PRELIMINARY SURVEY OF ZOOXANTHELLATE ZOANTHIDS (CNIDARIA: HEXACORALLIA) OF THE GALAPAGOS, AND ASSOCIATED SYMBIOTIC DINOFLAGELLATES (*SYMBIODINIUM* SPP.)

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SUMMARY

Despite their presence in almost all marine ecosystems, the zoanthids (Cnidaria: Hexacorallia: Zoantharia) are poorly studied, in large part due to a lack of useful morphological identification characters. Recent research combining morphology with DNA markers has begun to shed new light on diversity and distribution of the order Zoantharia. Here, preliminary findings on the diversity and distribution of zooxanthellate zoanthid species from the genera *Zoanthus* and *Palythoa* are presented, documenting these genera in the Galapagos for the first time. A brief description of the species found is provided. *Zoanthus* and *Palythoa* appear to be limited in the Galapagos to rocky shores in warm shallow sublittoral and infralittoral waters (minimum temperature >18°C), isolated from the colder water that dominates much of the archipelago. Preliminary results from the internal transcribed spacer region of ribosomal DNA sequences of symbiotic dinoflagellates suggest that both *Zoanthus* and *Palythoa* spp. in the Galapagos possess only *Symbiodinium* clade C. Brief descriptions of the zooxanthellate zoanthid species found in the Galapagos are provided.

RESUMEN

Estudios preliminares de zoántidos zooxantelados (Cnidaria: Hexacorallia) de las Galápagos, y dinoflagelados simbiontes asociados (*Symbiodinium* spp.). A pesar de su presencia en casi todos los ecosistemas marinos, los zoántidos (Cnidaria: Hexacorallia: Zoantharia) están pobremente estudiados, en gran parte debido a una falta de caracteres de identificación morfológica útiles. Investigaciones recientes combinando morfología con marcadores de DNA han empezado a dar nuevas luces sobre la distribución y diversidad del orden Zoantharia. Aquí son presentados hallazgos preliminares sobre la distribución y diversidad de especies de zoántidos zooxantelados de los géneros *Zoanthus* y *Palythoa*, documentando estos géneros en las Galápagos por primera vez. Una breve descripción de las especies encontradas es proporcionada. *Zoanthus* y *Palythoa* parecen estar limitados en las Galápagos a costas rocosas del sublitoral e infralitoral en aguas cálidas y someras (temperatura mínima >18°C), aisladas de las aguas más frías que dominan mayormente el archipiélago. Resultados preliminares de la región de los espaciadores internos transcriptos de la secuencia del ADN ribosomal de los dinoflagelados simbióticos sugieren que ambos *Zoanthus* y *Palythoa* spp. en las Galápagos poseen solamente *Symbiodinium* clado C. Se presentan breves descripciones de las especies de zoántidos zooxantelados en las Galápagos.

INTRODUCTION

The Galapagos Islands are world-famous for their terrestrial biodiversity and wealth of endemic species. However, the biological richness of the Galapagos marine ecosystem is equally exceptional but only recently has begun to be comprehensively investigated. In particular, marine invertebrate diversity was understudied until recently. While some groups of Anthozoans (Cnidaria) such as scleractinian corals (Cairns 1991, Glynn 2003), hydroids (Calder *et al.* 2003) and anemones (Fautin *et al.* 2007) are now relatively well documented in the Galapagos, others such as the order Zoantharia (zoanthids) remain to be described. Zoanthids, characterized by two rows of tentacles, one siphonoglyph and a (usually) colonial way of life, have long been taxonomically neglected, due in large part to their lack of useful morphological identification characters (Reimer *et al.* 2004, Sinniger *et al.* 2005). Additionally, most zoanthids use mineral particles (sand and detritus, foraminifer tests, spicules from sponges, sclerites from octocorals, *etc.*) taken from the water column to help make their structure, making dissection and cross-sectioning to access the few useful identification characters (sphincter muscle structure, mesentery count *etc.*) even more difficult.

Recent integrated taxonomic approaches combining traditional morphology with molecular DNA markers have proven useful in documenting the diversity of zoanthids (Reimer *et al.* 2004, 2006b, 2006c), examining their phylogeny (Sinniger *et al.* 2005, 2007) and describing new taxa (Reimer *et al.* 2006b, 2007a). Here, preliminary integrated taxonomic results from surveys undertaken between 2001 and 2007 are presented, documenting for the first time the presence of the genera *Zoanthus* and *Palythoa* in the Galapagos. Additionally, zoanthid distribution patterns are examined, as well as symbiotic dinoflagellate diversity (*Symbiodinium* spp.).

MATERIALS AND METHODS

Zooxanthellate zoanthids were searched for by hand intertidally or by SCUBA on most major islands in the Galapagos, and specimens collected from three locations (Fig. 1) in 2001, 2002 and 2007. Before collection, *in situ* photographs were taken to assist in identification and for morphological data (oral disk/polyp diameter, color, polyp form *etc.*). Specimens were then stored in 75% alcohol at ambient temperature. Samples were assigned numbers based on the sample year; thus: sample 07-01 is sample 1 from 2007.

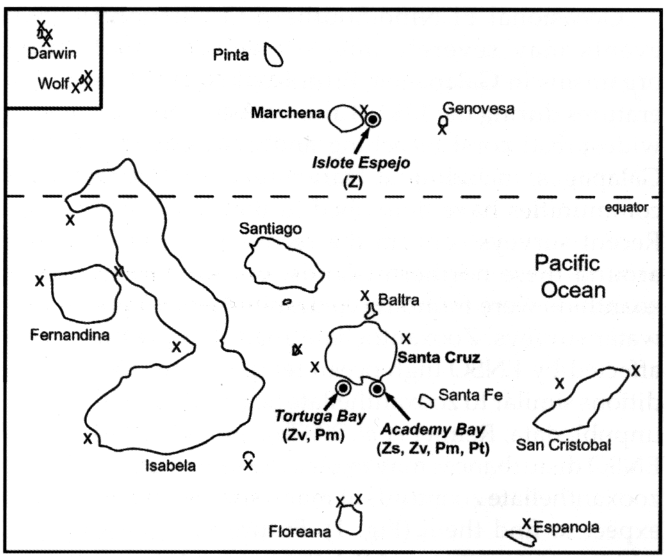

Figure 1. Map of Galapagos and sampling locations. Filled circles indicate presence of zooxanthellate zoanthids (with observed species in parentheses), "X" indicates locations examined where no zooxanthellate zoanthids found. Zs = *Zoanthus* cf. *sansibaricus*, Zv = *Z.* cf. *vietnamensis/kuroshio*, Z = unidentified *Zoanthus* sp., Pm = *Palythoa* cf. *mutuki*, Pt = *P.* cf. *tuberculosa*.

All specimens were identified by morphology, following descriptions in Reimer *et al.* (2006b) and Reimer (2007). Seventeen samples were also examined by using two mitochondrial DNA markers, cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (mt 16S rDNA), following procedures for DNA extraction, PCR amplification, and subsequent analyses outlined in Reimer *et al.* (2004, 2006c).

Species identifications are preliminary, as few records of *Zoanthus* and *Palythoa* exist for the E Pacific, where undescribed species with morphological and DNA characteristics matching those of the species listed here might exist. There are also poorly described Pacific species for which no specimens are available (thus no DNA and no good morphological information). Specimens and DNA data from the W coast of S America and other islands in the E Pacific would help to identify the Galapagos specimens with more confidence; nevertheless, these are at least closely related to the species listed. Descriptions of the species to which Galapagos specimens are referred are given in Appendix 1.

No means exist for reliably identifying *Symbiodinium* spp. clades by morphology, so 15 samples had their *Symbiodinium* spp. internal transcribed spacer of ribosomal DNA (ITS-rDNA) sequences examined (see Rowan & Powers 1991). *Symbiodinium* DNA extraction, ITS-rDNA PCR amplification, and subsequent analyses followed procedures explained in Reimer *et al.* (2006e). All *Symbiodinium* names follow LaJeunesse (2005).

Novel DNA sequences for both host zoanthid specimens (mitochondrial 16S ribosomal DNA, cytochrome oxidase subunit I, and ITS-rDNA) and *Symbiodinium* spp. (ITS-rDNA) will be discussed in detail elsewhere.

RESULTS

Zooxanthellate zoanthids of the genera *Zoanthus* (family Zoanthidae) and *Palythoa* (Sphenopidae) were found at only three and two sites respectively (Fig. 1): Tortuga Bay and Academy Bay on Santa Cruz Island (both genera at both sites), and Islote Espejo, Marchena Island (*Zoanthus* only). No zooxanthellate zoanthids were found at most sites investigated, although some locations were investigated only by SCUBA and not intertidally. All specimens were found in the low infralittoral zone or shallow sublittoral zone, often in tidepools (Table 1).

All *Zoanthus* found were attached to hard substrate, usually large rocks, in the low infralittoral to shallow sublittoral (<1.5 m depth) zones. Species found were *Zoanthus* cf. *sansibaricus* (Tortuga Bay only) and *Zoanthus* cf. *vietnamensis/kuroshio* species group (all three sites). Specimens of *Zoanthus* cf. *vietnamensis/kuroshio* species group were quite common at Academy Bay and Tortuga Bay, usually in locations with warm ocean temperatures (>18ºC), high wave or current activity and high light levels.

Palythoa spp. in Galapagos were mostly found in the same warm, high wave/current, high light levels and

Table 1. Zooxanthellate zoanthid specimens collected from the Galapagos. Sites are: AB = Academy Bay (Santa Cruz Island); TB = Tortuga Bay (Santa Cruz); E = Islote Espejo (Marchena Island). *P. m.* = *Palythoa mutuki; P. t.* = *P. tuberculosa; Z. s.*= *Zoanthus sansibaricus; Z. v./k.* = *Z. vietnamensis/kuroshio.* Pre-2007 collections were by CPH, 07-30 by JDR and B. Riegl, all others by JDR.

Specimen number	Site	Date	Depth (m)	Morphological identification	COI identification	mt 16S rDNA identification	*Symbiodinium* clade	Identity conclusion
01-05	TB	14 Jun 2001	Low infralittoral tidepool	*Z.* sp.	*Z. s.*	*Z. s.*	C1/C3 related	*Z.* cf. *s.*
01-105	AB	22 Jul 2001	Low infralittoral	*Z.* sp.	*Z. v./k.*	*Z. v./k.*	C1/C3 related	*Z.* cf. *v./k.*
01-106	AB	22 Jul 2001	Low infralittoral	*P. t.*	*P.* spp.	*P. t.*	C1/C3 related	*P.* cf. *t.*
02-122	TB	27 Jun 2002	Low infralittoral tidepool	*P. m.*	*P.* spp.	*P. m.*	C1/C3 related	*P.* cf. *m.*
07-01	TB	1 Mar 2007	+0.5	*Z.* sp.				*Z.* cf. *v./k.*
07-02	TB	1 Mar 2007	0	*Z.* sp.				*Z.* cf. *v./k.*
07-03	TB	1 Mar 2007	0	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-04	TB	1 Mar 2007	0	*Z.* sp.			C	*Z.* cf. *v./k.*
07-05	TB	1 Mar 2007	0	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-06	AB	2 Mar 2007	+0.5	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-07	AB	2 Mar 2007	0	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-08	AB	2 Mar 2007	0	*Z.* sp.	*Z. v./k.*			*Z.* cf. *v./k.*
07-09	AB	2 Mar 2007	0	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-10	AB	2 Mar 2007	0	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-11	AB	2 Mar 2007	0	*Z.* sp.	*Z. v./k.*		C	*Z.* cf. *v./k.*
07-12	AB	2 Mar 2007	0	*Z.* sp.	*Z. v./k.*			*Z.* cf. *v./k.*
07-13	AB	2 Mar 2007	0	*P. m.*	*P.* spp.		C1/C3 related	*P.* cf. *m.*
07-14	AB	2 Mar 2007	0	*P. m.*	*P.* spp.		C1/C3 related	*P.* cf. *m.*
07-15	AB	2 Mar 2007	0	*P. m.*	*P.* spp.		C1/C3 related	*P.* cf. *m.*
07-15b	AB	2 Mar 2007	0	*P. m.*	*P.* spp.			*P.* cf. *m.*
07-30	E	3 Mar 2007	-1.0	*Z.* sp.				*Z.* sp.

hard substrate conditions as *Zoanthus*. Species were *Palythoa* cf. *tuberculosa* (infralittoral zone in Academy Bay, only observed in 2001, not found in 2007), and *Palythoa* cf. *mutuki* (Academy Bay and Tortuga Bay), which was sympatric with but not as common as *Z.* cf. *vietnamensis/ kuroshio* (Fig. 2b).

All specimens examined, regardless of host species or genus, possessed *Symbiodinium* clade C (Table 1).

DISCUSSION

Distribution in Galapagos

Although the presence of zooxanthellate zoanthids at both Academy Bay and Tortuga Bay had been known for many years (Hedgpeth 1969), they had not been conclusively identified to species. The presence of *Zoanthus* and *Palythoa* was not unexpected, because they are by far the two most common zooxanthellate zoanthid genera, found worldwide in subtropical and tropical waters. Both genera possess *Symbiodinium* and are thus limited to ocean waters above *c.* 15–16°C (Reimer *et al.* 2006e, 2007b), similar to reef-building corals. Although the Galapagos Islands are situated over the equator, the cold Peru Oceanic Current brings water as cold as 18°C and, in the western archipelago, the Equatorial Undercurrent produces localized upwelling as cold as 14°C on the west coasts of Fernandina and Isabela (Chávez & Brusca 1991). Thus, zooxanthellate corals and other zooxanthellate colonial anthozoans are most likely limited to shallow, warm (>18°C) waters more common in the eastern archipelago and/or east coasts of islands, as well as the northern islands of Darwin and Wolf.

Occasional El Niño-Southern Oscillation (ENSO) events may severely affect zooxanthellate-hosting organisms in Galapagos. Protracted elevated sea temperatures during the 1982–3 and 1997–8 events resulted in widespread coral bleaching and mortality throughout Galapagos, including at Darwin and Wolf where coral communities have flourished in the past (Glynn 2003). Recent surveys confirm the recovery of reef structure around these northern islands, but no zooxanthellate zoanthids were found there despite numerous shallow-water surveys. Zooxanthellate zoanthids are negatively affected by ENSO high ocean temperature (>30°C) conditions similar to zooxanthellate Scleractinia (S. Ono *et al.* unpubl. data, Reimer *et al.* 2007b), and it is possible that ENSO disturbances may explain the apparent absence of zooxanthellate zoanthids at many sites where we should expect to find them (Fig. 1). Future surveys may find additional *Zoanthus* and *Palythoa* populations in shallow or infralittoral waters with rocky substrates in warm-water areas.

Origin of Galapagos zooxanthellate zoanthids

While data on mobile azooxanthellate larvae of *Palythoa* (zoanthellae) are sparse, it is known that *Zoanthus* *sansibaricus* in Japan reproduces sexually during summer in apparent mass spawning events (Ono *et al.* 2005), with swimming azooxanthellate larvae (zoanthinae) that survive at least 60 days without settling (S. Ono pers. comm.). Such larvae may have been able to colonize Galapagos from the west coast of S or central America. Unfortunately, there are few historical records on the distribution of *Zoanthus*, *Palythoa* and other zooxanthellate zoanthids from the E Pacific: *Palythoa* and *Zoanthus* from Easter Island (Carlgren 1922), *Palythoa* and *Zoanthus* from the Bay of Panama (Verrill 1869, Carlgren 1951), and *Palythoa* from French Polynesia (Boone 1938), the west coast of El Salvador and Mexico (Verrill 1869). Therefore comparisons of species diversity are not yet possible.

All specimens examined possessed *Symbiodinium* of clade C, as previously seen in *Palythoa* spp. (Reimer *et al.* 2006d) and *Zoanthus* spp. (Reimer *et al.* 2006e, 2007b). In particular, several specimens had *Symbiodinium* C1/C3, which is a host generalist found in a wide variety of corals throughout the Pacific and Atlantic Oceans (LaJeunesse 2005), and is also theorized to be an environmental generalist. *Symbiodinium* such as C1/C3 may be suited for the unusually variable environments found in the Galapagos.

Both *Zoanthus* spp. and the majority of *Palythoa* spp. have azooxanthellate larvae, acquiring *Symbiodinium* at settlement and attachment (both genera) or during the free-swimming zoanthinae stage (*Zoanthus* only) (Ryland *et al.* 2000). The Galapagos results suggest symbiont flexibility for both *Z.* cf. *sansibaricus* and *Z.* cf. *vietnamensis/kuroshio*, as specimens of both species groups possessed generalist C1/C3 or closely related types, which were not previously seen in *Zoanthus* spp. in Japan. *Z. sansibaricus* has previously been found to be flexible in its association with *Symbiodinium*, either possessing a specific subclade of *Symbiodinium* (designated C1z: C1/C3-related but different from types seen here, see Reimer *et al.* 2006e, 2007c), or clade A (Reimer *et al.* 2006e), while *Z. vietnamensis* in Japan possesses subclade C15 and related types (Reimer *et al.* 2007c). It may be that azooxanthellate larvae of *Zoanthus* spp. acquired C1/C3, the most common type of *Symbiodinium* in the Pacific (LaJeunesse 2005), upon colonization of the Galapagos.

Palythoa specimens also possessed C1/C3 *Symbiodinium*, as previously seen in *Palythoa* spp. (Reimer *et al.* 2006d), and thus it is impossible to speculate if these species arrived in the Galapagos as a result of sexual or asexual reproduction. However, these preliminary results reinforce findings from Japan that in the Pacific *P. tuberculosa* and *P. mutuki* associate only with C1/C3 (Reimer *et al.* 2006d).

Conclusions and future research

An examination of other locations in the Galapagos and the west coast of S and central America would help clarify our understanding of the distribution and diversity of these understudied genera (and their *Symbiodinium* spp.). As global warming increases, such records may also help in documenting the potential spread of these warm-water (>18ºC) organisms, as seen with the first sighting of the Crown-of-thorns starfish *Acanthaster planci* at Darwin Island in 1995, a species that may have arrived with the ENSO event of 1982–3 (Hickman 1998).

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APPENDIX 1

DESCRIPTIONS OF ZOOXANTHELLATE ZOANTHIDS IN THE GALAPAGOS (ADAPTED FROM REIMER 2007)

FAMILY ZOANTHIDAE

The only family within the Order Zoantharia not to be sand-encrusted. All three genera (*Zoanthus*, *Isaurus*, *Acrozoanthus*) are zooxanthellate.

Genus *Zoanthus*
Distinguished in Galapagos by its lack of sand and/or detritus. *Zoanthus* polyps are uniformly smooth on the outer surface. Can also often be distinguished from *Palythoa* by often having brightly colored oral disks, although green and brown forms (similar to *Palythoa* spp.) exist. The external surface of polyps and the coenenchyme is mainly light to dark purple, although pale green polyps are sometimes observed.
***Zoanthus* cf. *sansibaricus* Carlgren 1900 (Fig. 2a).** Often forms large colonies with polyps well clear and free of the coenenchyme ("liberae") (Reimer *et al.* 2006b; see Pax 1910). Adult polyps 3–12 mm in diameter, up to 20 mm in length. External polyp surface generally uniform light to dark purple, no markings, sometimes slightly paler around edge of oral disk. Tentacles 40–58, mesenteries 48–54. Wide variation in oral disk color: red, green (most common), brown, purple, white, blue (common), often fluorescent. Genetic data show it to consist of many different color morphotypes (Reimer *et al.* 2004, 2006b).

***Zoanthus* cf. *vietnamensis* Pax and Mueller 1957/*Zoanthus* cf. *kuroshio* Reimer and Ono 2006 species group.** *Z.* cf. *vietnamensis* has polyps up to 30 mm in length, up to 20 mm in diameter (Uchida 2001, Reimer *et al.* 2006a). Polyps "liberae". Oral disk pale to dark pink, occasionally mint green, often with white oral opening. Tentacles 55–64. Morphs of *Z.* cf. *vietnamensis* with pink or green oral disk often are very hard to distinguish from *Z.* cf. *sansibaricus*.

Molecular data suggest that *Z.* cf. *kuroshio* is simply a morphotype of *Z.* cf. *vietnamensis* (Reimer *et al.* 2006a), although their morphology is clearly different. *Z.* cf. *kuroshio* usually has oral disk pale pink, although white and pale blue varieties observed. Tentacles 50–64, mesenteries 42–48. Polyps deeply embedded, barely extend from coenenchyme ("immersae"). Oral disk 6–12 mm in diameter when expanded. Polyps narrower in diameter towards oral opening than at base. Edge of coenenchyme tongue-like in form. Colonies often intertidal and in tide pools on wave-exposed shoreline, can be very large and encrusting, forming a mat over substrate (rock or dead coral).

FAMILY SPHENOPIDAE

Differs from Zoanthidae in that it is sand-encrusted. Includes the colonial genus *Palythoa* with many species worldwide, as well as the solitary genus *Sphenopus*.

Genus *Palythoa*

Palythoa species can be distinguished from *Zoanthus* in Galapagos by their sand and detritus structures. Colony and polyp tissues are usually tan or brown, sometimes green. Patchy bleaching may sometimes be observed in larger *P. tuberculosa* colonies.

***Palythoa* cf. *tuberculosa* Delage and Herouard 1901.** Polyps immersae, barely extending above large, well-developed coenenchyme (Uchida 2001, Reimer *et al.* 2006c). Oral disks up to 20 mm in diameter, though often closed in daytime. Coenenchyme dark brown, generally uniform although some patchiness often observed. Colonies small to large, encrust substrate. Tolerates more marginal environments (reef lagoons, tide pools *etc.*) than many other colonial cnidarians (Reimer *et al.* 2006d).

***Palythoa* cf. *mutuki* Carlgren 1937 (Fig. 2b).** Polyps liberae, up to 40 mm in length (Ryland & Lancaster 2003, Reimer *et al.* 2006c). Oral disk up to 30 mm diameter, green. Radii often visible (white, pale brown). Colonies generally small (<100 polyps). Closely related to *P. tuberculosa*, and may have undergone reticulate evolution with it in the past (Reimer *et al.* 2007d).

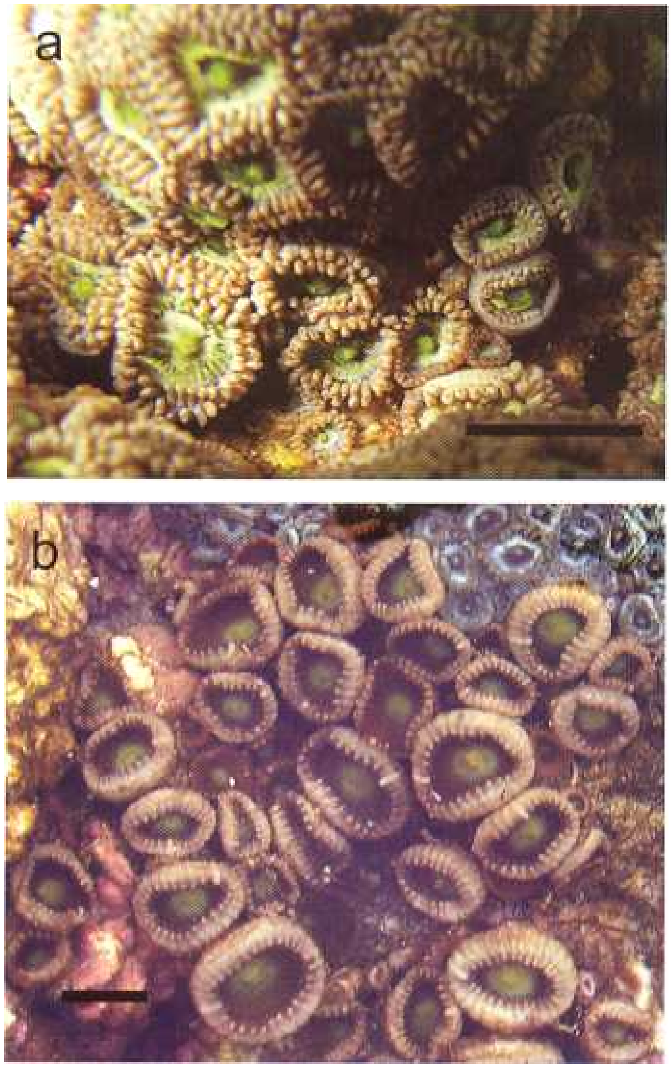

Figure 2. Zooxanthellate zoanthids *in situ* in the Galapagos, March 2007: (a) *Zoanthus* cf. *vietnamensis/kuroshio* (depth 0 m), Tortuga Bay, Santa Cruz Island; (b) *Palythoa* cf. *mutuki* (with interspersed *Z.* cf. *vietnamensis/kuroshio* at top of image), tidepool at low tide, Academy Bay, Santa Cruz Island. Black bars = 1 cm.