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ECOLOGICAL SURVEY OF ZOOXANTHELLATE ZOANTHID DIVERSITY (HEXACORALLIA: ZOANTHARIA) FROM KAGOSHIMA, JAPAN

By

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Abstract

Between 2003 and 2006 zooxanthellate zoanthid (Anthozoa: Zoantharia) species diversity was investigated at 39 locations in Kagoshima Prefecture, Japan. Our results show four species of *Zoanthus*, one species of *Isaurus*, and four species of *Palythoa* (total nine species) present in the shallow waters (< 20 m depth) of Kagoshima. In particular, *Zoanthus sansibaricus* was very common at the Hakamagoshi, Sakurajima and Tomori, Amami Oshima locations, and *Z. kuroshio* at Sangohama, Kurio, Yakushima and Tomori, Amami Oshima locations that are characterized by strong waves. *Palythoa* spp. were common in locations from Yakushima southwards, and particularly *P. tuberculosa*, which was noted at 10 locations.

Introduction

Zooxanthellate zoanthids (Anthozoa: Zoantharia) from three genera (*Zoanthus* and *Isaurus* in the family Zoanthidae; *Palythoa* in the family Sphenopidae) have been reported from the waters of Japan. Recent investigations have begun to examine the molecular phylogenetic diversity of these genera, and taxonomy and distribution of zoanthids in Japan is becoming standardized (i.e. Reimer *et al*. 2006a; 2007a; 2008). Additionally, Reimer (2007) examined the distribution of zooxanthellate zoanthids in southern Shikoku and found eight species. Other recent zoanthid research has focused on the sexual reproductive patterns of *Z. sansibaricus* (Ono *et al*. 2005) and long-term and seasonal changes in distribution at a monitored site at Hakamagoshi, Sakurajima, Kagoshima Prefecture (Ono *et al*. 2002; 2003). Despite the recent increase in research, questions remain regarding zooxanthellate zoanthids in southern Japan. One problem of particular importance is the almost complete lack of distributional information for southern Japan asides from Shikoku, with few detailed reports for Kagoshima Prefecture in southern Kyushu since 1969 (e.g. Hirata & Osako 1969). As Kagoshima Prefecture spans from the Kuroshio Current-influenced southern Nansei Islands to the temperate waters of Nagashima Island (Fig. 1), such data are critical in making assessments on the distribution and subsequent conservation of zoanthids.

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Much recent global research has focused on global warming and associated problems facing coral reefs (such as bleaching) and their overall decline, particularly of hard coral (Scleractinia) species (e.g. Brown 1997). Problems such as bleaching also impact zooxanthellate soft-bodied groups such as anemones (Dunn *et al*. 2002), zoanthids (Ono *et al*. 2003) (all in Hexacorallia) and soft corals (Octocorallia) (Michalek-Wagner & Willis 2001). It is expected that as global warming progresses distributions of such benthic zooxanthellate cnidarian groups will be impacted (Hoegh-Guldberg 1999). As zoanthids and these other taxa do not leave skeletal remains (unlike Scleractinia) when they die, no record of past distributions can be elucidated at future times. Thus, unless such distributional data are collected now, comparisons and monitoring changes in the future become impossible. This report will examine and collate information from many recent reports focused on species diversity in an attempt to understand and report on the distribution of *Zoanthus*, *Isaurus* and *Palythoa* spp. from Kagoshima, Japan.

Materials and Methods

Field surveys: Thirty-nine locations in Kagoshima Prefecture were investigated between 2003 and 2006 (Fig. 1). At each location, wave and tidal current activity, depth, substrate type and ocean temperature were recorded. Each individual location was investigated by either SCUBA or snorkeling for at least one hour, with zooxanthellate zoanthids species' data recorded. Data included relative abundance, depth, colony size, and microenvironment (shaded/non-shaded, current/wave activity, etc.). Digital images were taken *in situ* of colonies for further morphological analyses (see below). Samples of each species were collected from all locations and were preserved in 80-100% ethanol at –30°C for further morphological and phylogenetic analyses.

Morphological analyses: Digital photographs of all zoanthid specimens were examined, and the following morphological data collected: oral disk/polyp diameter, oral disk color(s), tentacle count, polyp and coenenchyme form, polyp color. Morphological examination of samples followed the procedure described in Ono *et al*. (2005), with samples fixed in Bouin's fluid and embedded in paraffin. Samples were cross-sectioned into 8-μm thick sections, stained with Azan, and observed under the microscope. Data were collected on polyp structure, diameter, and height; mesogleal thickness; and number of mesenteries (complete/incomplete).

Ocean temperature data: We obtained ocean temperature data (1981-2006) from the Kagoshima Prefectural Fisheries Experimental Center (KPFEC) for several locations very close to many of the investigated locations. KPFEC data were obtained from a depth of 5 m. Additional ocean temperature data were obtained from the Japan Meteorological Agency (2000-2006, depth = surface).

DNA extraction, PCR amplification, and sequencing/Phylogenetic analyses: DNA extraction, PCR amplification and subsequent sequencing of target molecular markers for zoanthid specimens collected are detailed in previous studies (Reimer *et al.* 2004; 2006a; 2006c; 2007a; 2007b).

For this study, previously obtained (see studies mentioned above) mitochondrial 16S ribosomal DNA (mt 16S rDNA) sequences for the species *P. tuberculosa*, *P. mutuki*, *P*. sp. sakurajimensis, *P. heliodiscus*, *Z. sansibaricus*, *Z. gigantus*, *Z. kuroshio*, *Z.* aff. *vietnamensis* and *I. tuberculatus* were aligned with an outgroup sequence from *Parazoanthus gracilis* by using CLUSTAL X version 1.8 (Thompson *et al*. 1997). The alignments were inspected by eye and manually edited. All ambiguous sites of the alignments were removed from the dataset for phylogenetic analyses. Consequently, an alignment dataset was generated of 810 sites of ten sequences. The alignment data are available on request from J. D. Reimer.

Maximum-likelihood (ML) analyses were performed using PhyML (Guindon & Gascuel 2003). PhyML was performed using an input tree 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, a 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 trees.

The neighbour-joining (NJ) method was performed using PAUP* Version 4.0 (Swofford 1998), with Kimura-2 parameter model (Saitou & Nei 1987). NJ bootstrap trees (500 replicates) were constructed using the same model.

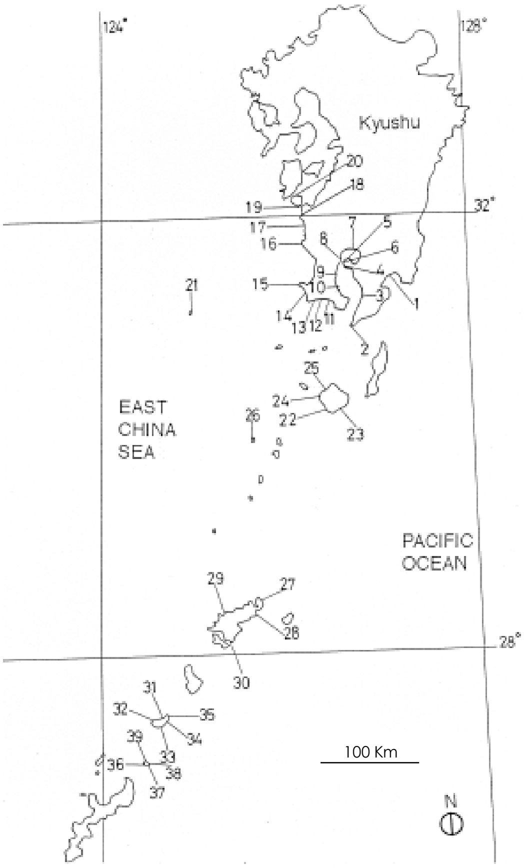

Fig. 1. Map of locations in Kagoshima Prefecture, Japan, where zooxanthellate zoanthid species diversity was investigated.

Results and Discussion

Study sites

The study area (Kagoshima Prefecture) covers a relatively large area, especially north to south (approximately 600 km). The sampling locations of this study (n = 39) are shown in Fig. 1, and environmental characteristics of each location in Table 1. A distribution map of zooxanthellate zoanthid species is shown in Fig. 2.

Kagoshima Mainland – Osumi Peninsula Pacific Coast (locations 1-2)

Both of these locations are located on the open Pacific coast (Fig. 1), and are strongly influenced by intense wave activity. From our investigations, we were only able to locate four species (*Z. sansibaricus, Z. kuroshio, P. tuberculosa, P. mutuki*) in this area, and all species were low in colony number. Further investigations are likely to increase the number of zooxanthellate zoanthid species found in this region.

Kagoshima Mainland - Kagoshima Bay (locations 3-10)

Kagoshima Bay (Fig. 1) is generally protected from the strong waves of the Pacific Ocean, and somewhat sheltered from the warm Kuroshio Current, and is thus generally much colder in winter than locations directly outside the bay (approximately 15°C opposed to 18°C outside the bay, KPFEC/JMA data).

Of particular interest is Hakamagoshi, Sakurajima (location 6 – Fig. 1). As detailed in Ono *et al.* (2003; 2007), this site was formed during the Taisho Eruption of the volcano Sakurajima in 1913. The inter- and sub-tidal lava flow with numerous fissures and cracks provides an ideal substrate for hard corals and zoanthids, which were among the first marine invertebrates to settle in this area.

Within Kagoshima Bay, three zooxanthellate zoanthid species were noted (*Z. sansibaricus, Z. vietnamensis, P.* sp. sakurajimensis). In particular, *Z. sansibaricus* had the highest abundance, with many colonies over a meter in diameter recorded from Hakamagoshi (location 6). Additionally, very high cover and colony numbers of *Z. sansibaricus*, as well as hard corals and other colonal benthic cnidarians, were seen at Hirakawa (location 9). This location is located directly next to a yacht harbor, and in summer months the visibility is very poor (approximately 0.5 to 1.0 m). Yet despite this, cnidarians seem to thrive at this location. *Z. sansibaricus* colonies of 30-50 cm in diameter were found at Hirakawa.

Kagoshima Mainland - South Satsuma Coast (locations 11-15)

The generally rocky southern coast of Satsuma Peninsula is exposed to the East China Sea and is strongly influenced by wave activity. Previously, the presence of hard corals was known at location 12 (Higashi Shioya), but in our surveys these corals were seen to have died due to some environmental disturbance. After the death of these hard corals, it appears that soft corals have increased in number (S. Ono, data not shown). Three species of zooxanthellate zoanthids (*Z. sansibaricus, P. tuberculosa, P. mutuki*) were found in small numbers along this coast.

Kagoshima Mainland North Satsuma Coast (locations 16-20)

The northwestern coast of Satsuma Peninsula, similar to Kagoshima Bay, has much colder waters in winter (i.e. < 18°C) than the other areas in this study as it is more removed from the warming influences of the Kuroshio Current. While much of this coastline is sandy, there are rocky areas. Several colonies of only one zoanthid species, *Z. sansibaricus*, have been confirmed from Takejima, Nagashima (location 20).

Uji Islands (location 21)

The Uji Islands are uninhabited rocky islets to the southeast of Satsuma Peninsula, and are very much exposed to the open ocean. Several colonies of both *Z. kuroshio* and *P. tuberculosa* were observed and collected from these islands, with *Z. kuroshio* found in the intertidal zone, the most northerly record of this occurrence.

Yakushima Island and outlying islets (locations 22-26)

Yakushima Island's coast is characterized by being exposed to waves from both the Pacific (east coast) and East China Sea (west coast), with the southern coast almost directly in the path of the Kuroshio Current. Sangohama, Kurio (location 22) in particular has a high diversity of both hard corals and zooxanthellate zoanthids, with seven species recorded from this location, including the relatively rare and cryptic *I. tuberculatus* (Reimer *et al.* 2008), the only known record of this species in Kagoshima Prefecture. Sangohama is also the type locality for *Z. gigantus* (Reimer *et al*. 2006a).

Uninhabited Gaja Island (location 26) lies to the southwest of Yakushima Island in the Tokara Island chain. Like Sangohama, this island is directly exposed the Kuroshio Current and high wave activity. However, unlike Sangohama and many other sites in the Nansei Island chain, the coastal platform on Gaja Island is very poorly developed, and there are not many areas suitable for benthic cnidarian settlement. Despite this, many *P. tuberculosa* colonies were present at Gaja Island, but no other zooxanthellate zoanthid species.

Amami Oshima Island (locations 27-30)

Approximately 200 km south of Yakushima Island, Amami Oshima Island marks the start of well-developed coral reefs in the Nansei Island chain. While locations on the west side of the island often do not appear to have a high diversity level of zooxanthellate zoanthid species, locations on the east coast, where coral reefs are more developed, have a high number of both zoanthid species and colony numbers.

In particular, the zoanthid species at Tomori (location 27) have been well investigated, and *Z. sansibaricus* and *Z. gigantus* are very common, with *Z. kuroshio* also in abundance. *Palythoa* spp. can also be found along the west coast, especially on the more exposed outer edges of reefs in shallow water. Intertidal *Z. sansibaricus* colonies exposed directly to sunlight at Tomori have been speculated to have a more light resistant strain of zooxanthellae (*Symbiodinium* spp.) than most *Z. sansibaricus* specimens (Reimer *et al*. 2006e), and investigations into the mechanisms behind this are ongoing.

Table 1. Abundance of zooxanthellae Zoantharia species in Kagoshima by location.

Region / site[1] (with number of species in area)	Site type[2]	Substrate	Wave/ current activity	*Zoanthus sansibaricus*	*Zoanthus gigantus*	*Zoanthus* aff. *vietnamensis*	*Zoanthus kuroshio*	*Isaurus tuberculatus*	*Palythoa tuberculosa*	*Palythoa mutuki*	*Palythoa* sp. Sakurajimensis	*Palythoa heliodiscus*
Mainland Kagoshima (6 species)				★		★	★		★	★	★	
1. Daguri Misaki, Osumi Peninsula	Bay	Rock/ Sand	Medium	○	×	×	×	×	×	×	×	×
2. Satamisaki Daygo Land Harbor, Osumi Peninsula	Sheltered	Rock	Medium	○	×	×	○	×	○	○	×	×
3. Takasu Coast, Kagoshima Bay	Bay	Rock/ Sand	Low	×	×	×	×	×	×	×	×	×
4. Furusato Onsen, Sakurajima, Kagoshima Bay	Bay	Rock	Low	×	×	×	×	×	×	×	×	×
5. Wariishisaki, Sakurajima, Kagoshima Bay	Bay	Rock/ Sand	Low	×	×	×	×	×	×	×	×	×
6. Hakamagoshi, Sakurajima, Kagoshima Bay	Bay	Rock	Low	●●	×	●●	×	×	×	×	1	×
7. Wakamiko, Kagoshima Bay	Bay	Rock	Low	◎	×	○	×	×	×	×	×	×
8. Iso Beach, Kagoshima Bay	Bay	Sand	Medium	×	×	×	×	×	×	×	×	×
9. Hirakawa, Kagoshima Bay	Bay	Sand/ Rock	Low	●●	×	×	×	×	×	×	×	×
10. Maenohama, Kagoshima Bay	Bay	Sand	Low	×	×	×	×	×	×	×	×	×
11. Kaimondake West, South Satsuma	Outside	Rock	High	○	×	○	×	×	○	×	×	×
12. Higashishioya, South Satsuma	Outside	Rock	High	○	×	×	×	×	○	×	×	×
13. Satumaitashiki, South Satsuma	Outside	Rock	High	○	×	×	×	×	○	○	×	×
14. Bonotsu, South Satsuma	Outside	Rock	High	○	×	×	×	×	○	×	×	×
15. Nomaike Tachibanejima, South Satsuma	Outside	Rock	High	×	×	×	×	×	×	×	×	×
16. Power Plant, North Satsuma	Outside	Rock	Medium	×	×	×	×	×	×	×	×	×

17. Sendai Tachibanajima, North Satsuma	Outside	Rock	Medium	×	×	×	×	×	×	×	×	×
18. Sagatabana, North Satsuma	Outside	Rock	High	×	×	×	×	×	×	×	×	×
19. Akune Sagatabana North, North Satsuma	Outside	Rock	Low	×	×	×	×	×	×	×	×	×
20. Nagashima, North Satsuma	Bay	Rock/ Sand	Low	○	×	×	×	×	×	×	×	×
21. Uji Islands	Outside	Rock	High	×	×	×	○	×	○	×	×	×
Yakushima (7 species)				★	★	★	★	★	★	★		
22. Sangohama, Kurio	Outside	Rock/ Coral	High	●	◎	◎	●●	○	●●	●●	×	×
23. Haruta	Outside	Rock	High	○	×	×	○	×	○	×	×	×
24. Isso West -tidal only	Outside	Rock	Medium	◎	×	×	◎	×	◎	×	×	×
25. Shishiko	Bay	Rock/ Coral	Medium	◎	×	◎	◎	×	◎	○	×	×
26. Gaja Island	Outside	Rock	High	×	×	×	×	×	●●	×	×	×
Amami Oshima (6 species)				★	★	★	★		★	★		
27. Tomori	Outside	Coral	High	●●	●	◎	●●	×	●●	●	×	×
28. Bashayama	Outside	Coral	Medium	●	×	◎	○	×	●	○	×	×
29. Sakibaru	Sheltered	Coral/ Sand	Low	×	×	×	×	×	○	×	×	×
30. Yadorihama	Bay	Coral/ Rock	Medium	◎	×	×	○	×	●	×	×	×
Okinoerabu Island (8 species)				★	★	★	★		★	★	★	★
31. Okidomari	Outside	Coral	Medium	●	×	◎	●	×	●●	◎	×	×
32. Kaito	Outside	Coral	Medium	×	×	×	◎	×	●●	×	×	◎
33. Sumiyoshi	Outside	Coral	High	◎	○	●●	●	×	●●	×	×	×
34. Wanjo	Bay	Coral	Medium	◎	×	◎	●	×	●●	●	×	×
35. Wanjo North	Bay	Coral	Medium	◎	×	◎	●	×	●●	●	**1**	×
Yoron (5 species)				★	★		★		★	★		
36. Shin's Reef (Nama)	Outside	Coral	High	●	○	×	◎	×	●●	●	×	×
37. Chabana Port	Harbor	Sand	Low	×	×	×	×	×	●	×	×	×
38. Shigo-Higashi	Outside	Coral	High	×	×	×	○	×	●●	○	×	×
39. Nama Beach	Outside	Coral	High	×	×	×	×	×	×	×	×	×

[1] Site numbers correspond to site numbers in Fig. 1.
[2] Outside: exposed to open ocean, Bay: exposed to open ocean but partially sheltered, Sheltered: largely protected from open ocean waves and more sheltered than "Bay", Harbor: within breakwaters, no waves.
★ = species present in region. Abundance symbols: ●● = abundant, ● = common, ◎ = uncommon, ○ = rare, **1** = single colony present, × = not present.

Okinoerabu Island (locations 31-35)

As Okinoerabu Island is much smaller than Amami Oshima, there are relatively few locations sheltered from wave activity from both the Pacific and East China Sea. Eight species of zooxanthellate zoanthids were found at locations on Okinoerabu Island, including the northernmost record of *P. heliodiscus* (Kaito, location 32). As well, a single specimen of *P.* sp. sakurajimensis was found in the intertidal zone at Wanjo North (location 35). *Z. sansibaricus*, *Z. kuroshio*, *P. tuberculosa* and *P. mutuki* were very common at many locations, particularly towards reef edges. Additionally, at Sumiyoshi (location 33), numerous *Palythoa* specimens with an intermediate morphology between *P. tuberculosa* and *P. mutuki* were observed. These unusual *Palythoa* may be either hybrids or a new species that may be the product of reticulate evolution (see Reimer *et al*. 2007a).

Yoron Island (locations 36-39)

All locations investigated at Yoron were inside the reef, and consisted of a substrate of dead coral from the 1998 ENSO-related bleaching event. *Zoanthus* spp. were not very common, but *P. tuberculosa* colonies were very common at some locations. Again, potential hybrid *Palythoa* were seen at one location (Shin's Reef, location 36).

Zooxanthellate zoanthid species of Kagoshima

Zoanthus sansibaricus

Z. sansibaricus was particularly common at Hakamagoshi, Sakurajima (location 6), Hirakawa (location 9), and Tomori, Amami Oshima (location 27). At Hakamagoshi, colonies were common in subtidal in shallow waters less than 10 m in depth, and were not seen at depths deeper than 15 m. Hakamagoshi and Hirakawa, being in Kagoshima Bay, do not have much wave activity. Interestingly, *Z. sansibaricus* colonies in Kagoshima Bay were found only attached to the lava rock substrate and to dead hard coral colonies.

Winter ocean temperatures are very cold in Kagoshima Bay (approximately 15°C, KPFEC data), and *Z. sansibaricus* polyps are completely closed during for approximately four months during winter. In contrast, at Tomori, *Z. sansibaricus* were most common near the reef edge exposed to waves. While found at other locations, *Z. sansibaricus* were generally present in low numbers.

Zoanthus gigantus

This recently described species was found only at four locations; Sangohama (location 22), Tomori (location 27), Sumiyoshi (location 33) and Shin's Reef (location 36). However, as there are other records of this species in Japan from Kochi and Wakayama, it is known that *Z. gigantus* is not limited to southern waters, but instead probably prefers areas at the edge of coral reefs where wave activity and visibility are high. Despite being relatively uncommon in the locations investigated in this study, *Z. gigantus* is numerous in Okinawa (J. D. Reimer, personal observation) and Shikoku (Reimer 2007), and is not believed to be particularly rare.

Zoanthus kuroshio

Z. kuroshio was only found north of Yakushima at one location, Satamisaki (location 2), but was common in many southern locations. In particular, many colonies were seen at Sangohama (location 22) and Tomori (location 27). On Okinoerabu Island, colonies of *Z. kuroshio* were often seen both in reef lagoons and outside coral reefs.

Zoanthus aff. *vietnamensis*

Colonies of *Z.* aff. *vietnamensis*, which are at the least very closely related to *Z. kuroshio* (see Reimer *et al.* 2007b), were very common at Hakamagoshi (location 6) and Sumiyoshi (location 33). At Hakamagoshi colonies were often 20-50 cm in diameter, and were often found attached to both the lava substrate or dead *Porites* colonies. *Z.* aff. *vietnamensis* was most often seen in shallow subtidal waters.

Isaurus tuberculatus

In this study *I. tuberculatus* was only found at one location; Sangohama on Yakushima Island. As mentioned in Reimer *et al*. (2008), this species is only known from a few locations in Japan (Danjo Islands, Nagasaki, and Otsuki, Kochi). Like many other zooxanthellate zoanthid species, *I. tuberculatus* is most often found in shallow subtidal waters less than 5 m in depth. The specimen found at Sangohama, however, was in the low intertidal zone.

Palythoa tuberculosa

P. tuberculosa was found at a few locations north of Yakushima Island (Satamisaki, location 1; South Satsuma, locations 11-14), and was not recorded at all from Kagoshima Bay or North Satsuma. However, *P. tuberculosa* was very common from Yakushima south, especially at Sangohama (location 22), Gajajima (location 26), Tomori (location 27), Okinoerabu (locations 31-35), and Yoron Island (locations 36, 38); and overall was the most commonly seen zooxanthellate zoanthid in the study region. *P. tuberculosa* was most commonly found on hard substrates (dead coral, rocks) facing the open ocean and/or waves, although occasionally colonies were seen in lagoons and other areas (see Reimer *et al*. 2006d).

Palythoa mutuki

Similar to *P. tuberculosa*, *P. mutuki* was only seen at a few locations north of Yakushima Island; at Satamisaki (location 2) and Satsuma Itashiki (location 13). *P. mutuki* was particularly common at Sangohama (location 22), and also found at other locations on Amami Oshima, Okinoerabu and Yoron Islands (locations 27, 34-36). Although unconfirmed, there is some evidence that *P. mutuki* is found, on average, slightly higher in the intertidal zone than *P. tuberculosa* from Yakushima Island south (J. D. Reimer, data not shown).

Palythoa sp. sakurajimensis

This potentially new species is known only from three specimens, one from Hakamagoshi (location 6), one from Wanjo North (location 35); and one from Shirahama, Wakayama (outside of the range of this study) (Reimer *et al*. 2007a). The oral disk of *P.* sp. sakurajimensis is green and brown, and oral disk diameter is much larger than other *Palythoa* species in Japan (up to 35 mm). Clearly more research is needed to confirm the status of this potentially new species. It may be that this species is tolerant of colder temperatures than most *Palythoa* species, as it was found both in an intertidal tidepool in Wakayama (where winter air temperatures drop to < 5°C), and in Kagoshima Bay, where ocean temperatures may drop to approximately 15°C and no other *Palythoa* have ever been recorded.

Palythoa heliodiscus

P. heliodiscus was recorded only from Kaito (location 32), Okinoerabu Island in this study, at a depth of 19.0 m. This species' distribution is from sub-tropical Okinoerabu Island south, and is often found in deeper (> 5 m) waters in areas of high current.

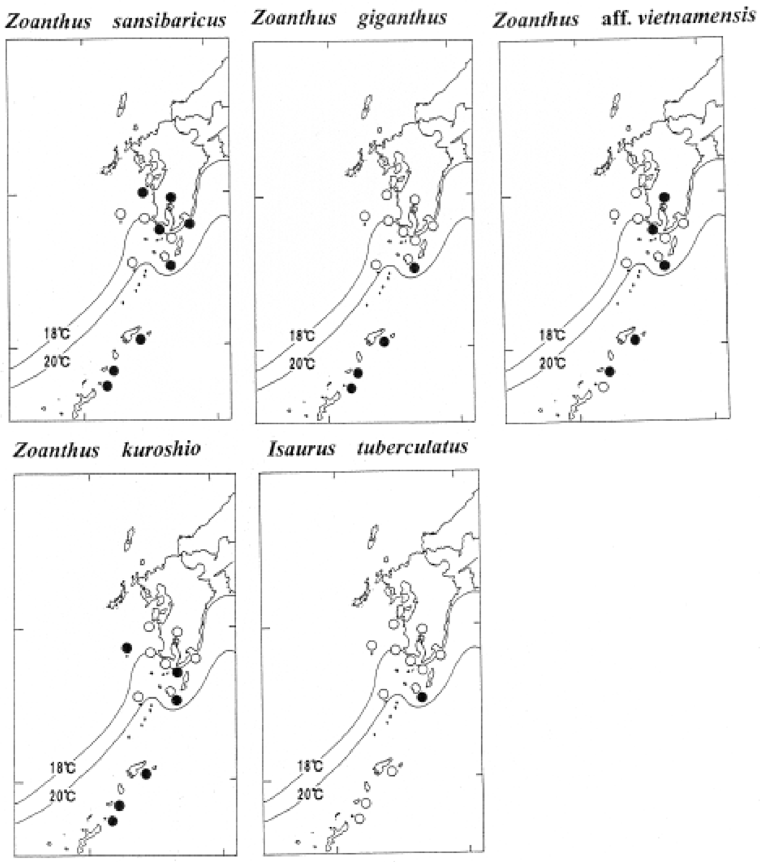

Fig. 2. Zooxanthellate zoanthid presence/absence at investigated locations in Kagoshima Prefecture, Japan. Black circles indicate the presence of zooxanthellate zoanthids. Ocean temperature data (winter minima) from Kagoshima Prefectural Fisheries Experimental Research Center (KPFEC) and the Japan Meteorological Agency (JMA) (depth = surface to 5 m) were used to calculate 18°C and 20°C thermoclines shown.

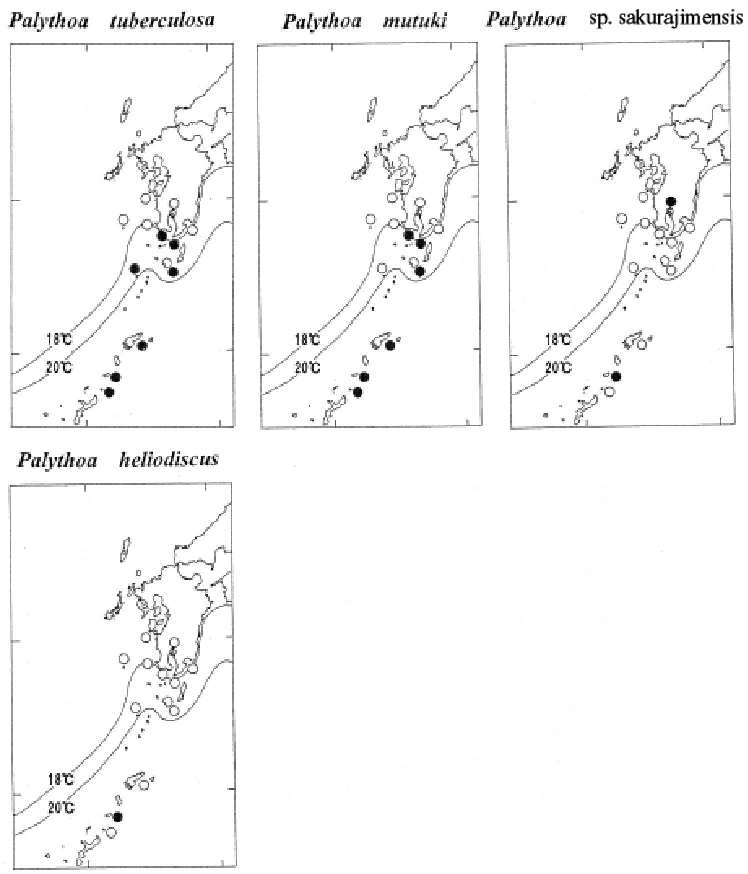

Fig. 2. continued

Phylogenetic overview of zooxanthellate zoanthids found in southern Japan

Previous studies (Reimer *et al*. 2004; 2006a; 2006b; 2006; 2007a; 2007b; 2008) have largely described the molecular phylogeny of zooxanthellate zoanthids from Japan. The maximum likelihood tree of the mt 16S rDNA phylogeny is shown in Fig. 3. In Japan, four species of *Palythoa*, four species of *Zoanthus*, and one species of *Isaurus* are known, and all species were found within the area examined in this study. Further study is needed to clarify whether *Z.* aff. *vietnamensis* and Z. *kuroshio* are separate species or not; whether hybridization occurs between *P. mutuki* and *P. tuberculosa*; and whether *P*. sp. sakurajimensis is a new species or not, but the overall phylogeny and diversity of Japanese zooxanthellate zoanthids is largely known. Future studies on potential new undescribed species are planned.

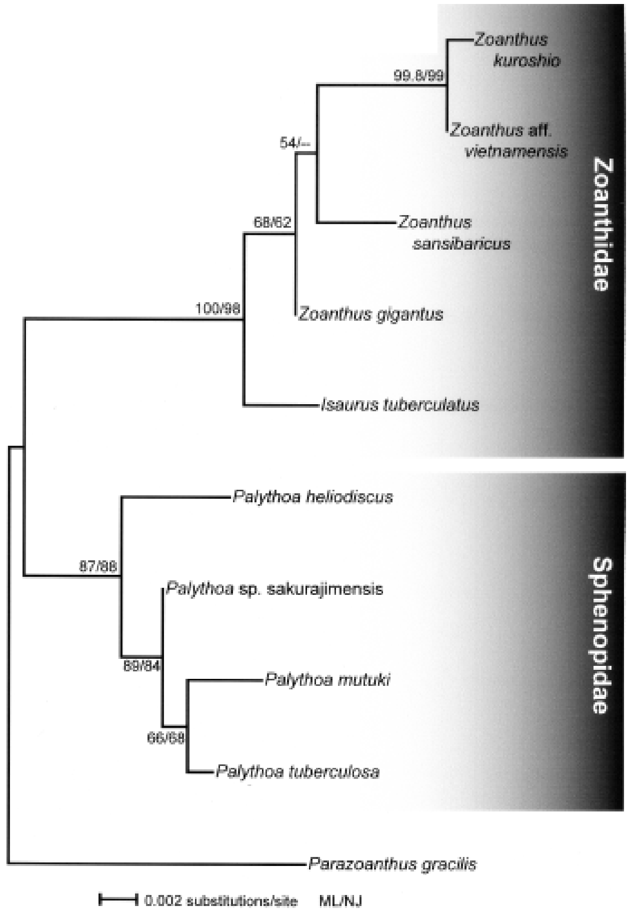

Fig. 3. Maximum likelihood tree of mitochondrial 16S ribosomal DNA (mt 16S rDNA) sequences showing phylogenetic relationships between the nine zooxanthellate zoanthid species found in Kagoshima, Japan, with *Parazoanthus gracilis* as the outgroup. Values at branches represent ML and NJ bootstrap probabilities, respectively (> 50%).

Temperature and zooxanthellate zoanthid distribution

Zooxanthellate zoanthids are generally found from sub-tropical to tropical waters, often in shallow waters on rocky substrata, with a very similar distribution to scleractinian corals. This is thought to be due to the presence of *Symbiodinium* spp. (zooxanthellae). This symbiosis with *Symbiodinium* likely limits the distribution of zooxanthellate zoanthids to generally shallow waters (< 35 m).

Additionally, water clarity appears to be important in the distribution of most zooxanthellate zoanthids, with species diversity much higher and species distributed over greater depths at consistently clear locations as opposed to locations with turbid water. For example, while visibility often reaches 20 m at Hakamagoshi, the annual average visibility is 11.1 m (S. Ono, data not shown), and zoanthids are found from the extreme low tide line to 10 m (twice average visibility). It is believed zooxanthellate zoanthids cannot live below this depth at Hakamagoshi.

Water temperature is likely the most important factor in horizontal distribution of zooxanthellate zoanthids. At Hakamagoshi, long-term observations have shown that *Zoanthus* does not grow and *Symbiodinium* activity greatly decreases when winter water temperatures are 16°C or below (Ono *et al*. 2003). Additionally, in laboratory experiments, *Zoanthus* polyps kept for approximately 200 days at 16°C with 12:12 hour light/dark cycles did not grow at all (S. Ono, unpublished data). At Hakamagoshi, temperatures are around or below 16°C for 90-100 days every winter, and *Zoanthus* can survive these conditions.

During the summer 1998 ENSO-related high ocean temperatures observed worldwide, reductions in both size and number of *Zoanthus* colonies at Hakamagoshi were recorded (Ono *et al*. 2003). Ocean temperatures at Hakamagoshi were on average 2.0°C higher than expected summer maximums, and remained over 30°C for approximately one month. While *Z. sansibaricus* colonies often survived despite heavy damage, many *Z.* aff. *vietnamensis* colonies disappeared completely. Additionally, soft coral *Stereonepthea japonica* colony numbers greatly decreased. The differences in mortality between these different cnidarian species may partly be attributed to differences in the types of *Symbiodinium* they harbor.

An *in situ* experiment in Hawaii utilizing *Z. pacificus* (= *Z. sansibaricus*, see Reimer *et al*. 2004) and *P. vestitus* has shown that *P. vestitus* grows six times faster than *Z. pacificus* (Cooke 1976). In laboratory experiments, *Z. sansibaricus* took 120 days to grow from one polyp to two polyps at 22°C, but *in situ* at Hakamagoshi one polyp on average grew from a single polyp to 19 polyps over 57 days (S. Ono, unpublished data). These *in situ* experiments were conducted in September and October, when water temperatures were 22°C to 27°C. Based on the above results and data, *Z. sansibaricus* does not expand below 16°C and has trouble surviving above 30°C.

As shown in Fig. 2, *Z.* aff. *vietnamensis* had almost the same distribution as *Z. sansibaricus*, and was found at locations where winter ocean temperatures reach below 18°C. On the other hand, *Z. kuroshio* and *Z. gigantus* were only found at locations where temperatures stayed above 18°C year round. *P. tuberculosa* and *P. mutuki* had similar distributions to *Z. kuroshio* and *Z. gigantus*, and were found south from South Satsuma and Satamisaki. While these four species are also found further north (Reimer *et al*. 2006d; 2007a; Reimer 2007; in press) in Shikoku, Honshu, and the Izu Islands, their distributions appear to be limited to Pacific coast waters influenced by the northward flowing Kuroshio Current. Additionally, *Z. sansibaricus* appears to be more common in southern coral reef environments (from Amami Oshima Island south), and may be a species with a more southern distribution on average than *Z.* aff. *vietnamensis*.

Over the past twenty years the average water temperatures in Kagoshima Bay have risen by approximately 1°C (based on KPFEC data), and it is predicted ocean temperatures will continue to rise (discussed in Hoegh-Guldberg 1999). As coral reefs are found in waters with a minimum winter temperature of 18°C or higher, it is possible that coral reefs will appear at locations in South Satsuma and Satamisaki. In a similar manner, it is possible zooxanthellate zoanthid species distributions will also change in a similar manner, and continued long-term monitoring is necessary.

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References

Brown, B.E. 1997. Coral bleaching: causes and consequences. Coral Reefs, 16 S1: 129-138.

Cooke, W.J. 1976. Reproduction, growth and some tolerances of *Zoanthus pacificus* and *Palythoa vestitus* in Kaneohe Bay, Hawaii. In: Mackie G.O. (ed.) Coelenterata Ecology and Behavior. Plenum Press, New York, pp. 281-288.

Dunn, S.R., J.C. Bythell, M.D.A. Le Tissier, W.J. Burnett and J.C. Thomason. 2002. Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aipstasia* sp. J. Exp. Mar. Biol. Ecol., 272: 29-53.

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

Hirata, K. and Y. Osako. 1969. Marine Park report in Kagoshima Bay. Kagoshima Prefectural Repot, pp. 9-19 (in Japanease).

Hoegh-Guldberg, O. 1999 Climate change, coral bleaching and the future of the world’s coral reefs. Mar. Freshwater Res., 50 (8): 839-866.

Michalek-Wagner, K. and B.L. Willis. 2001. Impacts of bleaching on the soft coral *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. Coral Reefs, 19: 231-239.

Ono, S., J.D. Reimer and J. Tsukahara. 2003. Seasonal changes in *Zoanthus* spp. in the infra-littoral zone at Taisho Lava Field, Sakurajima, Kagoshima, Japan. South Pacific Study, 22 (2): 41-52.

Ono, S., J.D. Reimer and J. Tsukahara. 2003. Long-term changes of *Zoanthus* spp. in the infra-littoral zone at Taisho Lava Fieid, Sakurajima, Kagoshima, Japan. Galaxea, J.C.R.S., 5: 21-31.

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

Ono, S., J.D. Reimer and J. Tsukahara. 2007. Thriving cnidarian community on the volcanic coastline of Sakurajima Taisho Lava Field, southern Japan. Coral Reefs, 26: 95.

Pax, F. 1910 Studien an westindischen Actinien. Zool. Jahrb. Suppl., 11: 157-330.

Reimer, J.D. 2007. Preliminary survey of zooxanthellate zoanthid diversity (Hexacorallia: Zoantharia) from southern Shikoku, Japan. Kuroshio Biosphere, 3: 1-16.

Reimer, J.D. in press. Implications for different diversity levels of *Symbiodinium* spp. (Dinophyceae, Suessiales) within closely related host genera: zoanthids (Cnidaria: Hexacorallia: Anthozoa) as a case study. Galaxea, J.C.R.S.

Reimer, J.D., S. Ono, K. Takishita, Y. Fujiwara and J. Tsukahara 2004. Reconsidering *Zoanthus* spp. diversity: molecular evidence of conspecifity within four previously presumed species. Zool. Sci., 21: 517-525.

Reimer, J.D., S. Ono, A. Iwama, J. Tsukahara, K. Takishita and T. Maruyama. 2006a. Morphological and molecular revision of *Zoanthus* (Anthozoa: Hexacorallia) from southwestern Japan with description of two new species. Zool. Sci., 23: 261-275.

Reimer, J.D., S. Ono, A. Iwama, J. Tsukahara and T. Maruyama 2006b. High levels of morphological variation despite close genetic relatedness between *Zoanthus* aff. *vietnamensis* and *Zoanthus kuroshio* (Anthozoa: Hexacorallia). Zool. Sci., 23: 755-761.

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

Reimer, J.D., K. Takishita and T. Maruyama 2006d. Molecular identification of symbiotic dinoflagellates (*Symbiodinium* spp.) from *Palythoa* spp. (Anthozoa: Hexacorallia) in Japan. Coral Reefs, 25: 521-527.

Reimer, J.D., K. Takishita, S. Ono, T. Maruyama and J. Tsukahara. 2006e. Latitudinal and intracolony ITS-rDNA sequence variation in the symbiotic dinoflagellate genus *Symbiodinium* (Dinophyceae) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia). Phycol. Res., 54: 122-132.

Reimer, J.D., K. Takishita, S. Ono and T. Maruyama. 2007a. Diversity and evolution in the zoanthid genus *Palythoa* (Cnidaria: Hexacorallia) utilizing nuclear ITS-rDNA. Coral Reefs, 26: 399-410.

Reimer, J.D., K. Takishita, S. Ono, J. Tsukahara and T. Maruyama 2007b. Molecular evidence suggesting intraspecific hybridization in *Zoanthus* (Anthozoa: Hexacorallia). Zool. Sci., 24: 346-359.

Reimer, J.D., S. Ono, J. Tsukahara and F. Iwase. 2008. Molecular characterization of the zoanthid genus *Isaurus* (Anthozoa: Hexacorallia) and associated zooxanthellae (*Symbiodinium* spp.) from Japan. Mar. Biol., 153: 351-363.

Rodriguez, F., J.L. Oliver, A. Marin and J.R. Medina. 1990. The general stochiatic model of nucleotide substitution. J. Theoretical Biol., 142: 485-501.

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

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

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res., 25: 4876-4882.