	67
	C33 and related types	1	4	7	2	29
total	**NA**	**28[B]**	**100**	**61**	**20**	**33**

[A] From data in Reimer et al. (2006b, 2006c, 2007b).

[B] One colony of *Palythoa tuberculosa* had C1, C3, and C33 *Symbiodinium* present.

NA = not applicable

and Coffroth 2003; Loram et al. 2007). However, in order to explain the unusually high amounts of sequences of *Symbiodinium* ITS-rDNA from *Z. sansibaricus, Symbiodinium* subclade C1z in association with *Z. sansibaricus* must have much more copies of ITS-rDNA than *Symbiodinium* in *P. tuberculosa* (C1/C3 "generalist" *Symbiodinium*) and indeed all other *Symbiodinium* investigated up until now.

Loram et al. (2007) have shown that there may be differences in the number of ITS-rDNA copies between clades A (approximately 10 to 25 copies) and B (two to 10 copies) *Symbiodinium*, and also that different host species may also influence the number of copies. Similarly, Mieog et al. (2007), although ITS-1 copy numbers are only briefly discussed in their study, do mention that clade D has approximately three times the number of copies as clade C. However, what subclade of clade C was investigated was not determined. Thus, while it is entirely possible that the genome of subclade C1z has more ITS-rDNA copies than other *Symbiodinium*, until now there are no such examples of "subclades" or taxa within *Symbiodinium* possessing such an anomalously high amount (at least ＞170 observed) of ITS-rDNA copies. Therefore, I believe it is possible that although intragenomic ITS-rDNA variability may be somewhat higher in subclade C1z than in other *Symbiodinium*, it cannot explain the entire amount of ITS-rDNA sequence variability observed in *Z. sansibaricus.* In fact, few members of the phylum Alveolata (including *Symbiodinium*) are known to have ＞100 copies of ITS-rDNA (see discussion in Loram et al. 2007, but also high copy numbers in Miego et al. 2007). Additionally, higher than expected (100％ of ITS-rDNA sequences unique, n＝5 colonies, 20 sequences) microvariation was also observed in A1z *Symbiodinium* from *Z. sansibaricus,* further supporting the hypothesis that observed ITS-rDNA microvariation in *Z. sansibaricus* is not an artifact confined solely to clade C *Symbiodinium* (see Reimer et al. 2006c).

Thornhill et al. (2007) show in their recent study that ITS-rDNA pseudogenes are likely to contribute to observed in hospite diversity of *Symbiodinium*, estimating that failure to recognize such pseudogenes may result in a 4-10-fold overestimation in diversity levels. Even when taking the maximum value of overestimation from this study, this would leave approximately 17 *Symbiodinium* types within *Z. sansibaricus* from within subclade C1z (from an original 170 original ITS-rDNA sequences observed; see Table 2).

Thus, the unusually high amounts of *Symbiodinium* ITS-rDNA sequence variation observed are more likely a reflection of truly high levels of *Symbiodinium* genotypic biodiversity within *Z. sansibaricus.* This does not mean that *Z. sansibaricus* does not have any specificity for a certain type of *Symbiodinium*; *Z. sansibaricus* shows a strong specificity for *Symbiodinium* subclade C1z and possesses multiple types from within this unique subclade (see Table 1 and Reimer et al. 2006c, 2007b). The unique C1z and A1z *Symbiodinium* within *Z. sansibaricus* are a similar situation to the distinct *Symbiodinium* observed with the acroporid coral genus *Montipora* (van Oppen 2004), and suggest C1z and A1z have co-evolved with *Z. sansibaricus.*

In other words, the high levels of microvariation seen in *Z. sansibaricus* are at least partially due to the presence of multiple types of *Symbiodinium* (even within the same subclade). Therefore, this high level of *Symbiodinium* variation when compared to *Symbiodinium* in *P. tuberculosa* and other zoanthids and anthozoans is likely at least partially due to one or both of the hypotheses described in b) and c) below, although some of the microvariation observed is very likely due to ITS-rDNA multiple intragenomic copies/pseudogenes as discussed above.

b) Potential effects of sexual/asexual reproduction of zoanthids on *Symbiodinium* diversity

Z. sansibaricus is known to annually sexually reproduce in southern Japan (Ono et al. 2005), while *P. tuberculosa* and closely related species reproduce primarily through asexual reproduction in the form of fission (Cooke 1976; Acosta et al. 2005; Tanner 2002), although some sexual reproduction does occur (see Yamazato et al. 1973). As *Symbiodinium* transmission in these two zoanthid species is horizontal during sexual reproduction (i.e., larvae are azooxanthellate and must acquire new *Symbiodinium* from the environment just before, during or just after settlement; see Ryland et al. 2000; Ono et al. 2005; K. Iwao, pers. comm.), *Z. sansibaricus* (higher reliance on sexual reproduction) therefore has more chance than *P. tuberculosa* (higher reliance on asexual reproduction) to acquire many different types of *Symbiodinium*, and therefore to acquire different adapted and specialized symbionts. As environmental conditions at spawning and settlement times (i.e., currents, ocean temperatures, sunlight/UV levels) change year to year, it may be beneficial for *Z. sansibaricus* to possess a wide variety of "specialist" *Symbiodinium* adapted to a variety of different environmental conditions. In other words, *Z. sansibaricus* may trend towards possessing specialized *Symbiodinium* (niche specialization/adaptation), while *P. tuberculosa,* which depends more on asexual reproduction and therefore does not have as many opportunities to re-acquire *Symbiodinium* despite fluctuating environmental conditions, should tend towards possessing a *Symbiodinium* "generalist".

Additionally, *Z. sansibaricus,* being more reliant on sexual reproduction than *P. tuberculosa,* may be more "flexible" in its association with *Symbiodinium* (see LaJeunesse 2005); and this flexibility is demonstrated with *Z. sansibaricus* from shaded intertidal locations on Amami possesses subclade C1z types, while colonies exposed to the sun possess clade A1-related *Symbiodinium* (hereafter designated A1z) (Reimer et al. 2006c). This "flexibility" of *Symbiodinium* associated with *Z. sansibaricus* may also be demonstrated by the high amounts of diversity observed within subclade C1z. While *Z. sansibaricus* has specificity for subclade C1z, it can associate with multiple "types" within this subclade (Table 1, Table 2; Reimer et al. 2006c, 2007b). On the other hand, *P. tuberculosa* from a variety of locations and environments throughout Japan possess only one or a few "types" of subclade C1/C3, a theorized host (LaJeunesse 2005) and environmental (Reimer et al. 2006b) "generalist" *Symbiodinium*. Additionally, although examined specimen numbers are low, *P. tuberculosa* from other, more distant locations in the Pacific (the Galapagos, Saipan, etc.) also seem to possess C1/C3 (LaJeunesse 2002, 2005; Reimer and Hickman in press; J.D. Reimer unpublished data), although clade D has been seen from *Palythoa* in the Indian Ocean (Burnett 2002) and Singapore (Reimer and Todd, unpublished data).

These ideas relating *Symbiodinium* diversity to sexual and asexual reproductive patterns in zoanthid (and other host species) remain to be confirmed, and future detailed examinations of *Symbiodinium* in other zoanthid/host species for which reproductive data are known are of critical importance. If in fact differences in primary modes of reproduction (sexual vs. asexual) are important factors in zooxanthellate zoanthid *Symbiodinium* diversity, the situation would be opposite to acroporid corals, where the mode of zooxanthellae transmission has been shown to not affect *Symbiodinium* diversity between two genera (van Oppen 2004). Additionally, a more "closed system" with less chance of acquiring new *Symbiodinium* as seen in more primarily asexual *P. tuberculosa* may actually lead to a higher possible of co-evolution between the symbiont and host. Thus, while reproduction method and symbiont diversity may be related, no firm conclusions can be made at this point, and it remains to be conclusively seen how sexual/asexual reproduction affects *Symbiodinium* diversity in zooxanthellate zoanthids.

c) "Generalist" vs. "specialist" host species

The third and final factor that may explain differences between *Symbiodinium* ITS-rDNA sequence diversity in *Z. sansibaricus* and *P. tuberculosa* are their different environmental adaptations. While very similar to the hypothesis advanced in b) above, instead of tendencies towards reliance on either sexual or asexual reproduction being responsible for differences in *Symbiodinium* diversity, the diversity differences are simply the result of the two zoanthid species' different distribution patterns. In other words, while in b) reliance on either sexual or asexual reproduction drives the zoanthid host species to be "generalists" or "specialists" respectively, in this hypothesis the host species possess different *Symbiodinium* in response to their environment, regardless of reproductive patterns.

Z. sansibaricus inhabits a much more "specialized", extreme environment than *P. tuberculosa.* In Okinawa and the Nansei and Ryukyu Islands, *Z. sansibaricus* is found higher (colonies averaging approximately 0.3 m above extreme low tide line, and found up to 1.0 m above the extreme low tide line) in the intertidal zone than *P. tuberculosa* (0.0 m, at the extreme low tide line) (Table 1). As well, *Z. sansibaricus* appears to generally favor this environment, as few colonies are found in subtidal areas below −2.0 m, although some colonies do exist at deeper (to ~30 m) depths similar to *P. tuberculosa. Z. sansibaricus* is adapted for the higher and less "stable" intertidal zone, with its longer periods of exposure to the air and strong UV rays during low tides, and subsequent winter cold (minimum monthly mean air temperatures of 7.3℃ [Yakushima] −13.1℃ [Naha], data from Japan Meteorological Agency [JMA]). (Note: monthly mean daily minimum and maximum temperature data were obtained from JMA for every month between January 1996 and December 2006. Numerous stations within Japan are listed in the JMA data, and for this study Yakushima [Station 47836, Lat. 30°23.1′N, Lon. 130°39.5′E]) and Naha [Station 47936, Lat. 26°12.4′N, Lon. 127°41.1′ E] stations' data were used.) and summer hot air temperatures (maximum monthly mean air temperatures 32.2℃ [Yakushima] −33.1℃ [Naha], JMA). The motile larvae (zoanthinae) of *Zoanthus* spp. are also known to be tolerant of ocean temperatures lower than 18℃ (Ryland et al. 2000), further demonstrating the potential adaptation of this group to areas such as the infralittoral zone.

On the other hand, *P. tuberculosa,* although common in the Okinawan intertidal zone, is not found as high up as *Z. sansibaricus* (see Fig. 4), and is also more commonly found at much deeper depths at most locations (to approximately −30.0 m; Table 1), with a much more "usual" zooxanthellate anthozoan distribution similar to *Acropora* spp. and other hard corals. From distribution data, it appears that *P. tuberculosa* is a "generalist" capable of inhabiting a variety of environments from the low intertidal to deeper waters. Additionally, the motile larvae of *Palythoa* (zoanthellae), unlike *Zoanthus* spp. larvae, have never been seen in waters with temperatures below 18°C (Ryland et al. 2000), further demonstrating the lack of adaptation to cooler environments (such as higher in the infralittoral zone where *Z. sansibaricus* is found). Thus, while *P. tuberculosa* is often found in areas where many other colonial anthozoans are not found (Reimer et al. 2006b) such as inside reef lagoons, this is a pattern consistent with other "generalist" species, while *Z. sansibaricus* consistently inhabits a "niche" that is more indicative of specialized adaptation. The differing *Symbiodinium* diversity patterns of these two species may be a reflection of such adaptations, with *P. tuberculosa* possessing a single or few "generalist" C1/C3 types, and *Z. sansibaricus* possessing a wide variety of "types" from within C1z (and A1z) to deal with both hotter and colder temperatures, as well as longer exposures to the open air.

Conclusions

The three hypothesized factors above may each be partially responsible for the observed differences in previous studies of *Symbiodinium* ITS-rDNA diversity between *Z. sansibaricus* and *P. tuberculosa.* However, experimentally discerning between sexual/asexual reproductive effort effects explained in b) and potential environmental adaptations effect explained in c) may prove to be very difficult. Based on observations here and in the literature, it appears that a combination of multiple intragenomic copies of ITS-rDNA and niche specialization leading to the presence of multiple "specialist" *Symbiodinium* types for adaptation to different intertidal temperatures and light levels are most likely to be account for the unusually high levels of microvariation seen in *Symbiodinium* in *Z. sansibaricus* in southern Japan.

Initial results of *Symbiodinium* ITS-rDNA analyses from other zooxanthellate zoanthid species found lower in the intertidal and subtidal zones than *Z. sansibaricus* (*Palythoa mutuki* Haddon and Shackleton 1891 [Reimer et al. 2006b] and *Isaurus tuberculatus* Gray 1828 [Reimer et al. 2008]) suggest these species possess only one or a few related types of "generalist" C1/C3 *Symbiodinium*, similar to *P. tuberculosa.* The few examined specimens thus far of *Z. gigantus* also possess C1/C3 (Reimer, unpublished data). However, little is known on the reproductive characteristics and ecology of these three species, and therefore further detailed studies of these species are needed to increase our understanding of zoanthid-*Symbiodinium* diversity and flexibility.

In the future, detailed analyses of: a) intragenomic ITS-rDNA sequences within various *Symbiodinium* clades and subclades, b) *Symbiodinium* diversity in other host species (not only zoanthids) with known sexual reproductive patterns, and c) *Symbiodinium* diversity in host species that are either "generalists" or "specialists" adapted to niches (especially relatively high in the intertidal zone) will help confirm or refute the hypotheses I have put forward here.

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