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Four new species and one new genus of zoanthids (Cnidaria, Hexacorallia) from the Galápagos Islands

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Abstract

Recent research has confirmed the presence of several species of undescribed macrocnemic zoanthids (Cnidaria: Hexacorallia: Zoantharia: Macrocnemina) in the Galápagos. In this study four new species, including two belonging to a new genus, are described. Two species, *Terrazoanthus onoi* **sp. n.** and *Terrazoanthus sinnigeri* **sp. n.**, both belong within the recently erected family Hydrozoanthidae to the new genus *Terrazoanthus,* which can be distinguished from the type genus *Hydrozoanthus* by being attached to abiotic substrate as opposed to hydrozoans for *Hydrozoanthus*. Each new species of zoanthid can be clearly distinguished by a number of characters. *Antipathozoanthus hickmani* **sp. n.** is distinguished by its exclusive association with the antipatharian *Antipathes galapagensis*, and has approximately 40 tentacles. *Parazoanthus darwini* **sp. n.** is distinguished by its frequent association with sponges, with approximately 24–30 tentacles and polyps embedded in a well-developed coenenchyme. *T. onoi* **sp. n.** is distinguished by its bright red oral disk color, 32–40 tentacles, and has only basitrichs and mastigophores present in the pharynx. *T. sinnigeri* **sp. n.** is distinguished by usually occurring on the underside of rubble and rocks on sandy bottoms, showing 30–36 tentacles, and numerous nematocyst types in the pharynx. The two *Terrazoanthus* species, although divergent in both morphology and ecology, are apparently very closely related, with identical mitochondrial 16S ribosomal DNA and cytochrome oxidase subunit I sequences. These two species can be molecularly distinguished by their subtly different yet distinct sequences of internal transcribed spacer of ribosomal DNA (ITS-rDNA).

Keywords
Key words: zoanthid, Galápagos, new genus, new species, ITS-rDNA, mt DNA

Introduction

The Galápagos Archipelago is a group of oceanic islands in the south-east Pacific. The islands are surrounded by warm and cold ocean currents that include upwelling currents. As a result of these currents, the marine ecosystems of the Galápagos islands are isolated from other regions, and there are high levels of diversity and unique fauna (Bustamante et al. 2000, 2002). The marine area of the Galapagos islands was inscribed as a UNSECO natural World Heritage Site in 1978. However, as the Galápagos islands are becoming increasingly famous and numbers of tourist visits rise, environmental pollution and other associated problems have arisen. Since 2007 the Galápagos have been designated as a World Heritage Site in danger.

Recently, the marine fauna of the Galápagos has begun to become more intensively investigated. Some results have been published as the Galápagos Marine Life Series field guides. One of these guides is focused on corals, zoanthids, octocorals and other benthic cnidarians of the Galápagos (Hickman 2008). The results of these investigations have demonstrated that there are many unidentified species in the order Zoantharia in the Galápagos (Reimer et al. 2008a).

However, zoanthids (Cnidaria, Anthozoa, Hexacorallia, Zoantharia) form a taxonomical group for which studies are lacking in general. Zoanthids are an order of benthic cnidarians that are found in most marine ecosystems (Sinniger et al. 2005; Reimer et al. 2007). Distinguishable by their two rows of tentacles, incorporation of sand into their mesoglea, and colonial nature (for most described species) (Sinniger et al. 2005), zoanthids are increasingly becoming a subject of research as they possess unique bioactive compounds and chemicals (Behenna et al. 2008). Despite this increase in research, the classification and identification of many zoanthids remains difficult due to a myriad of problems, including but not limited to morphological variation within species, a lack of research into their species diversity, and difficulty in internal morphological examinations due to the presence of sand and detritus (Reimer et al. 2010).Consequently, the true number of zoanthid species is currently unknown (Reimer et al. 2004).

However, studies using molecular techniques have begun to bring some standardization and reassessment to zoanthid taxonomy. Allozymes (Burnett et al. 1997), mitochondrial DNA (mt DNA) (Reimer et al. 2004; Sinniger et al. 2005) and nuclear DNA phylogenies (Reimer et al. 2007b; Swain 2009) combined with ecological data have resulted in the creation of new taxa (Sinniger and Häussermann 2008; Reimer et al. 2008a; Sinniger et al. 2009) and the merging of other taxa (e.g. Reimer et al. 2006).

With a molecular phylogenetic framework in place, recent research into zoanthids has focused on diversity in insular and previously under-investigated locations, such as New Caledonia (Sinniger 2008), the deep-sea (Reimer et al. 2007a), and the Galápagos (Reimer et al. 2008b, 2009).

Recent investigations into the diversity of zoanthids in the Galápagos revealed the presence of several new zoanthid species groups (Reimer et al. 2008b). In this study, four species and one new genus of zoanthids from the Galápagos are formally described, and the proposed future of zoanthid research in the southeastern Pacific is briefly discussed.

Materials and methods

Sample collection: Specimens were collected by hand intertidally, or by SCUBA or snorkeling from numerous sites at the Galápagos between June 2001 and March 2007. As specimens were collected, in situ digital images were taken to assist in identification and morphological analyses (oral disk/polyp diameter, color, polyp form, etc.). After collection specimens were further examined, photographed, and preserved in 75% ethanol. Specimens in this study consist of specimens initially described as *Parazoanthus* sp. G1, *Parazoanthus* sp. G2, and *Parazoanthus* sp. G3 in Reimer et al. (2008b, 2010).

DNA extraction and PCR amplification: DNA was extracted as described in Reimer et al. (2008a), using a spin-column Dneasy Blood and Tissue Extraction protocol (Qiagen, Santa Clarita, CA, USA). Mitochondiral 16S ribosomal DNA (mt 16S rDNA) was amplified using primers and protocol described in Sinniger et al. (2005), the cytochrome oxidase subunit I (COI) gene was amplified following Reimer et al. (2004, 2007a), and the internal transcribed spacer region of ribosomal DNA (ITS-rDNA) following Reimer et al. (2007b). PCR amplification procedures for each of the molecular markers were as given in the original references above. Amplified products were visualized by 1.5% agarose gel electrophoresis.

Phylogenetic analyses: New sequences obtained in the present study (Table 1) were deposited in DDBJ and GenBank (accession numbers GU357551-GU357567). By using CLUSTAL X version 1.8 (Thompson et al. 1997), the nucleotide sequences of mt 16S rDNA, COI, and ITS-rDNA from samples were aligned with previously published sequences (see Reimer et al. 2008b) from various zoanthid species representing the genera *Zoanthus*, *Savalia*, *Corallizoanthus*, *Mesozoanthus*, *Hydrozoanthus*, and *Parazoanthus*.

The outgroup sequences for both mt 16S rDNA and COI trees were from the genera *Zoanthus* and/or *Palythoa*. The only zoanthid genera not included in both analyses were *Epizoanthus*, previously shown to be basal in the order Zoantharia phylogeny for both mt 16S rDNA and COI (Sinniger et al. 2005; Reimer et al. 2007a); *Abyssoanthus,* demonstrated to be divergent from other zoanthids for both mt 16S rDNA and COI (Reimer et al. 2007a), and *Sphenopus*, which is in the same family as one of the out-groups of this study, *Palythoa* (family Sphenopidae). These represent the current full range of described zoanthid genera. New mt 16S rDNA sequences from specimens in this study were clearly divergent when compared with *Mesozoanthus* sequences, but *Mesozoanthus* sequences from Sinniger and Häussermann (2009) were not included in trees presented in this study due to their relatively short length (469 base pairs).

Hydrozoanthidae ITS-rDNA sequences (particularly ITS-1 and ITS-2 spacers) were highly divergent from other obtained ITS-rDNA sequences, and thus initially an ITS-rDNA alignment consisting only of Hydrozoanthidae sequences with sequence AB214161 from *Hydrozoanthus gracilis* Carlgren (Reimer et al. 2007c) plus two new *H. gracilis* specimen sequences as the outgroup was generated. The new Galapagos specimens were shown to form a very well supported monophyletic group, and therefore to improve resolution, subsequently an alignment was created with only these sequences. An ITS-rDNA phylogeny from a previous study shows the monophylies and positions of non-Hydrozoanthidae species discussed in this study (*Parazoanthus* sp. G1, *Parazoanthus* sp. G2; both *sensu* Reimer et al. 2008b; see Figure 7 in Reimer et al. 2008b).

All alignments were inspected by eye and manually edited by removing all ambiguous sites (if present) of the alignments (*e.g.* sites present in either only forward or reverse directions, not seen in any other sequence) from the dataset for phylogenetic analyses, and aligning mt 16S rDNA and ITS-rDNA indels as in previous studies (Reimer et al. 2008b; Sinniger et al. 2009). Consequently, three alignment datasets were generated: 1) 651 sites of 39 sequences (mt 16S rDNA); 2) 280 sites of 45 sequences (COI); and 3) 595 sites of 14 sequences (ITS-rDNA). The alignment data are available on request from the corresponding author.

The alignments of mt 16S rDNA, COI, and ITS-rDNA were tested for optimal fit of various nucleotide substitution models using jModelTest version 0.1.1 (Posoda 2008). For the mt 16S rDNA dataset, the general time reversible (GTR) model (Rodriguez et al. 1990) incorporating variable sites and a discrete gamma distribution (GTR + I + G) was suggested by jModelTest under Akaike Information Criterion (AIC), while the Hasegawa, Kishino and Yano model (Hasegawa et al. 1985) incorporating variable sites (HKY + I) was suggested for the COI dataset, and the K80 model (Kimura 1980) was suggested for the ITS-rDNA dataset. The maximum-likelihood (ML) analyses with PhyML (Guindon and Gascuel 2003) of these datasets were independently performed using an input tree generated by BIONJ (Gascuel 1997) with the models selected by jModelTest. PhyML bootstrap trees (1000 replicates) were constructed using the same parameters as the individual ML tree.

Bayesian trees were reconstructed by using the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) under the K80 model incorporating variable sites of nucleotide substitution (K80 + I) for the mt DNA 16S rDNA and COI datasets, and under the Jukes and Cantor model (JC69; Jukes and Cantor 1969) for the ITS-rDNA dataset [all models selected by jModelTest under Bayes Information Criterion]. One cold and three heated Markov chain Monte Carlo (MCMC) chains with default-chain temperatures were run for 20 million generations, sampling log-likelihoods (InLs), and trees at 1000-generation intervals (20,000 InLs and trees were saved during MCMC). The first

15% of all runs were discarded as "burn-in" for all datasets. The likelihood plots for all three datasets also showed that MCMC reached the stationary phase by this time. Thus, the remaining 17,000 trees (17 million generations) of mt 16S rDNA, COI and ITS-rDNA were used to obtain posterior probabilities and branch-length estimates, respectively.

Morphological analyses: The external morphology of specimens was examined using both preserved specimens and in situ images. Polyp dimensions (oral disk diameter, polyp height) for both in situ and preserved specimens were obtained, as were the following data: tentacle number, color of polyp, color(s) of oral disk, relative amount of sand encrustation, associated/substrate species. Additionally, the relative development of the coenenchyme was examined.

For internal morphological examinations, some specimens underwent initial decalcification followed by desilification as outlined in Reimer et al. (2010). After these treatments, specimens were dehydrated through an ethanol-xylene series. Some specimens in 100% ethanol were placed *in vacuo* for approximately 30 minutes to remove bubbles in the coelenteron. Then, they were embedded in paraffin. Serial sections of 5–10 µm thick were prepared with a rotary microtome and stained with Delafield's hematoxylin and eosin.

Obtained slides of HF-treated zoanthid specimens were examined with a light microscope (Nikon Express E50i). The following morphological characters and conditions were examined; mesentery condition, number, and form (in particular fifth mesentery from dorsal directive complete or incomplete); presence or absence of drag marks from debris; presence or absence of sand and debris in mesoglea; overall condition of tissue and cells; and in particular ectoderm and endoderm; any other morphological characters of note (e.g. presence of gametes, etc.) (described in Reimer et al. 2010).

Nematocyst observation: Undischarged nematocysts were measured from tentacles, column, actinopharynx, and mesenterial filaments of polyps (specimens examined n=2–4; polyp n=4–8) for all new species. 400x images of the nematocysts were obtained by optical microscope, and measured using the software ImageJ (National Institutes of Health, USA). Nematocyst nomenclature generally followed England (1991), however both Schmidt (1974) and Hidaka and co-workers (1987; 1992) have previously suggested basitrichs and mastigophores are same type of the nematocyst, and thus in this study, these two types were dealt with as the same type, unless they could be clearly distinguished from one another, in which case they were analyzed separately.

Abbreviations used:

USNM National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA

CMNH Coastal Branch of Natural History Museum and Institute, Chiba, Japan

MHNG Natural History Museum of Geneva, Switzerland

MISE Molecular Invertebrate Systematics and Ecology Laboratory, University of the Ryukyus, Nishihara, Okinawa, Japan

Systematics

Family Parazoanthidae Delage & Hérouard, 1901

Diagnosis: Macrocnemic zoanthids that have an endodermal sphincter muscle. Many species in this family associated with other organisms as substrate.

Genus *Antipathozoanthus* Sinniger, Reimer & Pawlowski, 2009

Type species: *Antipathozoanthus macaronesicus* (Ocaña & Brito, 2004)

Diagnosis (from Sinniger et al. 2009): Unlike other described zoanthid genera, *Antipathozoanthus* is found on living antipatharians (Hexacorallia: Antipatharia), and unlike gorgonian-associated *Savalia*, does not appear to secrete its own scleroproteinous axis. Colonial zoanthids, polyps linked together by a basal coenenchyme usually covering all of antipatharian substrate axis, size of expanded oral disks usually between 4–12 mm width and 4–15 mm high. Column lightly incrusted with fine sediments, not completely covering ectoderm. Column and tentacles often yellowish, pinkish, cream or red. Mesenteries follow macrocnemic organisation. Distributed in tropical and subtropical area at depths ranging from 10 m to 45 m.

Antipathozoanthus hickmani sp. n.

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Figures 1, 5, 7, 9, Tables 1, 2, 3

Etymology. Named after Dr. Cleveland Hickman, Jr., who graciously invited the first author to the Galápagos, and collected the first specimens of this new species. Noun in the genitive case.

Material examined. *Type locality*: Ecuador, Galapagos: Floreana I., La Batielle, 1.2904°S 90.4989°W.

Holotype: Specimen number MHNG-INVE-67495. Colony of approximately 40 polyps connected by well-developed coenenchyme on two branches of *Antipathes galapagensis* Diechmann, 1941 branches. Both branches approximately 7 cm long. Polyps approximately 1.5–4.0 mm in diameter, and approximately 1.0–6.0 mm in height from coenenchyme. Coenenchyme covers branches of antipatharian. Polyps and coenenchyme sand encrusted, cream-yellow in color. Collected from La Batielle, Floreana I., Galapagos, Ecuador, at 31.4 m by A. Chiriboga (AC), March 13, 2007. Preserved in 99.5% ethanol.

Paratypes (all from Galapagos, Ecuador):

Paratype 1. Specimen number CMNH-ZG 05883. Collected from Roca Onan, Pinzon I., at 27 m by AC, March 14, 2007.

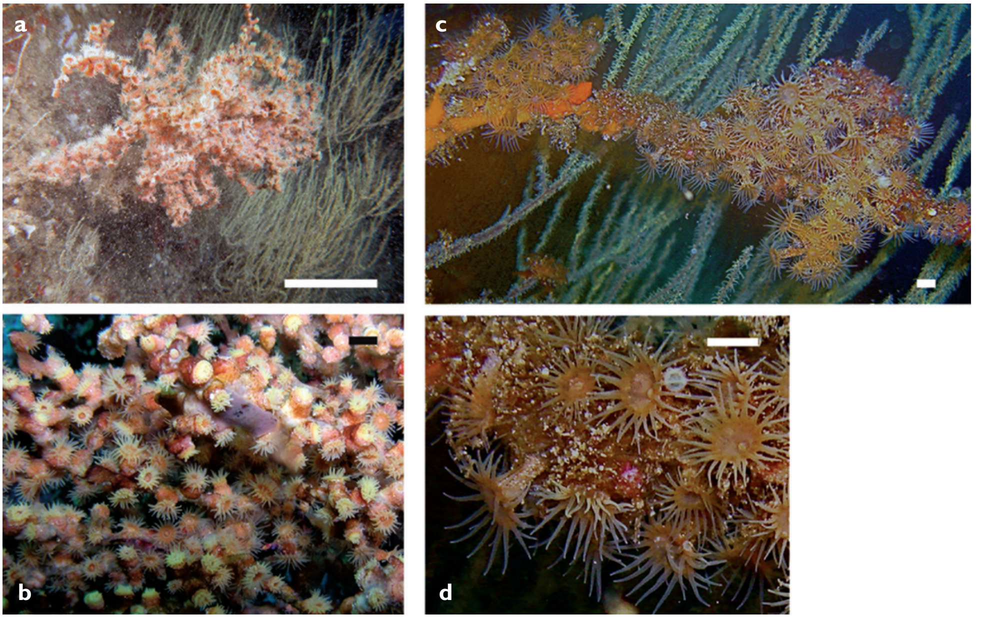


Figure 1. *Antipathozoanthus hickmani* sp. n. in situ in the Galapagos. **a** holotype MHNG-INVE-67495 showing the entire colony covering an *Antipathes galapagensis*, with living antipatharians visible in the background. Image by Angel Chiriboga (AC) **b** specimen MISE 441 at Don Ferdi, Bainbridge Rocks, Santiago I., at 23 m by JDR, March 9, 2007 **c** and **d** specimen MISE 474, Roca Onan. Pinzon I., at 35 m by AC. All scale bars: 1 cm except in **a** (10 cm).

Paratype 2. Specimen number USNM 1134064. Collected from Cousins Rock, at 28 m by James D. Reimer (JDR), March 10, 2007.

Other material (all from Galapagos, Ecuador): MISE 03-221, Cousins Rock, at 12 m by AC on October 9, 2003; MISE 03-539, Cousins Rock, at 20 m by CH on November 11, 2003; MISE 03-549, Cousins Rock, at 23 m by CH on November 11, 2003; MISE 04-341, Elizabeth Bay, Isabela I., at 25 m by G. Edgar (GE) on December 2, 2003; MISE 440, Don Ferdi, Bainbridge Rocks, at 22 m by JDR, March 9, 2007; MISE 441, Don Ferdi, Bainbridge Rocks, at 23 m by JDR, March 9, 2007; MISE 444, Cousins Rock, Galapagos, Ecuador, at 21 m JDR, March 10, 2007; MISE 474, La Batielle, Floreana I., at 35 m by AC, March 14, 2007.

Sequences. See Table 1.

Description. *Size*: Polyps in situ approximately 4–12 mm in diameter when open, and approximately 4–15 mm in height.

Morphology: *Antipathozoanthus hickmani* has approximately 40 bright yellow and/or red tentacles, with long red, yellow, or cream-colored polyps that extend well clear of the coenenchyme (Figure 1). Tentacles are almost always longer than the expanded oral disk diameter.

Cnidae: Basitrichs and microbasic p-mastigophores (often difficult to distinguish), holotrichs (large and medium), spirocysts (see Table 2, Figure 9).

Table 1. Examined zoanthid specimens for new species from the Galapagos Islands, and GenBank Accession Numbers.

Collection no.[a]	Collection locality	Latitude[b]	Longitude[b]	Date collected	Depth (m)	Collected by[c]	Oral disk color	Substrate	COI Accession Number	mt 16S rDNA Accession Number	ITS-rDNA Accession Number	Taxonomic identification
03–221	Cousins Rock	-0.2370	-90.5731	2003.10.9	12	AC	Yellow	*Antipathes galapagensis*	NA	EU333755	EU333797	*Antipathozoanthus hickmani*
03–539	Cousins Rock	-0.2370	-90.5731	2003.11.11	20	CH	Cream yellow	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
03–549	Cousins Rock	-0.2370	-90.5731	2003.11.11	23	CH	Yellow	Dead *Antipathes galapagensis*	EU333783	EU333756	EU333798	*Antipathozoanthus hickmani*
04–341	Isabela: Elizabeth Bay	-0.5996	-91.09059	2004.12.2	25	GE	NA	Dead *Antipathes galapagensis*	EU333790, EU333796	EU333757	NA	*Antipathozoanthus hickmani*
440	Bainbridge Rocks: Don Ferdi	-0.2100	-90.567	2007.3.9	22	JDR	Red	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
441	Bainbridge Rocks: Don Ferdi	-0.2100	-90.567	2007.3.9	23	JDR	Red	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
USNM 1134064	Cousins Rock	-0.2370	-90.5731	2007.3.10	28	JDR	Red	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
444	Cousins Rock	-0.2370	-90.5731	2007.3.10	21	JDR	Red	Dead *Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
MHNG 67495	Floreana: La Botella	-1.2904	-90.4989	2007.3.13	31	AC	Pale yellow	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
474	Pinzon: Roca Onan	-0.5909	-90.6860	2007.3.14	35	CH, RP, AC	Red	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*
CMNH ZG05883	Pinzon: Roca Onan	-0.5909	-90.6860	2007.3.14	27	AC	Red	*Antipathes galapagensis*	NA	NA	NA	*Antipathozoanthus hickmani*

Collection no.[a]	Collection locality	Latitude[b]	Longitude[b]	Date collected	Depth (m)	Collected by[c]	Oral disk color	Substrate	COI Accession Number	mt 16S rDNA Accession Number	ITS-rDNA Accession Number	Taxonomic identification
03–47	Isabela: Punta Vicente Roca	-0.0558	-91.5604	2003.1.16	2	CH	Pink	Lava rock	EU333778	EU333749	EU333801	*Parazoanthus darwini*
03–177	Gordon Rocks	-0.5660	-90.1406	2003.1.22	18	CH	Pink	Lava rock	EU333781	EU333753	EU333799	*Parazoanthus darwini*
03–290	Fernandina: Cape Douglas	-0.30037	-91.6483	2003.8.25	6	AC	Red-yellow	Rock/debris	EU333782	EU333748	EU333800	*Parazoanthus darwini*
03–652	Isabela: Tagus Cove	-0.2677	-91.3723	2003.11.16	13	CH	NA	Live red sponge	EU333786	EU333752	NA	*Parazoanthus darwini*
04–155	Floreana: La Botella	-1.2904	-90.4989	2004.5.25	12	AC	Cream yellow	Lava rock	EU333788	EU333754	NA	*Parazoanthus darwini*
04–184	Isabela: Las Marielas	-0.5996	-91.0906	2004.12.2	6	AC	Pink-red	Live sponge & rock	GU357559	NA	NA	*Parazoanthus darwini*
04–328	Isabela: Las Marielas	-0.5996	-91.0906	2004.12.2	3	AC	NA	Live sponge	EU333789	EU333750	NA	*Parazoanthus darwini*
04–348	Isabela: Caleta Iguana	-0.9945	-91.4454	2004.12.3	9	CH	NA	Rock	EU333795	EU333751	EU333802	*Parazoanthus darwini*
412	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	6	JDR, CH, FL	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
413	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	7	JDR, CH, FL	Yellow	Rock	NA	NA	NA	*Parazoanthus darwini*
USNM 1134065	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	8	JDR, CH, FL	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
CMNH ZG05884	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	8	JDR, CH, FL	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
MHNG 67497	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	8	JDR, CH, FL	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
417	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	NA	JDR, CH, FL	Yellow	Rock	NA	NA	NA	*Parazoanthus darwini*

Collection no.[a]	Collection locality	Latitude[b]	Longitude[b]	Date collected	Depth (m)	Collected by[c]	Oral disk color	Substrate	COI Accession Number	mt 16S rDNA Accession Number	ITS-rDNA Accession Number	Taxonomic identification
424	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	9	JDR. FL, BR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
425	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	9	JDR. FL, BR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
428	Wolf: El Elefante	1.3726	-91.8249	2007.3.5	24	DR, FR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
429	Wolf: El Elefante	1.3726	-91.8249	2007.3.5	21	DR, FR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
430	Wolf: El Elefante	1.3726	-91.8249	2007.3.5	22	JDR, OB, BR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
431	Wolf: El Elefante	1.3726	-91.8249	2007.3.5	21	JDR, OB, BR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
450	Gordon Rocks	-0.5660	-90.1406	2007.3.11	17	JDR	Tan	Sponge	NA	NA	NA	*Parazoanthus darwini*
451	Gordon Rocks	-0.5660	-90.1406	2007.3.11	21	JDR	Tan	Sponge	NA	NA	NA	*Parazoanthus darwini*
452	Gordon Rocks	-0.5660	-90.1406	2007.3.11	17	AC	Tan	Sponge	NA	NA	NA	*Parazoanthus darwini*
456	San Cristobel: Whale Rock	-0.8868	-89.6308	2007.3.12	17	JDR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
458	San Cristobel: Kicker Rock	-0.7797	-89.5219	2007.3.12	15	JDR	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
461	Espanola: Baho Garden	-1.3646	-89.6353	2007.3.12	10	MV	NA	Rock	NA	NA	NA	*Parazoanthus darwini*
466	Floreana: Gardner	-1.3329	-90.2953	2007.3.13	15	JDR, AC	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
468	Floreana: Gardner	-1.3329	-90.2953	2007.3.13	14	JDR, CH	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*

Collection no.[a]	Collection locality	Latitude[b]	Longitude[b]	Date collected	Depth (m)	Collected by[c]	Oral disk color	Substrate	COI Accession Number	mt 16S rDNA Accession Number	ITS-rDNA Accession Number	Taxonomic identification
470	Floreana: Devil's Crown	-1.2144	-90.4238	2007.3.13	9	JDR, MV	Yellow	Sponge	NA	NA	NA	*Parazoanthus darwini*
01–61	Isabela: Punta Vicente Roca	-0.0558	-91.5604	2001.6.19	6	CH	Red	Dead giant barnacle	EU333770	EU333761	EU333803	*Terrazoanthus onoi*
02–27	Darwin: Arch	1.6725	-91.9900	2002.5.18	6–8	CH	Red	Dead coral	EU333774	EU333760	EU333810	*Terrazoanthus onoi*
02–59	Isabela: Punta Vicente Roca	-0.0558	-91.5604	2002.5.20	9	CH	Red	Lava rock	EU333775	EU333758	EU333806	*Terrazoanthus onoi*
03–46	Isabela: Punta Vicente Roca	-0.0558	-91.5604	2003.1.16	2	CH	Red	Lava rock	EU333777	NA	NA	*Terrazoanthus onoi*
03–135	Pinzon:Roca Onan	-0.5909	-90.6860	2003.1.20	NA	LV	Red	Rock	EU333780	EU333762	EU333809	*Terrazoanthus onoi*
03–566	Marchena: Punta Espejo	0.3113	-90.3984	2003.11.12	9	CH	Red	Rock and shells	EU333784	EU333767	EU333808	*Terrazoanthus onoi*
03–641	Isabela: Punta Vicente Roca	-0.0558	-91.5604	2003.11.15	NA	CH	Red	Lava rock	EU333785	EU333759	EU333805	*Terrazoanthus onoi*
04–140	Floreana: La Botella	-1.2904	-90.4989	2004.2.8	8	AC	Red	Dead giant barnacle	GU357558	NA	NA	*Terrazoanthus onoi*
04–343	Isabela: Caleta Iguana	-0.9945	-91.4454	2004.12.3	NA	GE	Red	Dead antipatharian	EU333791	EU333768	NA	*Terrazoanthus onoi*
04–345	Isabela: Caleta Iguana	-0.9945	-91.4454	2004.12.3	8	CH	Red	Rock	EU333792	EU333766	NA	*Terrazoanthus onoi*
04–346	Isabela: Elizabeth Bay	-0.5996	-91.09059	2004.12.2	25	GE	Red	Dead antipatharian	EU333793	EU333763	EU333804	*Terrazoanthus onoi*
04–347	Isabela: Elizabeth Bay	-0.5996	-91.09059	2004.12.2	2	CH	Red	Rock	EU333794	EU333764	EU333807	*Terrazoanthus onoi*
04–641	Isabela: Punta Vicente Roca	-0.0558	-91.5604	2004	NA	NA	Red	Rock	GU357557	NA	NA	*Terrazoanthus onoi*
CMNH ZG05885	Darwin: Glynn's Reef	1.6731	-91.9981	2007.3.8	13	FL, AC	Red	Rock	NA	NA	NA	*Terrazoanthus onoi*

Collection no.[a]	Collection locality	Latitude[b]	Longitude[b]	Date collected	Depth (m)	Collected by[c]	Oral disk color	Substrate	COI Accession Number	mt 16S rDNA Accession Number	ITS-rDNA Accession Number	Taxonomic identification
USNM 1134066	San Cristobel: Whale Rock	-0.8868	-89.6308	2007.3.12	21	JDR	Red	Rock	NA	NA	NA	*Terrazoanthus onoi*
MHNG 67496	Espanola: Anchorage	-1.3646	-89.6353	2007.3.12	Inter-tidal	AC	Red	Rock	NA	NA	NA	*Terrazoanthus onoi*
467	Floreana: Gardner	-1.3329	-90.2953	2007.3.13	14	JDR, CH	Red	Rock	NA	NA	NA	*Terrazoanthus onoi*
469	Floreana: Devil's Crown	-1.2144	-90.4238	2007.3.13	12	JDR, MV	Red	Rock	NA	NA	NA	*Terrazoanthus onoi*
473	Floreana: La Botella	-1.2904	-90.4989	2007.3.13	12–15	AC	Red	NA	NA	NA	NA	*Terrazoanthus onoi*
475	Pinzon: Roca Onan	-0.5909	-90.6860	2007.3.14	8	AC	Red	Rock	NA	NA	NA	*Terrazoanthus onoi*
02–09	Genovesa: Entrance	0.3019	-89.9440	2002.5.13	9	CH	White	Empty mollusk shell facing downwards	EU333773	EU333765	NA	*Terrazoanthus sinnigeri*
03–560	Marchena: Punta Espejo	0.3113	-90.3984	2003.11.12	7	CH	White	Under rock	NA	NA	NA	*Terrazoanthus sinnigeri*
418	Marchena: Punta Espejo	0.3113	-90.3984	2007.3.3	7	JDR, CH, FL	Brown/ clear	Under rock	GU357567	NA	GU357553	*Terrazoanthus sinnigeri*
MHNG 67498	Marchena: Roca Espejo	0.3125	-90.4012	2007.3.3	9	JDR, Fl, BR	White	Under rock	GU357564	NA	GU357551	*Terrazoanthus sinnigeri*
CMNH ZG05886	Darwin: Glynn's Reef	1.6731	-91.9981	2007.3.7	13	AC, FL	Brown	Under rock	GU357562	NA	NA	*Terrazoanthus sinnigeri*
434	Darwin: Glynn's Reef	1.6731	-91.9981	2007.3.7	13	AC, FL	White	Under rock	GU357560	NA	NA	*Terrazoanthus sinnigeri*
438	Darwin: Glynn's Reef	1.6731	-91.9981	2007.3.7	10	JDR, CH, FL	Brown/ clear	Under rock	NA	NA	NA	*Terrazoanthus sinnigeri*
USNM 1134067	Darwin: Glynn's Reef	1.6731	-91.9981	2007.3.7	10	JDR, CH, FL	Brown/ clear	Under rock	GU357565	NA	NA	*Terrazoanthus sinnigeri*

Collection no.[a]	Collection locality	Latitude[b]	Longitude[b]	Date collected	Depth (m)	Collected by[c]	Oral disk color	Substrate	COI Accession Number	mt 16S rDNA Accession Number	ITS-rDNA Accession Number	Taxonomic identification
442	Bainbridge Rocks: Don Ferdi	-0.2100	-90.567	2007.3.9	25	AC	Brown	Rock	GU357561	NA	GU357555	*Terrazoanthus sinnigeri*
445	North Seymour	-0.4118	-90.2871	2007.3.10	15	MV	Brown	Rock, bottom of ledge	NA	NA	GU357556	*Terrazoanthus sinnigeri*
464	Floreana: Gardner	-1.3329	-90.2953	2007.3.13	27	JDR, AC	Brown	Under rock	GU357563	NA	GU357554	*Terrazoanthus sinnigeri*
471	Floreana: Devil's Crown	-1.2144	-90.4238	2007.3.13	7	JDR, AC	Brown	Under rock	GU357566	NA	GU357552	*Terrazoanthus sinnigeri*

NA = not available or data not acquired.

[a]Specimens with the designations such as 03-560 are from 2001-2004 surveys (see Reimer et al. 2008b). Other specimens are from 2007 and have either specimen numbers (e.g. 471) in JDR's collection, or museum type specimen numbers as given. Abbreviations: USNM: National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA, CMNH: Chiba Prefectural Natural History Museum, Japan, MHNG: Natural History Museum of Geneva, Switzerland, MISE: Molecular Invertebrate Systematics and Ecology Laboratory, University of the Ryukyus, Nishihara, Okinawa, Japan.

[b]Latitude and longitude values that are negative represent South and West values respectively, while positive values (latitude only) represent North values.

[c]Collector abbreviations: CH = C. Hickman, Jr., LV = L. Vinueza, AC = A. Chiriboga, GE = G. Edgar, JDR = JD Reimer, RP = R. Pepolas, FL = F. Liss, BR = B. Riegl, DR = D. Ruiz, FR = F. Riveiria, OB = O. Breedy, MV = M. Vera.

Differential diagnosis. Differs from *Antipathozoanthus macaronesicus* (Ocaña & Brito, 2004) (with regards to distribution; Galapagos as opposed to Cape Verde), coloration (no red or cream colors observed in *A. macaronesicus*), substrate (*Antipathes galapagensis* as opposed to *Tanacetipathes cavernicola* Opresko, 2001).

Other morphologically similar and undescribed zoanthids (epizoic on antipatharians, similar sizes, yellowish in color) have been recorded from Madagascar and Japan (specimens in JDR's collection), although these other specimens were found on different antipatharian species than *Antipathozoanthus hickmani*, and were never red or cream in color.

Antipathozoanthus hickmani is the only zoanthid in the Galápagos found on living *Antipathes galapagensis* (Table 3).

Habitat and distribution. All collected samples from Galapagos were on the black coral *Antipathes galapagensis,* at depths of 12 m to 35 m. Although *A. galapagensis* is found throughout the archipelago, *Antipathozoanthus hickmani* colonies were observed only at Santiago, Floreana, Isabela and Pinzon Islands, and it may be that this genus has a patchy distribution in the Galápagos. *A. hickmani* is potentially also found at Isla del Coco (Costa Rica) on the same antipatharian species, based on Museo de Zoologia, University of Costa Rica specimen UCR 827, although this has yet to be confirmed with detailed examinations.

Biology and associated species. *Antipathozoanthus hickmani* may cover only a portion of a living *Antipathes galapagensis* black coral colony, or cover the entire colony, suggesting this species may be parasitic. Some *A. hickmani* specimens were found on completely dead *A. galapagensis* colonies or branches.

Notes. Previously mentioned in Reimer et al. (2008b, 2010) and Hickman (2008) as *Parazoanthus* sp. G1.

Genus *Parazoanthus* Haddon & Shackleton, 1891

Type species: *Parazoanthus axinellae* Schmidt, 1862

Diagnosis. Colonial zoanthids characterised by a mesogleal lacuna and by canals forming a 'ring sinus' in distal part of polyp. Fine mineral particles incorporated in polyps.

Parazoanthus darwini sp. n.

urn:lsid:zoobank.org:act:9CE65167-B6F8-4CF7-BCF2-BCAF277F9AAC

Figures 2, 5, 9, Tables 1, 2, 3

Etymology. Named after Charles Darwin, whose 200th birthday was celebrated in 2009. Noun in the genitive case.

Material examined. *Type locality*: Ecuador, Galapagos: Marchena I., Roca Espejo, 0.3125°N 90.4012°W.

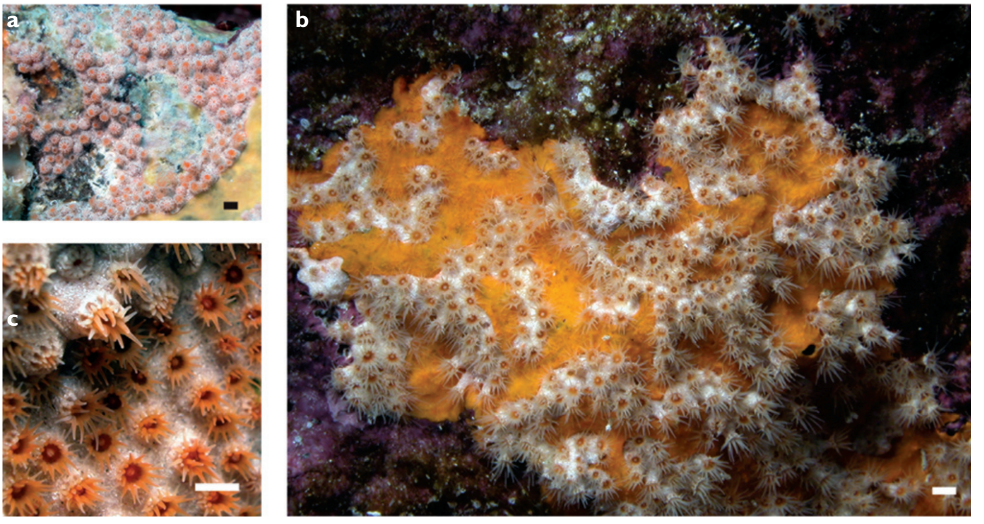


Figure 2. *Parazoanthus darwini* sp. n. in situ in the Galapagos. **a** specimen MISE 451 at Gordon Rocks, depth 21 m, by JDR, March 11, 2007 **b** specimen 456 on orange sponge at Whale Rock, San Cristobel I., depth 17 m, by JDR, March 12, 2007 **c** close up of specimen 03-290, Cape Douglas, Fernandina Island, 6 m, by Angel Chiriboga, August 8, 2003. All scale bars: 1 cm.

Holotype. MHNG-INVE-67497. Colony divided into three pieces, on rocks of approximately 1.5 × 2.0 cm, with heights of approximately 1.0 cm. Total of approximately 90 polyps conn0ected by very well-developed coenenchyme on top of a white sponge. Polyps approximately 1.0–3.0 mm in diameter, and completely embedded in coenenchyme to 2.0 mm in height from coenenchyme. Polyps and coenenchyme encrusted with black sand, which appear as specks, tissue of polyps and coenenchyme light brown in color. Collected from Roca Espejo, Marchena I., Galapagos, Ecuador, at 8 m, collected by JDR and C. Hickman, Jr. (CH), March 3, 2007. Preserved in 99.5% ethanol.

Paratypes (all from Galapagos, Ecuador):

Paratype 1. Specimen number CMNH-ZG 05884. Roca Espejo, Marchena I., at 8 m, collected by JDR and CH, March 3, 2007.

Paratype 2. Specimen number USNM 1134065. Roca Espejo, Marchena I., at 8 m, collected by JDR and CH, March 3, 2007.

Other material (all from Galapagos, Ecuador): MISE03-47, Punta Vincente Roca, Isabela I., at 2 m, collected by CH, January 16, 2003; MISE03-177, Gordon Rocks, at 18 m, collected by CH, January 22, 2003; MISE03-290, Cape Douglas, Fernandina I., at 6 m, collected by AC, August 25, 2003; MISE03-652, Tagus Cove, Isabela I., at 13 m, collected by CH, November 16, 2003; MISE04-155, La Botella, Floreana I., at 12 m, collected by AC, May 25, 2004; MISE04-184, Las Marielas, Isabela I., at 6 m, collected by AC, December 2, 2004; MISE04-328, Las Marielas, Isabela I., at 3 m, collected by AC, December 2, 2004; MISE04-348, Caleta Iguana, Isabela I., at 9 m, collected by CH, December 3, 2004; MISE 412, Roca Espejo,

Table 2. Nematocyst types and sizes of different areas of polyps of new zoanthid species in this study.

Location	Zoanthid species	Nematocyst Type(s)	Length (max-min) (μm)	Width (max-min) (μm)	Frequency within samples[a] (number of specimens found in/total specimens examined)
tentacles	*P. darwini*	Basitrich & Mastigophores	13–23	3–5	Common (2/2)
		Holotrich M	15–22	7–9	Occasional (1/2)
		Spirocysts	8–18	2–3	Numerous (2/2)
	A. hickmani	Basitrich & Mastigophores	14–20	3–4	Occasional (2/2)
		Holotrich M	11–21	6–10	Numerous (2/2)
		Spirocysts	12–22	2–4	Numerous (2/2)
	T. sinnigeri	Basitrich & Mastigophores	7–15	2–3	Common (3/3)
		Holotrich M	17–31	7–14	Rare (2/3)
		Spirocysts	10–17	1–4	Numerous (3/3)
	T. onoi	Basitrich& Mastigophores	8–16	2–4	Numerous (2/2)
		Holotrich L	37–43	14–18	Rare (1/2)
		Holotrich M	17–19	6–7	Rare (1/2)
		Spirocysts	10–18	2–3	Numerous (2/2)
pharynx	*P. darwini*	Basitrich & Mastigophores	13–25	3–6	Numerous (2/2)
		Holotrich M	28	11	Rare (1/2)
		Holotrich S	12	5	Rare (1/2)
		Spirocysts	13–24	2–4	Occasional (2/2)
	A. hickmani	Basitrich & Mastigophores	14–22	3–5	Common (2/2)
		Holotrich M	13–26	5–10	Numerous (2/2)
		Spirocysts	13–23	2–4	Common (2/2)
	T. sinnigeri	Basitrich & Mastigophores	11–17	2–4	Numerous (3/3)
		Holotrich L	23–40	7–15	Occasional (2/3)
		Holotrichs M	10–16	4–8	Rare (1/3)
		Holotrich S	7–14	3–7	Occasional (2/3)
		Spirocysts	10–18	2–4	Occasional (2/3)

Location	Zoanthid species	Nematocyst Type(s)	Length (max-min) (μm)	Width (max-min) (μm)	Frequency within samples[a] (number of specimens found in/total specimens examined)
	T. onoi	Basitrich & Mastigophores	13–17	2–4	Numerous (2/2)
		Holotrichs M	24	7–8	Rare (1/2)
		Spirocysts	10–15	2–3	Occasional (1/2)
filament	*P. darwini*	Basitrich & Mastigophores	8–21	2–5	Numerous (2/2)
		Holotrich M	12–25	5–12	Occasional (2/2)
		Holotrich S	5–7	2–4	Occasional (1/2)
		Spirocysts	7–18	1–3	Occasional (2/2)
	A. hickmani	Basitrich & Mastigophores	14–18	3–5	Occasional (2/2)
		Holotrich L	24–32	9–12	Numerous (2/2)
		Holotrich M	12–22	5–10	Numerous (2/2)
		Spirocysts	15–21	3–4	Occasional (1/2)
	T. sinnigeri	Holotrich L	27–45	11–19	Common (2/3)
		Holotrich M	9–20	6–10	Common (3/3)
		Holotrich S	8–13	3–6	Numerous (1/3)
		Mastigophores	8–20	4–6	Numerous (2/3)
	T. onoi	Spirocysts	9–24	2–4	Rare (2/2)
		Holotrich L	32–43	12–16	Numerous (1/2)
		Basitrich & Mastigophores	9–17	2–5	Numerous (2/2)
column	*P. darwini*	Holotrich M	10–27	5–26	Numerous (2/2)
		Holotrich S	4–10	2–5	Occasional (2/2)
	A. hickmani	Basitrich & Mastigophores	13–17	4–5	Occasional (1/2)
		Holotrich M	10–18	5–10	Common (2/2)
	T. sinnigeri	Holotrich L	14–48	5–16	Occasional (3/3)
		Holotrich M	13–18	6–9	Common (2/3)
		Holotrich S	6–12	3–7	Numerous (1/3)
	T. onoi	Holotrich L	36–45	14–19	Occasional (2/2)
		Holotrich M	11–24	6–20	Common (2/2)

[a]Frequency, in decreasing order; Numerous, Common, Occasional, Rare.

Marchena I., 6 m, collected by JDR and CH, March 3, 2007; MISE413, Roca Espejo, Marchena I., 7 m, collected by JDR and CH, March 3, 2007; MISE417, Roca Espejo, Marchena I., no depth given, collected by CH, March 3, 2007; MISE 424, Roca Espejo, Marchena I., 9 m, collected by JDR, F. Liss (FL), B. Riegl (BR), March 3, 2007; MISE425 Roca Espejo, Marchena I., 9 m, JDR, FL, BR, March 3, 2007; MISE428 El Elefante, Wolf I., 24 m, collected by D. Ruiz (DR) and F. Riveira (FR), March 5, 2007; MISE429 El Elefante, Wolf I., 21 m, collected by DR and FR, March 5, 2007; MISE430 El Elefante, Wolf I., 22 m, collected by JDR, O. Breedy (OB), BR (FR), March 5, 2007; MISE431 El Elefante, Wolf I., 22 m, collected by JDR, OB, BR, March 5, 2007; MISE450 Crater at Gordon's Rock, 17 m, collected by JDR, March 11, 2007; MISE451 Crater at Gordon's Rock, 21 m, collected by JDR, March 11, 2007; MISE452 Crater at Gordon's Rock, 17 m, collected by AC, March 11, 2007; MISE456 Whale Rock, San Cristobel I., 17 m, collected by JDR, March 12, 2007; MISE458 Kicker Rock, San Cristobel I., 15 m, collected by JDR, March 12, 2007; MISE461 Baho Garden, Espanola I., 10 m, collected by M. Vera (MV), March 12, 2007; MISE466 Gardner, Floreana I., 15 m, collected by JDR and AC, March 13, 2007; MISE468 Gardner, Floreana I., 14 m, collected by JDR and CH, March 13, 2007; MISE470 Devil's Crown, Floreana I., 9 m, collected by JDR and MV, March 13, 2007.

Sequences. See Table 1.

Description. *Size*: Polyps are approximately 3–6 mm in diameter when open, and approximately 2–6 mm in height. Colonies may be very small (a few cm^2 in area), or extend to cover large areas over a square meter in area.

Morphology: *Parazoanthus darwini* polyps have yellow, orange, or cream tentacles, and a red, yellow, or light yellow oral disk, with a light tan, light pink, or cream coenenchyme. Polyps have between 24 and 30 tentacles that are usually longer than expanded oral disk diameter. Although polyps extend clear of the coenenchyme, when contracted the polyps are mere bumps on the surface of the coenenchyme.

Cnidae: Basitrichs and microbasic p-mastigophores (often difficult to distinguish), holotrichs (medium and small), spirocysts (see Table 2, Figure 9).

Differential diagnosis. In *P. darwini,* the polyps are much more embedded in the coenenchyme than those of both *A. hickmani* and Hydrozoanthidae species of the Galápagos (Table 3). Whereas many *Parazoanthus* species from other areas of the world are epizoic on sponges, none are morphologically similar (color, size, polyp shape; see below) to *P. darwini*. *Parazoanthus axinellae* and *P. catenularis* Duchassaing de Fonbressin & Michelotti 1860 from the Caribbean are found on clearly different host sponges from *P. darwini*. *Parazoanthus swiftii* Duchassaing de Fonbressin & Michelotti, 1860 and *P. parasiticus* Duchassaing de Fonbressin & Michelotti, 1860 are somewhat similar to *Parazoanthus darwini* in both polyp and coenenchyme color, but have fewer tentacles (maximum to 28; *sensu* Swain 2009) than *P. darwini*, and clearly different mitochondrial 16S ribosomal DNA (Reimer et al. 2008b).

Table 3. Differences between four new species of zoanthids from the Galapagos.

Species	Polyp length (mm)	Oral disk diameter (mm)	Number of tentacles	Color	Substrate, microhabitat	Other distinguishing features
Antipathozoanthus hickmani	6–15	6–12	Approx. 40	Cream, yellow, or red	*Antipathes galapagensis*	
Parazoanthus darwini	2–6	3–6	24–30	Cream, yellow, light pink, or light red	(Usually) sponges	Polyps embedded in well-developed coenenchyme
Terrazoanthus onoi	Up to 20	4–12	32–40	Bright red	Upper surfaces of rock, non-living substrate	Only basitrichs and mastigophores in pharynx; unique ITS-rDNA from *T. sinnigeri*.
Terrazoanthus sinnigeri	<10	2–8	30–36	Brown, white, or transparent	Undersides of rocks or other non-living substrate	Many types of nematocysts in pharynx; unique ITS-rDNA from *T. onoi*

Habitat and distribution. Similar to *Terrazoanthus onoi* sp. n. (below), specimens of *Parazoanthus darwini* are found on rock walls, in crevices, or at the base of rocks, and were found from depths of 2 m to ~30 m, and may extend deeper. Colonies of *P. darwini* were seen at Wolf, Marchena, Isabela, Fernandina, Santa Cruz, San Cristobal, Española and Floreana Islands, and its range is likely throughout the archipelago.

Biology and associated species. Collected *Parazoanthus darwini* specimens from Galapagos are often (but not always) associated with different species of bright yellow-orange or red sponges, possibly in the groups Poecilosclerida and/or Hadromerida (T. Swain, personal communication). *P. darwini* colonies often grow in patches over the sponge, or may even cover it entirely, and often extend to surrounding rock substrate. Despite being covered by *P. darwini*, the sponge is always alive, suggesting this association may be symbiotic.

Notes. Despite COI and mt 16S rDNA sequences of this species being identical to sequences from *Parazoanthus swiftii* from the Caribbean (Figures 5a, 5b), slightly longer mt 16S rDNA sequences (Figure 2 in Reimer et al. 2007) were not identical. Additionally, due to the morphology of *P. swiftii* (rarely not on sponges, relatively shorter tentacles, large [6 mm] diameter polyps that often extend well out from coenenchyme) and large geographic distances between *P. swiftii* and *P. darwini* localities, it is clear that these are two different species.

Previously mentioned in Reimer et al. (2008b) and Hickman (2008) as *Parazoanthus* sp. G2.

Family Hydrozoanthidae Sinniger, Reimer & Pawlowski, 2009

Diagnosis (from Sinniger et al. 2009):

Tropical and sub-tropical macrocnemic zoanthids associated with hydrozoans or associated with non-living substrate. Includes former Parazoanthidae species sharing specific insertions and deletions in mt 16S rDNA, especially in the V5 region (as defined in Sinniger et al. 2005) of this gene. Phylogenetically species are more closely related to brachycnemic zoanthids (especially from the genus *Palythoa* Lamouroux 1816) than to other parazoanthids.

Genus *Terrazoanthus* gen. n.

urn:lsid:zoobank.org:act:BFED371E-2284-4B8A-82F1-0D53A5AE0B5F

Type species: (present designation): *Terrazoanthus onoi* sp. n.

Diagnosis. Sub-tropical to tropical Hydrozoanthidae that are found on rocky substrates, (e.g., not obligate symbionts with hydrozoans). Some species in this genus are brightly colored.

Etymology. Named for the latin "terra" meaning "rock", the substrate on which species of this genus are commonly found on, with ending in concordance to other zoanthid genera. Gender neuter, as with other zoanthid genera ending in "-zoanthus".

Terrazoanthus onoi sp. n.

urn:lsid:zoobank.org:act:429212C7-BC17-4ECC-BC66-85465AFE7C83
Figures 3, 5, 6, 8, 9, Tables 1, 2, 3

Etymology. This species is named in honor of Dr. Shusuke Ono, who introduced the first author to zoanthids and has played a major role in zoanthid research in Japan. Noun in the genitive case.

Material examined. *Type locality*: Ecuador, Galapagos: Espanola I., Anchorage, 1.3646°S 90.2953°W.

Holotype: MHNG-INVE-67496. Colony on rock, approximately 3.0 × 6.0 cm. Total of approximately 130 polyps connected by well-developed coenenchyme. Polyps approximately 1.0–3.0 mm in diameter, and approximately 0.5–2.0 mm in height from coenenchyme. Polyps and coenenchyme encrusted with sand, tissue of polyps and coenenchyme dark brown in color. Collected from Anchorage, Espanola I., Galapagos, Ecuador, at low tide line, collected by AC, March 12, 2007. Preserved in 99.5% ethanol.

Paratypes (all from Galapagos, Ecuador):

Paratype 1. Specimen number CMNH-ZG 05885. Glynn's Reef, Darwin I., at 13 m, collected by FL and AC, March 8, 2007.

Paratype 2. Specimen number USNM 1134066. Whale Rock, San Cristobel I., at 21 m, collected by JDR, March 12, 2007.

Other material (all from Galapagos, Ecuador): MISE 02-59, Punta Vincente Roca, Isabela I., at 9 m, collected by CH, May 20, 2002; MISE 03-46, Punta Vincente Roca, Isabela I., at 2 m, collected by CH, January 16, 2003; MISE 03-135, Roca Onan, Pinzon I., depth not available, collected by L. Vinueza (LV), January 20, 2003; MISE 03-566, Punta Espejo, Marchena I., at 9 m, collected by CH, November 12, 2003; MISE 03-641,

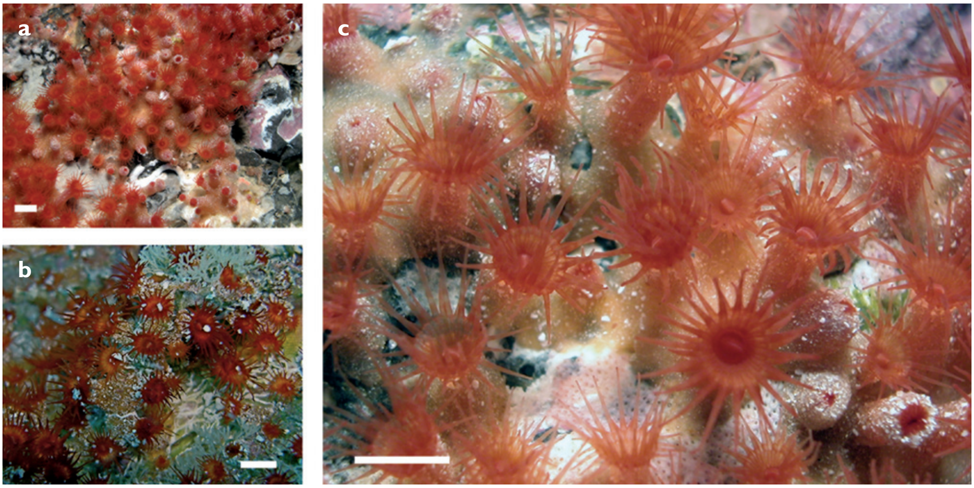


Figure 3. *Terrazoanthus onoi* sp. n. in situ in the Galapagos. **a** and **c** paratype USNM 1134066, at Whale Rock, San Cristobel I., depth 21 m, by JDR, March 12, 2007 **b** paratype CMNH-ZG 05885, Glynn's Reef, Darwin I., depth 13 m, by Fred Liss, March 8, 2007. All scale bars: 1 cm.

Punta Vincente Roca, Isabela I., depth not available, collected by CH, November 15, 2003; MISE 04-140, La Botella, Floreana I., at 8 m, collected by AC, February 8, 2004; MISE 04-343, Caleta Iguana, Isabela I., depth not available, collected by GE, December 3, 2004; MISE 04-345, Caleta Iguana, Isabela I., at 8 m, collected by CH, December 3, 2004; MISE 04-346, Elizabeth Bay, Isabela I., at 25 m, collected by GE, December 2, 2004; MISE 04-347, Elizabeth Bay, Isabela I., at 13 m, collected by CH, December 2, 2004; MISE 467, Gardner, Floreana I., 14 m, collected by JDR and CH, March 13, 2007; MISE 469, Devil's Crown, Floreana I., 12 m, collected by JDR and MV, March 13, 2007; MISE 473, La Botella, Floreana I., at 12–15 m, collected by AC, March 13, 2007; MISE 475, Roca Onan, Pinzon I., 8 m, collected by AC, March 14, 2007

Sequences. See Table 1.

Description. *Size*:

Polyps are approximately 4–12 mm in diameter when open, and rarely more than 20 mm in height. Colonies may reach sizes of over a meter in diameter.

Morphology: *Terrazoanthus onoi* has bright red or red-brown oral disks and the outer surface of polyps is tan to dark brown, with polyps relatively clear of the coenenchyme. *T. onoi* has 32 to 40 tentacles that are almost as long as the diameter of the expanded oral disk (Figure 3).

Cnidae: Basitrichs and microbasic p-mastigophores (often difficult to distinguish), holotrichs (large, medium, and small), spirocysts (see Table 2, Figure 9).

Differential diagnosis. In the Galápagos, *Terrazoanthus onoi* differs from *Parazoanthus darwini* and *Antipathozoanthus hickmani* by substrate preference (rock as opposed to sponges and anthipatharians, respectively), as well as from *Terrazoanthus sinnigeri* sp. n. (below) by both color (bright red as opposed to brown, white or transparent) and habitat ecology (exposed rock surfaces as opposed to under rocks and rubble).

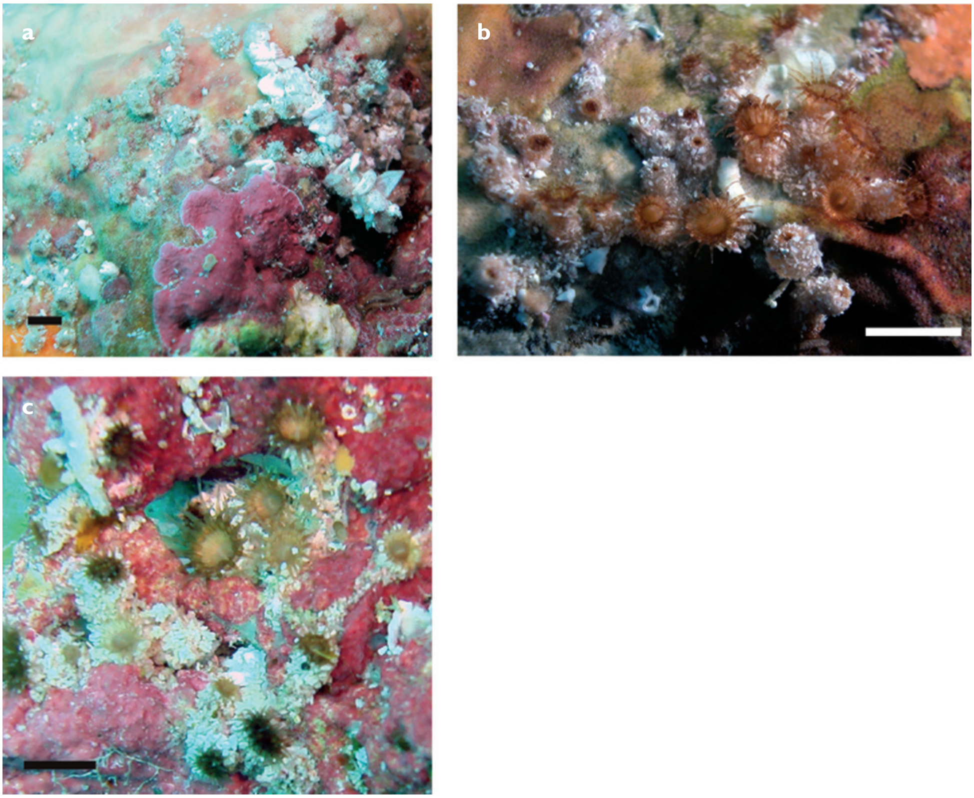


Figure 4. *Terrazonthus sinnigeri* sp. n. in situ in the Galapagos. **a** specimen MISE 442, depth 25 m, Don Ferdi, Bainbridge Rocks, Santiago I., by Angel Chiriboga, on March 9, 2007 **b** specimen MISE 445, Gardner, Floreana I., depth 15 m, by Mariana Vera, on March 10, 2007 **c** specimen MISE 438, Glynn's Reef, Darwin I., depth 10 m, by JDR , March 7, 2007. All scale bars: 1 cm.

In addition, *T. onoi* is bigger (oral disk diameter and polyp height) than *T. sinnigeri*, and forms much larger colonies (Table 3). *T. onoi* commonly has only basitrichs and microbasic p-mastigophores in its pharynx, and no large or small holotrichs at all, unlike *T. sinnigeri* (Table 2).

Phylogenetically, *Terrazoanthus onoi* is very closely related to *T. sinnigeri*, with identical COI and mt 16S rDNA sequences, but consistently differs by four base pairs in ITS-rDNA, and forms a clade separate from *T. sinnigeri*.

An extensive literature search revealed no other described Parazoanthidae species from the Pacific that are non-epizoic and bright red in color. An undescribed zoanthid species inhabiting rock and coral reef substrata from Indonesia often referred to as "yellow polyps" (*sensu* Sinniger et al. 2005) is likely also a *Terrazoanthus* sp., but is distinct from *T. onoi* in terms of color and distribution, and is phylogenetically different.

Habitat and distribution. Specimens of *Terrazoanthus onoi* were found on rock substrate in areas of high current (i.e., the base of large rocks, rock walls, etc.). Colonies were found at Darwin, Marchena, Genovesa, Isabela, Pinzon, Española, and Floreana

Islands, and it is likely *T. onoi* is found throughout the archipelago. This species has been found from the low infra-littoral to depths of over 35 m, and is likely to be at even deeper depths.

Biology and associated species. Found on the top surfaces of rocks and biogenic non-living substrate, *Terrazoanthus onoi* is often found close to sponges, seaweed, and other benthos, but is not epizoic and does not have an association with any particular species.

Notes. Previously mentioned in Reimer et al. (2008b, 2010) and Hickman (2008) as *Parazoanthus* sp. G3, except for specimen MISE 02-27 mentioned below.

It should be noted that specimen MISE 02-27 was found to have an ITS-rDNA sequence inconsistent with other *Terrazoanthus onoi* specimens (Figure 6), although other data (morphology, mt 16S rDNA and COI data) fit well with *T. onoi*. For these reasons, this specimen has not been conclusively assigned to *T. onoi* or to the other new *Terrazoanthus* species below. These results indicate there may be other *Terrazoanthus* species in the Galápagos that await discovery and description.

Terrazoanthus sinnigeri, sp. n.

urn:lsid:zoobank.org:act:2B865570-1FD7-4FB6-BF86-81B5DEDA2289
Figures 4, 5, 6, 9, Tables 1, 2, 3

Etymology. This species is named for Dr. Frederic Sinniger, who has greatly helped spur the recent phylogenetic reexamination of zoanthid taxonomy. Noun in the genitive case.

Material examined. *Type locality*: Ecuador, Galapagos: Marchena I., Roca Espejo, 0.3125°N 90.4012°W.

Holotype: MHNG-INVE-67498. Colony divided into three pieces, on rocks of approximately 2.5 × 2.5 cm, 2.5 × 1.0 cm, and 2.0 × 1.5 cm, with heights of approximately 1.0 cm. Total of approximately 40 polyps connected by stolons. Polyps approximately 1.5–2.0 mm in diameter, and approximately 1.0–2.0 mm in height from coenenchyme. Polyps and coenenchyme encrusted with relatively large pieces of sand clearly visible to the naked eye, tissue of polyps and coenenchyme light brown/grey in color. In situ, colony was on bottom of rock. Collected from Roca Espejo, Marchena I., Galapagos, Ecuador, at 9.1 m, collected by JDR, FL, and BR, March 3, 2007. Preserved in 99.5% ethanol.

Paratypes (all from Galapagos, Ecuador):

Paratype 1. Specimen number CMNH-ZG 05886. Glynn's Reef, Darwin I., at 13 m, collected by FL and AC, March 7, 2007.

Paratype 2. Specimen number USNM 1134067. Glynn's Reef, Darwin I., at 10 m, collected by JDR, FL, CH, March 7, 2007.

Other material (all from Galapagos, Ecuador):

MISE 464, Gardner, Floreana I., 27 m, collected by JDR and AC, March 13, 2007; MISE 471, Devil's Crown, Floreana I., 7 m, collected by JDR and AC, March 13, 2007; MISE 418, Punta Espejo, Marchena I., 7 m, collected by JDR, FL, CH,

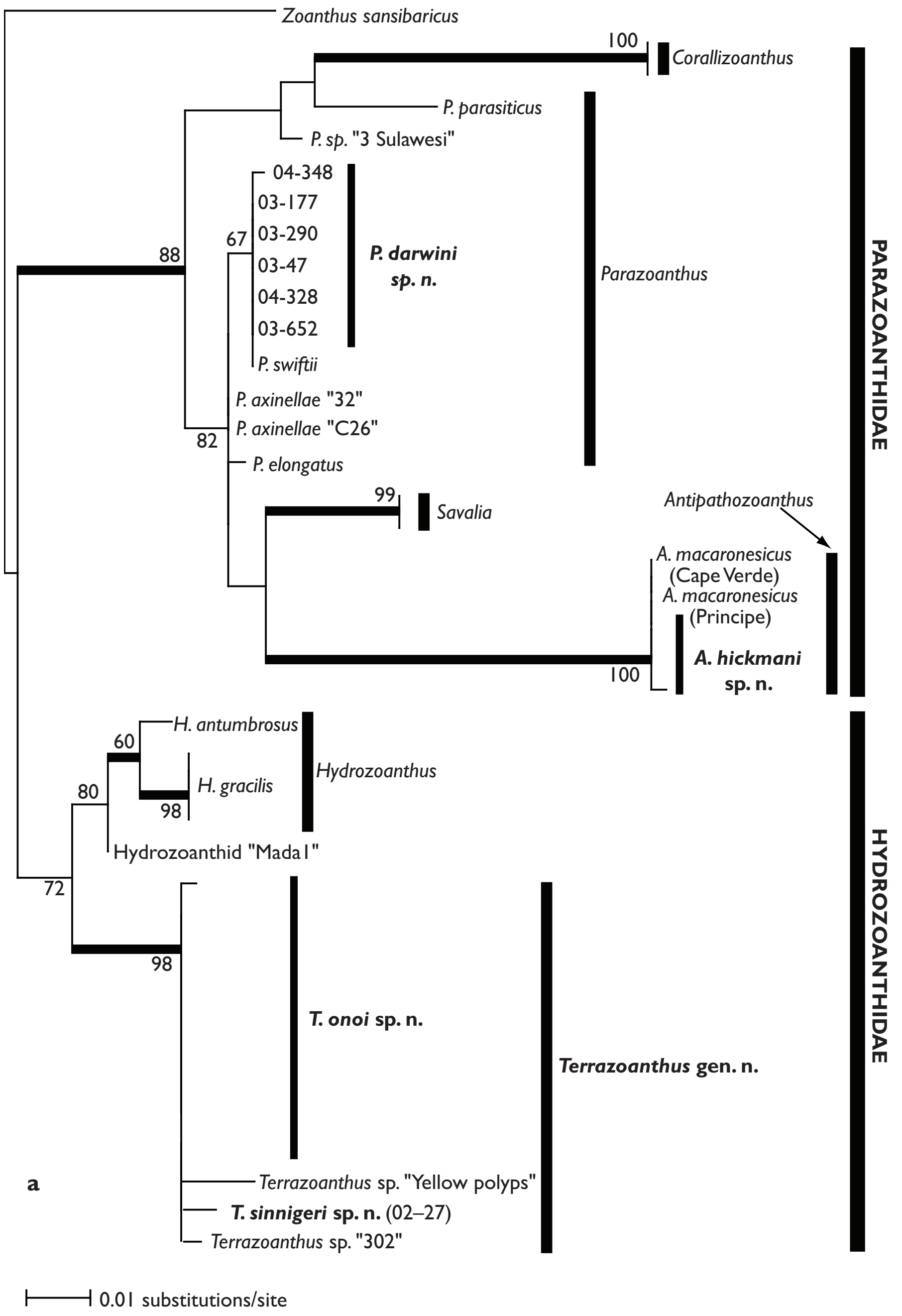
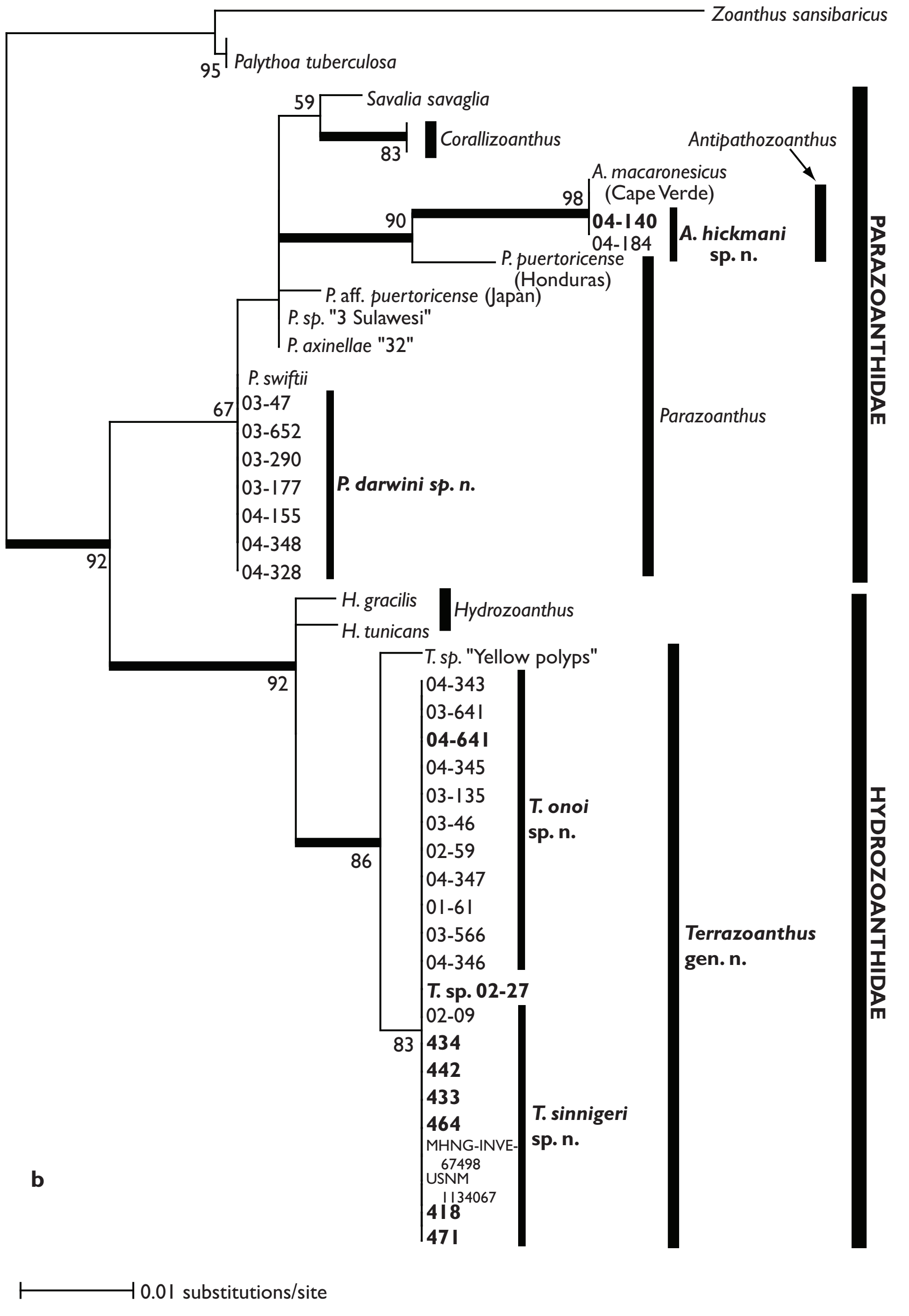


Figure 5. Maximum likelihood (ML) trees of **a** mitochondrial 16S ribosomal DNA, and **b** cytochrome oxidase subunit I (COI) sequences for zoanthid specimens. Values at branches represent ML probabilities (>50%). Monophylies with more than 95% Bayesian posterior probabilities are shown by thick branches. Sequences for new species in this study in larger font; sequences newly obtained in this study and new taxa described in this study in bold. Sequences/species names from previous studies in regular font. For specimen information see Table 1.


Zoanthus sansibaricus
95
Palythoa tuberculosa
59
Savalia savaglia
83
Corallizoanthus
Antipathozoanthus
A. macaronesicus
(Cape Verde)
98
90
04-140
04-184
A. hickmani
sp. n.
P. puertoricense
(Honduras)
P. aff. puertoricense (Japan)
P. sp. "3 Sulawesi"
P. axinellae "32"
P. swiftii
67
03-47
03-652
03-290
03-177
04-155
04-348
04-328
P. darwini sp. n.
Parazoanthus
PARAZOANTHIDAE
92
H. gracilis
H. tunicans
Hydrozoanthus
T. sp. "Yellow polyps"
92
04-343
03-641
04-641
04-345
03-135
03-46
02-59
04-347
01-61
03-566
04-346
T. onoi
sp. n.
86
T. sp. 02-27
02-09
83
434
442
433
464
MHNG-INVE-
67498
USNM
1134067
418
471
T. sinnigeri
sp. n.
Terrazoanthus
gen. n.
HYDROZOANTHIDAE
b
0.01 substitutions/site

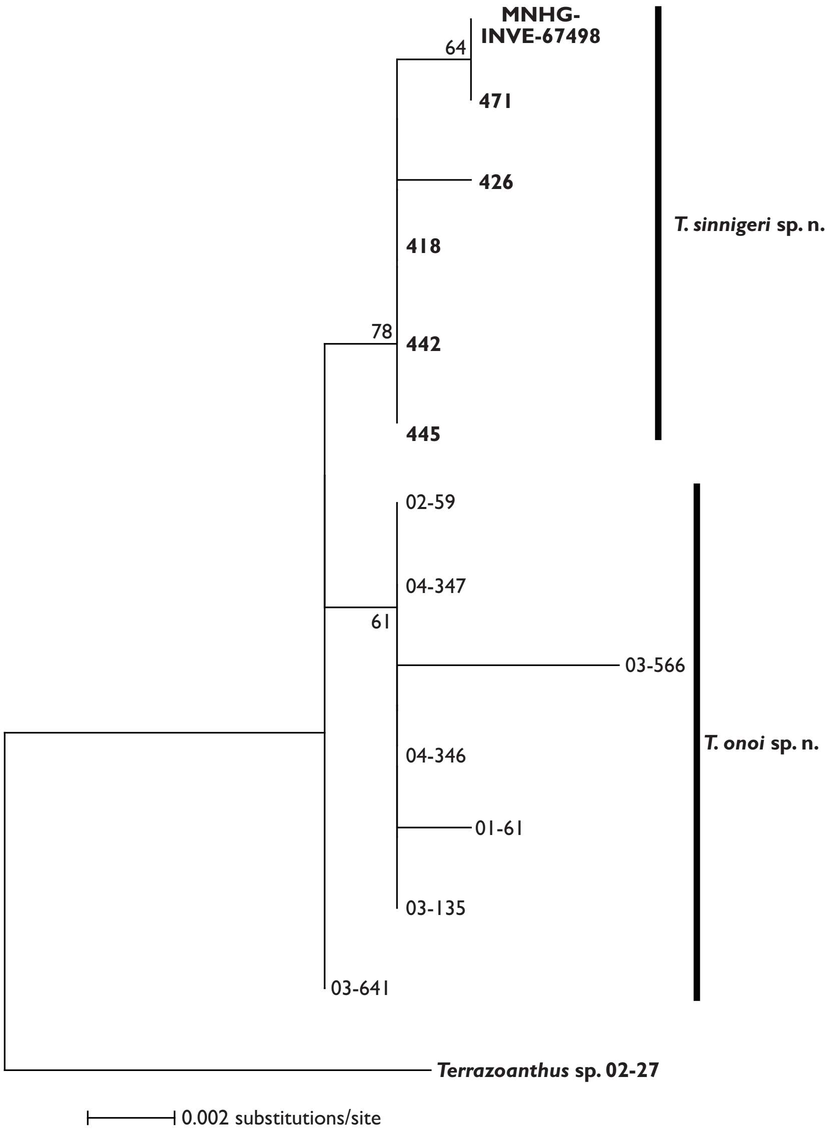


Figure 6. Maximum likelihood (ML) tree of internal transcribed spacer of ribosomal DNA (ITS-rDNA) for *Terrazoanthus* specimen sequences. Values at branches represent ML probabilities (>50%). Monophylies with more than 95% Bayesian posterior probabilities are shown by thick branches. For specimen information see Table 1.

March 3, 2007; MISE 02-09, Entrance, Genovesa I., at 9 m, collected by CH, May 13, 2002; MISE 03-560, Punta Espego, Marchena I., 7 m, collected by CH, November 12, 2003; MISE 434, Glynn's Reef, Darwin I., 13 m, collected by AC and FL, March 7, 2007; MISE 442, Don Ferdi, Bainbridge Rocks, 25 m, collected by AC, March 9, 2007; MISE 445, North Seymour I., 15 m, collected by MV, March 10, 2007.

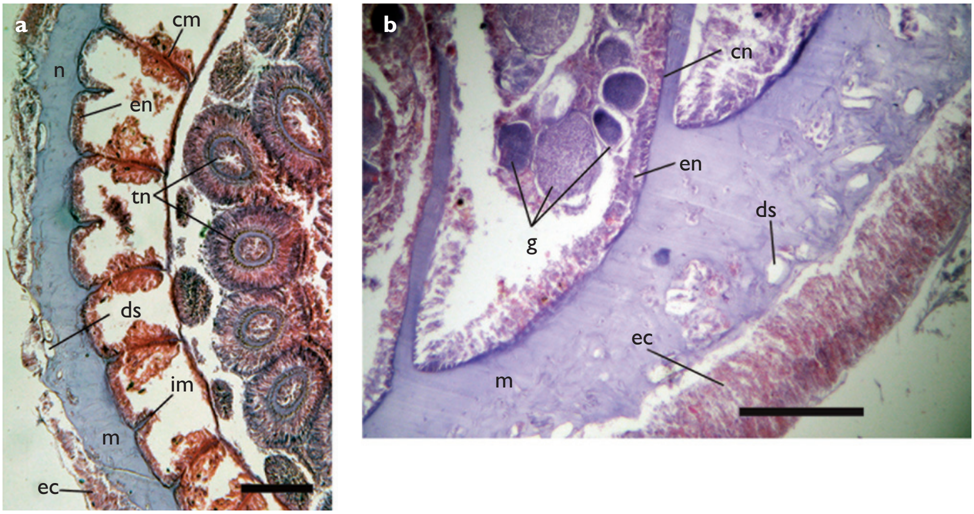


Figure 7. a–b Cross sections of *Antipathozoanthus hickmani* sp. n., MISE specimen 04-345 (details in Table 1) at the actinopharynx region showing preserved histological features. Abbreviations: cm=complete mesentery, **ds**=dissolved sand "holes", **ec**=ectoderm, **en**=endoderm, **g**=gonads, **im**=incomplete mesentery, **m**=mesoglea, **tn**=tentacles. Scales = a) 100 µm; b) 50 µm.

Sequences: See Table 1.

Description. *Size*: Polyps are approximately 2–8 mm in diameter when open, and rarely more than 10 mm in height. Colonies small, consisting of one polyp (unitary) to less than 50 polyps.

Morphology: *Terrazoanthus sinnigeri* has dull brown, white, or clear oral disks and the outer surface of polyps is heavily encrusted with large particles, with polyps clear of the stolon. Stolons are also heavily encrusted, and approximately the width of polyp diameters. *T. sinnigeri* has 30 to 36 tentacles that are almost as long or sometimes longer as the diameter of the expanded oral disk (Figure 4). Tentacles often much more transparent than oral disks (when colored).

Cnidae: Basitrichs and microbasic p-mastigophores (often difficult to distinguish from each other), holotrichs (large, medium), spirocysts (Table 2, Figure 9).

Differential diagnosis. In the Galápagos, *Terrazoanthus sinnigeri* differs from *Parazoanthus darwini* and *Antipathozoanthus hickmani* by substrate preference (rock as opposed to sponges and anthipatharians, respectively), as well as from *Terrazoanthus onoi* sp. n. (above) by both color (brown, white or transparent as opposed to bright red) and microhabitat (under rocks and rubble as opposed to exposed rock surfaces). In addition, *T. sinnigeri* is smaller (oral disk diameter and polyp height) than congener *T. onoi*. *T. sinnigeri* colonies are stoloniferous and generally much smaller than colonies of *T. onoi* (Table 3). *Terrazoanthus sinnigeri* can be further distinguished from *T. onoi* by the presence of many types of nematocysts in the pharynx, unlike *T. onoi*, which only commonly possesses basitrichs and microbasic p-mastigophores with rare medium-sized holotrichs in the pharynx (Table 2). *Terrazoanthus sinnigeri* also has small holot-

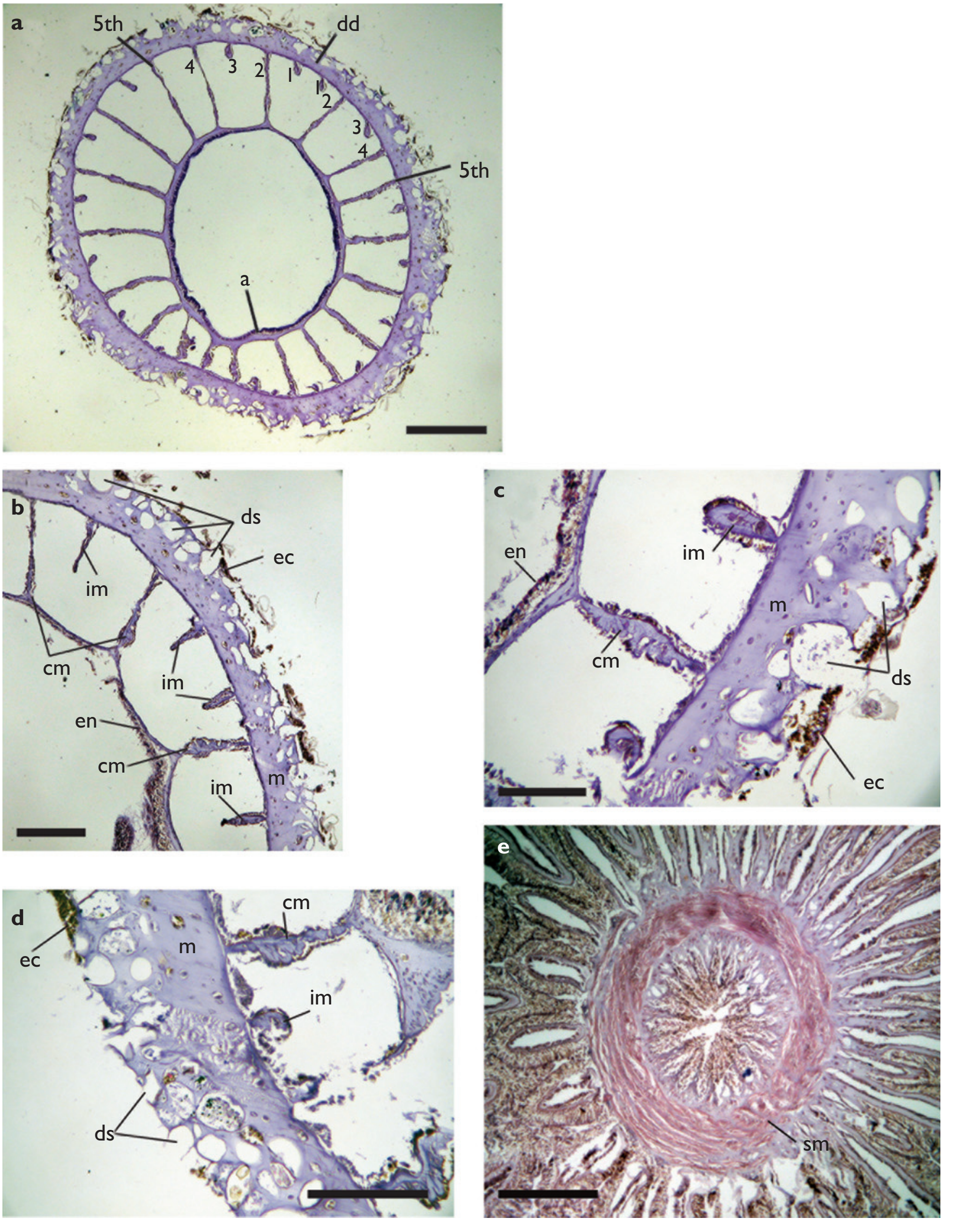


Figure 8. Cross-sections of *Terrazoanthus onoi* sp. n., MISE specimen 03-539 (details in Table 1) **a** Cross-section at the actinopharynx region demonstrating some major features of zoanthids. Note fifth mesentery is complete and therefore specimen is in the suborder Macrocnemina **b** to **e** various cross sections of *T. onoi* at the actinopharynx region showing preserved histological features. Abbreviations: **a**=actinopharynx, **cm**=complete mesentery, **dd**=dorsal directives, **ds**=dissolved sand "holes", **ec**=ectoderm, **en**=endoderm, **im**=incomplete mesentery, **m**=mesoglea, **numbers**=mesentery numbers from the dorsal directive, **sm**=sphincter muscle, **5th**=fifth mesentery. Scales = a) 500 µm, b) and e) 100 µm, c) and d) 50 µm.

richs, while *T. onoi* does not (Table 2). Encrustations on the scapus of *T. sinngeri* are generally much larger than on *T. onoi* (compare Figures 3 and 4).

Terrazoanthus sinnigeri is phylogenetically very closely related to *T. onoi*, but has different and unique ITS-rDNA (see *T. onoi* description; Figure 6).

Similar to *Terrazoanthus sinnigeri,* there have been reports of other small zoanthids inhabiting cryptic habitats under coral rubble and rock from the Galápagos, Singapore and Japan (J.D. Reimer, T. Fujii, personal observation), but these zoanthids are clearly different in DNA sequence from all known Hydrozoanthidae and Parazoanthidae, and will be described elsewhere. Morphologically, these undescribed zoanthids look very similar to *T. sinnigeri*, but are often unitary (not colonial), are encrusted with very large pieces of sand, have very little coloring (usually lacking any color asides from around the oral opening) and have fewer tentacles (<26, usually 20–22; data not shown) than *T. sinnigeri*.

Habitat and distribution. Specimens located at depths of 7 to over 27 meters at Floreana, Marchena, Darwin, North Seymour Islands, and Bainbridge Rocks, with other potential specimens observed at other islands. It is likely that this species is widely distributed throughout the Galápagos, and its distribution may extend into deeper waters as it was often found at the lowest depth searched during collection dives. Generally found on the underside of rocks, rubble, or dead shells, often in small cracks or crevices.

Biology and associated species. Found under rocks and rubble, *Terrazoanthus sinnigeri* is often found nearby bryozoans and coralline algae, but appears to not be epizoic on any particular organism.

Notes. In Reimer et al. (2008b) it was originally thought that *Terrazoanthus sinnigeri* (specimens 02-09, 03-560) was a different, white morphotype of *T. onoi* (mentioned in the paper and Hickman (2008) as *Parazoanthus* sp. G3) based on COI and mt 16S rDNA sequence data, but given the species' divergent morphologies, cnidae, and ecologies, as well as different ITS-rDNA sequences, we describe them as closely related but distinct species. It is likely that these two sibling species have recently diverged from one another.

Although speculative, it may be that *Terrazoanthus sinnigeri*'s preferred habitat under rocks has resulted in its lack of bright pigmentation or occasional total lack of pigments compared to bright red *T. onoi,* which is found in areas more exposed to light, similar as to seen in subterranean invertebrates (e.g. Leys et al. 2003), and this should be investigated in the future.

Phylogenetic results

mt 16S rDNA

The phylogenetic tree from analyses of mt 16S rDNA showed two large clades; one consisting of Parazoanthidae specimens, and another of Hydrozoanthidae specimens

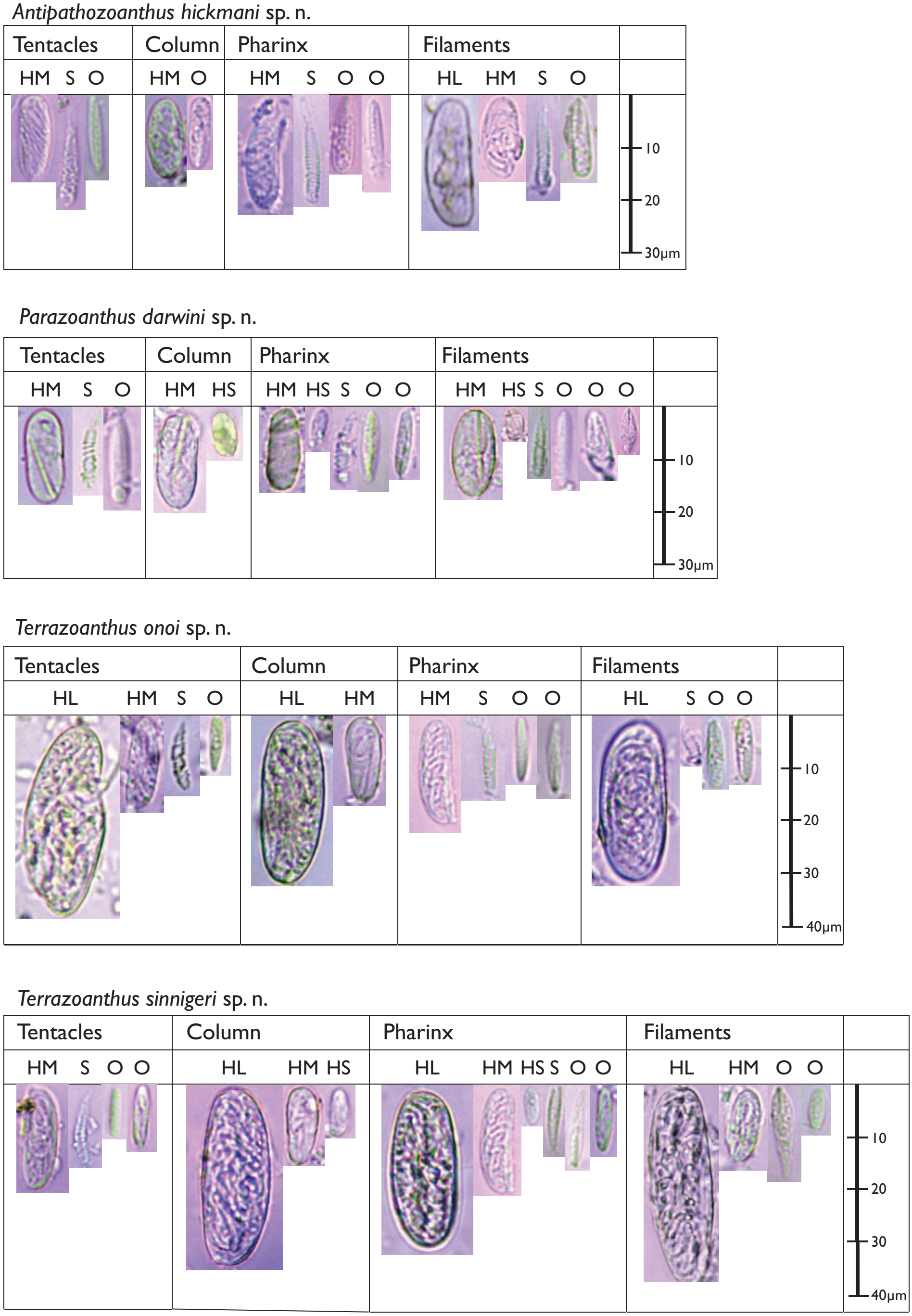


Figure 9. Cnidae in the tentacles, column, pharynx and filament of *Antipathozoanthus hickmani* sp. n., *Parazoanthus darwini* sp. n., *Terrazoanthus onoi* sp. n., and *Terrazoanthus sinnigeri* sp. n., respectively. Abbreviations: **HL**: large holotrich, **HM**: medium holotrich, **HS**: small holotrich, **O**: basitrichs or mastigophores, **S**: spirocysts.

(Figure 5a). Antipatharian-associated specimens previously referred to as *Parazoanthus* sp. G1 (04-140, 03-549, 04-341) formed a very well supported (ML=100%, Bayes=1.00) clade together with *Antipathozoanthus* specimens from Principe and from Cape Verde. This clade was sister to the genus *Savalia* Nardo 1814.

Sponge-associated specimens previously referred to as *Parazoanthus* sp. G2 (03-290, 03-47, 04-328, 03-652, 03-177) formed a moderately supported monophyletic group (ML=70%, Bayes=0.80) that also included *Parazoanthus swiftii*. This clade was derived from *Parazoanthus axinellae* (two specimens from Europe) and *P. elongatus* McMurrich 1904 from Chile.

Specimens previously designated *Parazoanthus* sp. G3 (03-566, 04-346, 02-59, 03-641, 02-27, 02-09, 01-61, 03-135, 04-345, 04-347, as well as 02-09) were within the large Hydrozoanthidae clade, within a subclade separate from *Hydrozoanthus* spp. sequences. The sequences formed a very well supported monophyletic group (ML=98%, Bayes=1.00) that also included "yellow polyps" *sensu* Sinniger et al. (2005) and "*Parazoanthus"* sp. 302, which both had slightly divergent sequences.

COI

COI phylogenetic results are shown in Figure 5b. The phylogenetic tree showed two large clades: one consisting of Parazoanthidae specimens, and another of Hydrozoanthidae specimens. Antipatharian-associated specimens previously informally described as *Parazoanthus* sp. G1 (04-140, 04-184) formed a well-supported (ML=98%, Bayes=1.00) clade together with an *Antipathozoanthus macaronesicus* specimen from Cape Verde. This clade was sister to *Parazoanthus puertoricense* West 1979 from Honduras.

Sponge-associated specimens previously informally described as *Parazoanthus* sp. G2 (03-290, Ang155, 03-47, 04-328, 04-348, 03-652, 03-177) formed a moderately supported monophyletic group (ML=67%, Bayes=0.92) that also included *Parazoanthus swiftii*. This clade was basal to all other Parazoanthidae.

Specimens previously designated *Parazoanthus* sp. G3 (04-343, 03-641, 04-461, 04-345, 03-46, 02-59, 04-347, 01-61, 03-566, 04-346, 02-09, 02-27) as well as new specimens (434, 442, 464, 426, 439, 418, 471) were within the large Hydrozoanthidae clade, within a subclade separate from *Hydrozoanthus* spp. sequences. The sequences formed a moderately well supported monophyletic group (ML=83%, Bayes=<0.50) sister to "yellow polyps" *sensu* Sinniger et al. (2005), which together formed a well-supported monophyletic group (ML=86%, Bayes=1.00).

ITS-rDNA

ITS-rDNA phylogenetic results are shown in Figure 6. The ITS-rDNA sequence from 02-27 is divergent from other sequences. Most sequences from brightly red-colored specimens previously designated *Parazoanthus* sp. G3 (02-59, 04-347, 01-61, 04-346, 03-566, 03-135) formed a poorly supported monophyletic group (ML=64%, Bayes=0.70). Basal to this was one sequence from specimen 03-641. Many specimens from 2007 collection trips (471, 464, 426, 418, 442, 445) that were similar morphologically to earlier specimens 02-09 and 03-560, being brown, white, or clear in color, and found on the undersides of rocks, formed a separate moderately supported monophyletic group (ML=76%, Bayes=0.78) sister to the red-colored specimen sequences. These 2007 sequences had four base pairs unique from the "red clade" sequences.

Discussion

"Parazoanthid" diversity

Traditionally, the higher-level taxonomy of zoanthids has relied on a wide variety of diagnostic characteristics, including mesenterial arrangement (Haddon and Shackleton 1891) and nematocysts (Schmidt 1974). Although suborders are organized based on the position of the sphincter muscle (mesodermal or endodermal), genera have been historically designated based on not only morphology, but also ecology and species associations. Thus, the recent reexamination and reclassification of zoanthid taxa utilizing DNA sequences along with their ecology as diagnostic characters is not without historical precedent. From the results of this study along with data in Sinniger et al. (2009), Sinniger and Häussermann (2009) and Reimer et al. (2008a) the "parazoanthids" (the family Parazoanthidae as it formerly existed) are now divided into two families and eight genera (Parazoanthidae, including *Parazoanthus* Haddon & Shackleton, 1891, *Savalia* Nardo, 1814, *Isozoanthus* Chun, 1903, *Corallizoanthus* Reimer et al., 2008, *Mesozoanthus* Sinniger & Häussermann, 2009, *Antipathozoanthus* Sinniger et al., 2009; and Hydrozoanthidae, including *Hydrozoanthus* Sinniger et al., 2009 and *Terrazoanthus* gen. n.), reflecting the formerly unknown levels of generic and family diversity that are present in this zoanthid group. It is apparent that Parazoanthidae as it formally existed was clearly a "catch-all" for many different zoanthid species, as previously hypothesized (Reimer et al. 2008a).

It is also becoming increasingly apparent that zoanthid diversity is higher than previously thought in "ignored" or understudied regions, ecosystems (Reimer et al. 2007a) or even microhabitats (Reimer et al. 2008b, this study). This is clearly demonstrated by *Terrazoanthus sinnigeri,* which inhabits the underside of rocks and dead coral, a very common yet relatively understudied microhabitat. In subtropical seas, biological studies of zoanthids have focused mainly on coral reef areas, and in contrast studies of boulder/rubble areas have been neglected. This microhabitat has also recently been shown to host other previously undescribed invertebrate species, such as the comatulid *Dorametra sesokonis* Obuchi, Kogo & Fujita, 2009 in southern Japan (Obuchi et al. 2009). Additional specimens collected from the Galápagos also found from the undersides of rocks (specimen 03-103 in Reimer et al. 2008b; specimens 427, 455, 460 in JDR's collection from the 2007 expedition) apparently belong to one or more other undescribed zoanthid taxa, and will be described elsewhere. Until the discovery of such zoanthids (Reimer et al. 2008b), no information on zoanthids found in such a cryptic microenvironment had been reported, and it may be that this and other understudied microhabitats also harbor zoanthid species new to science.

Proposed future zoanthid research in the southeastern Pacific

Despite a few reports, zoanthid diversity over the entire southeastern Pacific remains understudied. This study and recent investigations from Chile (Sinniger and Häussermann 2009) demonstrate that undescribed zoanthid diversity exists in this region. In particular, very little data exist for the Pacific coast of South and Central America, and efforts should be made to promote investigations in this area. With more knowledge of zoanthid diversity and distribution in the southeastern Pacific, more accurate biogeographical discussions of the evolution of parazoanthids and hydrozoanthids will become possible.

Conclusions

As shown by this research and other recent investigations (Sinniger et al. 2009), the levels of higher level (e.g. >genus) diversity of zoanthids are much higher than has previously been thought.

Insular and relatively unexplored marine regions of the world such as the Galapagos likely harbor many undiscovered and undescribed zoanthid species.

Phylogenetic analyses as performed here provide a powerful identification tool that can determine relative levels of relationships between zoanthids that would not be possible with only morphological and ecological data. This is most strongly demonstrated by the very close evolutionary relationship between the two new *Terrazoanthus* species, which are genetically very close and morphologically and ecologically quite distinct.

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