

Zoanths of the Cape Verde Islands and their symbionts: previously unexamined diversity in the Northeastern Atlantic

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Key words: Cape Verde Islands, Cnidaria, *Symbiodinium*, undescribed species, zoanthid

Abstract

The marine invertebrate fauna of the Cape Verde Islands contains many endemic species due to their isolated location in the eastern Atlantic, yet research has not been conducted on most taxa here. One such group are the zoanths or mat anemones, an order of benthic cnidarians (Hexacorallia: Zoantharia) common in many marine environments. In this study, the diversity of zoanths in the Cape Verde Islands is specifically examined for the first time. Field images and sampling are combined with molecular phylogenetic analyses utilizing two mitochondrial DNA markers (cytochrome oxidase subunit I and 16S ribosomal DNA) to determine the number of species present. Specimens from eight species (*Palythoa caribaeorum*, *Palythoa* sp. 265, *Zoanthus* aff. *pulchellus*, *Isaurus tuberculatus*, *Parazoanthus* sp. 269, *Parazoanthus* sp. 1401, *Antipathozoanthus macaronesicus*, *Terrazoanthus* sp. 276) were identified, including two to four species that are likely new to science. Additional examinations of *Symbiodinium* endosymbionts in zooxanthellate species showed that clades A, B, and C were present; this is the first report of clade B associating with a zoanthid species. An appendix describing the eight zoanthid species found in the Cape Verde Islands is included to provide a base for future zoanthid research in this region.

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Introduction

Zoanths are an order of benthic anthozoans found in most marine environments. Despite their presence in most marine ecosystems, zoanths remain a taxonomically neglected group due to a relatively simple body plan, a lack of proven species-level diagnostic morphological characteristics, high levels of intraspecific variation (Burnett *et al.*, 1997; Reimer *et al.*, 2004), and encrustations of sand and detritus in their mesoglea (body walls) making internal examinations very problematic (discussed in Reimer *et al.*, 2010). Thus, with a lack of information, overall levels of species diversity remain unknown (Burnett *et al.*, 1997; Reimer *et al.*, 2004).

However, recent examinations of zoanths from many different locations such as Australia (Burnett *et al.*, 1997), the Galapagos Islands (Reimer *et al.*, 2008c; Reimer and Hickman, 2009), Japan (Reimer, 2007), New Caledonia (Sinniger, 2006), Singapore (Reimer and Todd, 2009), and the Caribbean (Swain, 2009) have shown that a combination of careful in situ observation of substrate and habitat along with the utilization of molecular techniques can accurately ascertain taxonomic identifications and identify

potential undescribed species. Furthermore, examinations from insular and more isolated oceanic regions such as the Galapagos have demonstrated the presence of potentially endemic or rarely known zoanthids that were simply just in need of discovery (Reimer and Fujii, 2010).

The marine fauna and flora of the Cape Verde Islands is much more tropical in character than the West African coast at the same latitude, where cold upwelling occurs (Türkyay, 1982). Morri *et al.* (2000) gave a preliminary description of the marine zoogeography of the Cape Verde Islands. As pointed out by numerous authors (*e.g.* Laborel, 1974; Prud'homme van Reine and Van den Hoek, 1988; Wirtz, 2001 and references therein), the marine fauna and flora of the Cape Verde Islands shows a strong amphi-atlantic component. The molluscs (Rolán, 2005), fish (Reiner, 1996; Brito *et al.*, 1999) and some selected groups of Hexacorallia (Boekschoten and Borel Best, 1988) are some marine groups reasonably well studied. Türkyay (1982) and Wirtz and D'Udekem d'Acoz (2001) summarized the state of knowledge on decapod crustaceans; Núñez *et al.* (2000) gave a preliminary list of the polychaetes. Many other marine invertebrate groups lack comprehensive accounts and unrecorded

and undescribed species are constantly being discovered (*e.g.* Opresko, 2003; Freitas and Castro, 2005; Stokvis and van Ofwegen, 2006; Wirtz, 2009). Due to their isolated position about 600 km off the western tip of Africa, the Cape Verde Islands harbor numerous endemic species (see the species lists mentioned above for examples).

Morri and Bianchi (1995), Morri *et al.* (2000), and Monteiro *et al.* (2008) described epibenthic communities at Sal Island and recorded the depth distribution of *Palythoa caribaeorum* Duchassaing and Michelotti, 1864. There are two historical records of zoanthid species in the Cape Verde Islands, which are mentioned later, in the discussion part of this manuscript. Other recent records of zoanthids from the Cape Verde Islands include 1) xanthid crabs associated with *Palythoa* (Den Hartog and Holthuis, 1984; Den Hartog and Türkyay, 1991), 2) a mention of *Zoanthus* and *Palythoa* in Van der Land (1993), 3) a photo of *Isaurus tuberculatus* Gray, 1828 by the third author, taken near Tarrafal, Santiago Island (Wirtz and Debelius, 2003, page 63, bottom left), and 4) several records discussing the presence of *Antipathozoanthus macaronesicus* Ocaña and Brito, 2003 (Ocaña and Brito, 2003; Ocaña *et al.*, 2007; Sinniger *et al.*, 2010).

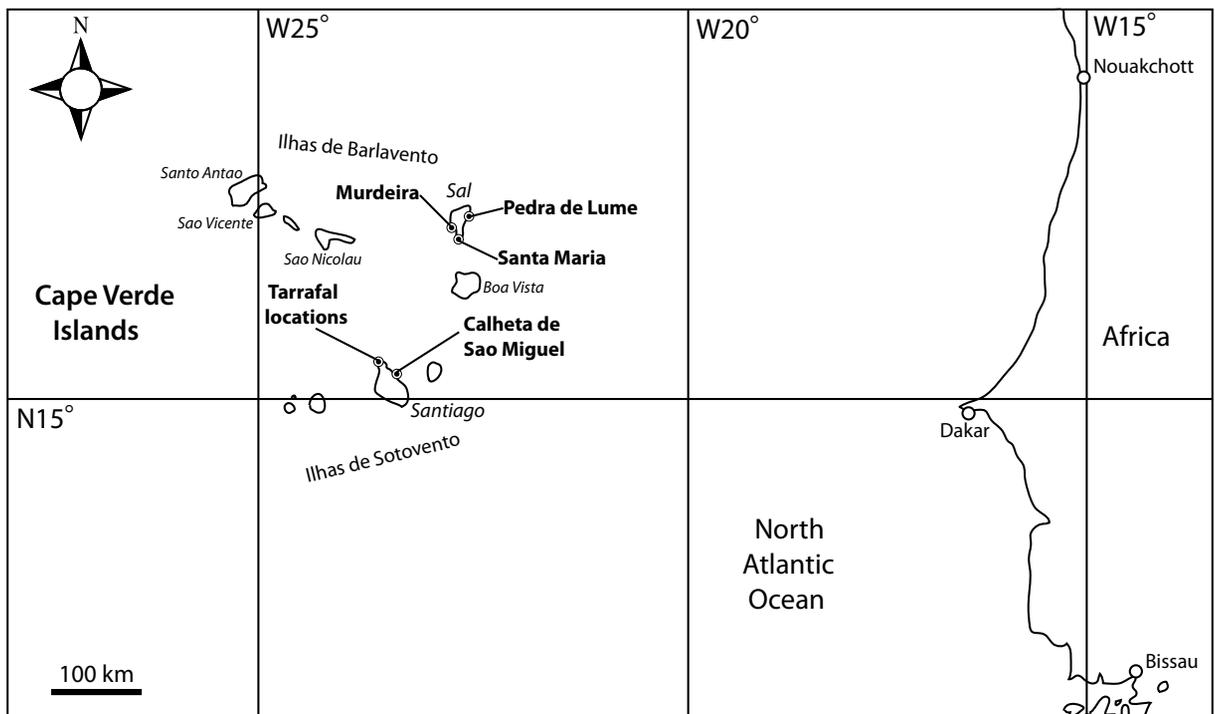


Fig. 1. Map showing location of zoanthid specimen sampling sites in the Cape Verde Islands. Sampling location names in bold, island names in italics.

Table 1. Zoantheid specimens from Cape Verde examined in this study, n/a = not available.

specimen no.	location	collection month	habitat	COI accession no.	16S accession no.	<i>Symbiodinium</i> clade	conclusion (identity)
264	Tarrafal Bay, Santiago I.	Aug. 07	tidal flat	HMI130482	HMI130463	n/a	<i>Palythoa caribaeorum</i>
265	Tarrafal Bay, Santiago I.	Aug. 07	tidepool	HMI130481	HMI130464	clade C	<i>Palythoa</i> sp. 265
266	Tarrafal Bay, Santiago I.	Aug. 07	low tide line	HMI130486	HMI130465	clade A	<i>Zoanthus</i> aff. <i>pulchellus</i>
267	Calheta de Sao Miguel, Santiago I.	Aug. 07	tidepool	HMI130483	HMI130466	clade C	<i>Palythoa caribaeorum</i>
268	Tarrafal Bay, Santiago I.	Aug. 07	<i>Tanacetipathes wirtzi</i> (antipatharian)	HMI130496	HMI130467	n/a	<i>Antipathozoanthus macaronensis</i>
269	Tarrafal Bay, Santiago I.	Aug. 07	inside cave, on sponge	HMI130497	HMI130468	n/a	<i>Parazoanthus</i> sp. 269
270	Tarrafal Bay, Santiago I.	Aug. 07	tidepool	HMI130492	HMI130469	clade A	<i>Zoanthus</i> aff. <i>pulchellus</i>
271	Tarrafal Bay, Santiago I.	Aug. 07	low tide line	HMI130487	n/a	clade A	<i>Zoanthus</i> aff. <i>pulchellus</i>
272	Tarrafal Bay, Santiago I.	Aug. 07	low tide line	HMI130488	n/a	clade A	<i>Zoanthus</i> aff. <i>pulchellus</i>
273	Santa Maria, Sal I.	Aug. 07	20 m	HMI130484	HMI130470	clade A	<i>Palythoa caribaeorum</i>
274	Pedra de Lume, Sal I.	Aug. 07	tidepool	HMI130489	HMI130471	clade A	<i>Zoanthus</i> aff. <i>pulchellus</i>
275	Pedra de Lume, Sal I.	Aug. 07	tidepool	HMI130490	HMI130472	clade A	<i>Zoanthus</i> aff. <i>pulchellus</i>
276	Murdeira, Sal I.	Aug. 07	intertidal, under stone	HMI130495	HMI130473	n/a	<i>Terrazoanthus</i> sp. 276
277	Murdeira, Sal I.	Aug. 07	tidepool	HMI130491	HMI130474	clade B	<i>Zoanthus</i> aff. <i>pulchellus</i>
278	Tarrafal Bay, Santiago I.	Oct. 09	18 m	HMI130485	HMI130475	clade C	<i>Isaurus tuberculatus</i>
1401	Lighthouse, Tarrafal Bay, Santiago I.	Mar. 10	overhanging rock faces, 14 m	HMI130498	HMI130478	n/a	<i>Parazoanthus</i> sp. 1401
1402	Lighthouse, Tarrafal Bay, Santiago I.	Mar. 10	overhanging rock faces, 14 m	HMI130499	HMI130479	n/a	<i>Parazoanthus</i> sp. 1401
1406	Seamount, Tarrafal Bay, Santiago I.	Mar. 10	18 m	n/a	n/a	n/a	<i>Isaurus tuberculatus</i>
1407	Seamount, Tarrafal Bay, Santiago I.	Mar. 10	18 m	n/a	n/a	n/a	<i>Isaurus tuberculatus</i>
1409	Seamount, Tarrafal Bay, Santiago I.	Mar. 10	dark tunnel, 20 m	HMI130500	HMI130480	n/a	<i>Parazoanthus</i> sp. 1401
1414	Tarrafal Bay, Santiago I.	Mar. 10	inside cave, on sponge	n/a	n/a	n/a	<i>Parazoanthus</i> sp. 269
1419	Kingfisher Bay, Santiago I.	Mar. 10	intertidal	n/a	n/a	n/a	<i>Zoanthus</i> aff. <i>pulchellus</i>
1424	Danger, Tarrafal Bay, Santiago I.	Mar. 10	cave wall, 20 m	n/a	n/a	n/a	<i>Parazoanthus</i> sp. 269
1425	Danger, Tarrafal Bay, Santiago I.	Mar. 10	9 m	n/a	n/a	n/a	<i>Palythoa</i> sp. 265
ZsC	Cahuíta, Costa Rica	Oct. 09	1 m	HMI130494	HMI130476	clade A	<i>Zoanthus sociatus</i>
Zs9	Cahuíta, Costa Rica	Oct. 09	1 m	HMI130493	HMI130477	clade A	<i>Zoanthus sociatus</i>



Fig. 2. Zoanths of the Cape Verde Islands examined in this study. a) *Palythoa caribaeorum* (specimen 264) in the intertidal zone at Tarrafal Bay, Santiago Island, b) *Palythoa* sp. 265 (specimen 265) in a tidepool at Tarrafal Bay, Santiago Island, c) *Zoanthus* aff. *pulchellus* (specimen 277) in a tidepool at Murdeira Bay, Sal Island, d) *Isaurus tuberculatus* (specimen not collected) approximately 2 km north of Tarrafal Bay, Santiago Island, e) *Antipathozoanthus macaronensis* (specimen 268) on the antipatharian *Tanacetipathes cavernicola* at Tarrafal Bay, Santiago Island, f) *Parazoanthus* sp. 269 (specimen 269) on a sponge at Tarrafal Bay, Santiago Island, g) *Parazoanthus* sp. 1401 (specimen 1401), at Lighthouse, Tarrafal Bay, Santiago Island, h) *TerraZoanthus* sp. 276 (with closed polyps; specimen 276) at Murdeira, Sal Island. Scale bars (except Fig. 2a) = 1 cm. For specimen information refer to Table 1.

In this study we specifically examine the diversity of zoanths in the Cape Verde Islands for the first time, using both morphologic and molecular techniques, and present a preliminary species list. Additionally, we present data on symbiotic dinoflagellates (*Symbiodinium* spp.) for zooxanthellate *Zoanthus*, *Isaurus* and *Palythoa* species, as the charac-

terization of *Symbiodinium* can allow us to understand the ecology and biogeography of the holobiont (host + symbiont) (Reimer and Todd, 2009). Our results should provide a basis for future zoanthid and *Symbiodinium* research in the islands, and allow future comparisons with data from other Atlantic locations.

Material and methods

Specimen collection and initial identification

Twenty-three zoanthid specimens (in this study specimens = colonies) representing all observed zoanthid morphotypes were collected from the intertidal zone or by snorkeling or SCUBA from several locations in the Cape Verde Islands (several Tarrafal Bay locations and Calheta de Sao Miguel on Santiago Island, and Santa Maria, Pedra de Lume and Murdeira on Sal Island – Fig. 1) in August 2007, September 2008, and March 2010 (Table 1).

Specimens were preliminarily identified using morphological characteristics used in past literature (e.g. sand-encrusted or not), oral disk color, polyp form, etc. (Table 1, Fig. 2) based on *in situ* photographs (taken with a Canon Powershot digital camera in an underwater housing) and *ex situ* physical examination. Specimens were subsequently stored in 96% ethanol at ambient temperature. All samples were finally deposited at the University of the Ryukyus (Nishihara, Okinawa, Japan) in 99.5% ethanol at -20°C .

Specimen nomenclature

Specimens were originally assigned names based on sampling location and order (Table 1).

DNA extraction, PCR Amplification, and sequencing

DNA was extracted from specimen portions (tentacles and column) weighing 5–20 mg using a spin-column Dneasy Animal Extraction protocol (Qiagen, Santa Clarita, CA, USA). PCR amplification using the genomic DNA as a template was performed using Hot-StarTaq DNA polymerase (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. Mitochondrial (mt) 16S rDNA was amplified following procedures outlined in Sinniger *et al.* (2005). COI was amplified using zoanthid-specific primer COIZoanF (Reimer *et al.*, 2007a) and general COI primer HCO2198 (Folmer *et al.*, 1994). PCR amplification was performed on the samples under the following conditions: an initial denaturing step at 95°C for 15 minute, followed by 35 cycles of 1 minute denature at 94°C , 1 minute annealing at 40°C , and 90 s extension at 72°C , followed by 7 minutes' extension at 72°C . The ITS-rDNA region of *Symbiodinium* was amplified following procedures outlined in Reimer *et al.* (2006c). The amplified products were visualized by 1.0% agar-

ose gel electrophoresis. PCR products were treated with Exonuclease I and Alkaline Phosphatase (Shrimp) (Takara) prior to sequencing reactions using DTCS Quick Start Master Mix (Beckman Coulter). The products were analyzed using a CEQ8800 (Beckman Coulter) automated DNA sequencing system.

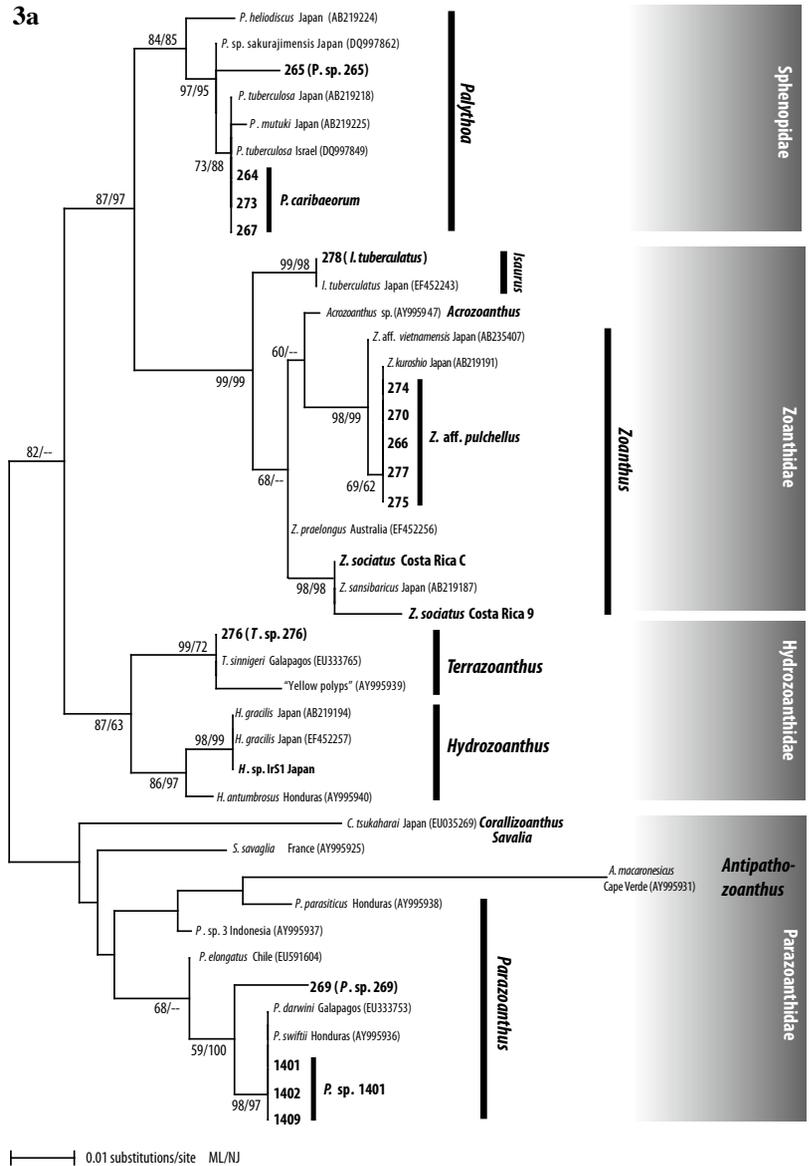
Phylogenetic analyses

New sequences obtained in the present study were deposited in GenBank (accession numbers HM130463–HM130514). Nucleotide sequences of mt 16S rDNA and COI from samples were manually aligned with previously published mt 16S rDNA and COI sequences from various zoanthid species representing the genera *Palythoa*, *Zoanthus*, *Acrozoanthus*, *Isaurus*, *Parazoanthus*, *Antipathozoanthus*, *Savalia*, *Corallizoanthus*, *Hydrozoanthus*, and *Terrazoanthus*. Originally, outgroup sequences for both mt 16S rDNA and COI trees were from the genus *Epizoanthus*, and once the monophyly of the remaining zoanthids was confirmed, final phylogenetic trees were made without *Epizoanthus* outgroups to improve resolution.

For *Symbiodinium* ITS-rDNA sequences, different *Symbiodinium* clade sequences are highly divergent from each other, and an alignment of ITS-rDNA was downloaded from the Scott Santos laboratory homepage (<http://www.auburn.edu/~santosr/>), and new sequences from other recent studies were added. This alignment consisted of mainly the second internal ribosomal spacer of ribosomal DNA (ITS2), which has been shown to have great utility in identifying *Symbiodinium* types (e.g. LaJeunesse, 2002). GenBank Accession Numbers of *Symbiodinium* sequences used are given in the resulting phylogenetic tree.

All alignments were inspected by eye and manually edited. All ambiguous sites of the alignments were removed from the dataset for phylogenetic analyses. Consequently, four alignment datasets were generated: 1) 651 sites of 42 sequences (mt 16S rDNA); 2) 300 sites of 49 sequences (COI); and 3) 336 sites of 59 sequences (*Symbiodinium* ITS-2). The alignment data are available on request from the corresponding author and at the homepage <http://web.me.com/miseryukyu/>.

For the phylogenetic analyses of the three alignments, the same methods were applied independently. Alignments were subjected to analyses with the maximum-likelihood (ML) with PhyML (Guindon and Gascuel, 2003) and neighbour-joining (NJ) methods. PhyML was performed using an input tree generated by BIONJ with the general time-reversible model (Ro-



driguez *et al.*, 1990) of nucleotide substitution incorporating invariable sites and a discrete gamma distribution (eight categories) (GTR + I + G). 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 tree. The distances were calculated using a Kimura's 2-parameter model (Kimura, 1980). Support for NJ branches was tested by bootstrap analysis (Felsenstein, 1985) of 1000 replicates. PAUP* version 4.0 was used for phylogenetic analyses (Swofford, 1998).

Results

In situ morphological zoanthid identification

The preliminary *in situ* identifications of the zoanthid specimens are summarized in Table 1, and images of all species shown in Fig. 2. Specimens 264, 267 and 273 were seen to be sand-encrusting *Palythoa* forms (=immersae, Pax 1910) with an extremely well developed coenenchyme, and thus likely *P. caribaeorum* (Fig. 2a). Specimens 265 and 1425 were also sand-encrusted, and 'intermediae' (Pax, 1910) in colony and

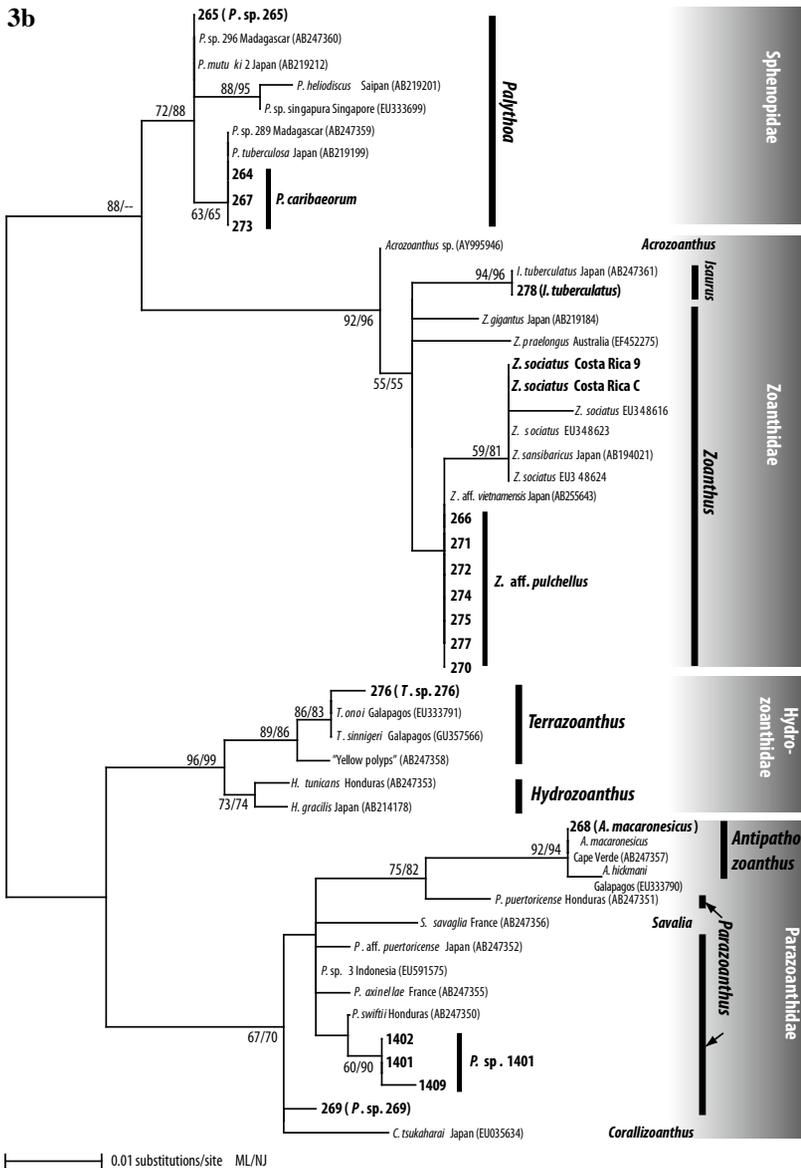


Fig. 3. Maximum likelihood (ML) trees of a) mitochondrial 16S ribosomal DNA, and b) cytochrome oxidase subunit I (COI) sequences for zoanthid specimens. Values at branches represent ML and neighbor-joining (NJ) probabilities (>50%), respectively. Sequences newly obtained in this study in bold. Sequences/species names from previous studies in regular font with GenBank Accession Numbers. For specimen information see Table 1.

polyp form, with approximately 60 relatively short tentacles (Fig. 2b). The oral disk patterning was purple-brown with white markings in primarily ring patterns, and septae were visible. This specimen did not match conclusively with any described Atlantic *Palythoa* species.

Several specimens were clearly members of the genus *Zoanthus*, and easily identifiable to genus level by the lack of sand encrustation and the smooth outer surfaces of polyps. Polyps were generally crowded together, and appeared to be ‘immersae’ or ‘intermediae’, growing from a well-developed coenenchyme (Fig.

2c). Tentacles numbered 50–64, and were all approximately half the length of the oral disk diameter. Most specimens examined were intertidal, and oral disk color varied widely from mint green (266), fluorescent green (270), dark purple with a fluorescent green oral opening (275), to pale pink (277). These *Zoanthus* specimens (also including specimen 1419) could not be conclusively identified to species level.

Specimens 278, 1406, and 1407 were clearly an *Isaurus* species due to the relatively large size of the polyps, a lack of sand encrustation and external tubercles on the polyps’ outer surface, and following Muirhead and

Ryland (1985) and Wirtz and Debelius (2003) the specimens were identified as *Isaurus tuberculatus* (Fig. 2d).

Specimen 268 was sand-encrusted, and found on the antipatharian *Tanacetipathes cavernicola* Opresko, 2001, and therefore preliminarily identified as *Antipathozoanthus macaronesicus* (Ocaña and Brito 2003; Ocaña et al., 2007; Sinniger et al., 2010) (Fig. 2e).

Specimen 269 (Fig. 2f) was also sand-encrusted but found associated with a sponge inside a cave; it was not identifiable to species level, although it was assigned to the revised genus *Parazoanthus* (Sinniger et al., 2010) based on this association. Specimens 1414 and 1424 were also assigned to this species.

Similarly, specimens 1401, 1402, and 1409 were found only in the vicinity of Tarrafal, generally in either dark (tunnel) or overhang locations, often but not always associated with either an orangish-red or yellow encrusting sponge (Fig. 2g). Again, based on the association with the sponge, it was assigned to the genus *Parazoanthus*.

Found during low tide on the underside of a large intertidal rock, the small sand-encrusted specimen 276 did not match with any known Atlantic zoanthid genus (Fig. 2h). Specimen 276 did, however, match in ecology to both undescribed zoanthids from the Pacific (Reimer et al., 2008c) and to one species of the newly described genus *Terrazoanthus* (Hydrozoanthidae), namely *T. sinnigeri* Reimer and Fujii, 2010. Due to this uncertainty, this specimen could not be identified even to family with initial examinations.

DNA sequence and phylogenetic identification - mt 16S rDNA

Mitochondrial 16S ribosomal DNA (mt 16S rDNA) phylogenetic results are shown in Fig. 3a. The phylogenetic tree from analyses was similar in topology to previous studies examining Zoantharia, with most families forming well-supported clades. The very strongly supported clade of the family Zoanthidae (ML=99%, NJ=99%) was seen to include identical sequences from specimens 266, 280, 274, 275, and 277 along with other *Zoanthus*, *Acrozoanthus*, and *Isaurus* sequences. Additionally, sequences from specimens 271 and 272 were identical to these sequences but shorter in length and were subsequently not used in phylogenetic analyses. These sequences were identical to sequence AB219191 from *Zoanthus kuroshio* Reimer and Ono, 2006 (in Reimer et al., 2006a), which together with AB235407 from *Zoanthus* aff.

vietnamensis Pax and Muller, 1957 formed a strongly supported group (ML=98%, NJ=99%). Specimen 278 formed a separate monophyly with *Isaurus tuberculatus* from Japan (IKO1) (ML=99%, NJ=98%).

Sphenopidae sequences formed a moderately well supported clade (ML=84%, NJ=85%) and included sequences from specimens 264, 267, 273 and 265. The first three specimens' sequences were identical to *Palythoa tuberculosa* Klunzinger, 1877 sequences (AB219218, DQ997849) from Japan and Israel, and together with *Palythoa mutuki* Haddon and Shackleton, 1891 (AB219225) formed a moderately well supported monophyly (ML=73%, NJ=88%). This subclade was sister to another group that included 265 and *Palythoa* sp. sakurajimensis, and these together formed a monophyly (ML=97%, NJ=95%) separate from *Palythoa heliodiscus* Ryland and Lancaster, 2003.

Specimens 269, 1401, 1402, and 1409 were within a moderately supported Parazoanthidae clade (ML=68%, NJ=<50%), and the latter three were part of a very well supported subclade (ML=98%, NJ=97%) including *P. darwini* Reimer and Fujii, 2010, and *P. swiftii* Duchassaing and Michelotti, 1860. The sequence from specimen 268 was too short to be included in the final phylogenetic analyses (approximately 400 bp in length), but was identical to a sequence from *Antipathozoanthus macaronesicus* (AY995930) previously collected from the Cape Verde Islands.

Specimen 276 was included in a monophyly (ML=87%, NJ=63%) of Hydrozoanthidae sequences, within a well-supported clade (ML=99%, NJ=72%) that was sister to another clade containing *Hydrozoanthus* sequences (ML=86%, NJ=97%). 276 was seen to be identical to a sequence obtained from *Terrazoanthus onoi* Reimer and Fujii, 2010 (EU333760) from the Galapagos Islands.

COI

Cytochrome oxidase subunit I (COI) phylogenetic results are shown in Fig. 3b. As seen with mt 16S rDNA results, the phylogenetic tree from analyses was similar in topology to previous studies examining Zoantharia, with each family forming a well-supported clade. The clade of the family Zoanthidae (ML=92%, NJ=96%) was seen to contain sequences from the largest number of specimens in this study. Sequences from specimens 266, 270, 271-275, and 277 were identical, and were also identical to GenBank sequence

AB255643 (*Zoanthus* aff. *vietnamensis*, Japan) and also EU348616 (*Z. sociatus*’ Ellis and Solander, 1786, Brazil), which together formed a group (ML=<50%, NJ=<50%) that was basal to another clade, containing other ‘*Z. sociatus*’, and *Z. sansibaricus* Carlgren, 1900 sequences ((ML=59%, NJ=81%). Specimen 278 formed a separate monophyly with *Isaurus tuberculatus* from Japan (IKO1) (ML=94%, NJ=96%).

Sphenopidae sequences formed another group (ML=72%, NJ=88%). Within this family, sequences from 264, 267, and 273 were seen to be identical to sequences from *Palythoa tuberculosa* (AB219199), and these sequences formed a moderately supported monophyly (ML=63%, NJ=65%). The COI sequence from 265 was identical to sequences from *P. mutuki* (AB219211), and these sequences were basal to the *P. tuberculosa* and related specimens and *P. heliodiscus* and related specimens’ clades.

Specimen 269 was placed with the moderately well supported Parazoanthidae clade (ML=67%, NJ=70%), although resolution and bootstrap support within Parazoanthidae was not strong. Sequences from specimens 1401, 402, and 1409 formed a subclade (ML=60%, NJ=90%) also within Parazoanthidae, derived from *P. swiftii*. Additionally, the COI sequence from specimen 268 was seen to be identical to a sequence from *Antipathozoanthus macaronesicus* from the Cape Verde Islands (AB247357), and together with *A. hickmani* Reimer and Fujii, 2010 (EU333790), formed a highly supported *Antipathozoanthus* subclade (ML=92%, NJ=94%).

The COI sequence from specimen 276 was placed within a well-supported Hydrozoanthidae clade (ML=96%, NJ=99%). Within Hydrozoanthidae, specimen 276 was most closely related to *Terrazoanthus onoi* (EU333791) and *T. sinnigeri* (GU357566) from the Galapagos Islands, forming a well-supported subclade (ML=86%, NJ=83%). Together with a sequence from *Terrazoanthus* sp. ‘yellow polyps’, these sequences formed a well-supported *Terrazoanthus* generic clade (ML=89%, NJ=86%) separate from a *Hydrozoanthus* clade.

Symbiodinium ITS-rDNA

Internal transcribed spacer 2 (ITS2) phylogenetic results are shown in Fig. 4. Resulting ITS-2 sequence data were clear in both forward and reverse directions with no ‘double-peaks’, and thus no cloning was performed. *Symbiodinium* ITS2 sequences from speci-

mens in this study were found to belong to three different *Symbiodinium* clades. Six of seven *Z. aff. pulchellus* colonies (266, 270, 271, 272, 274, 275) were found to contain clade A *Symbiodinium*, identical to the previously reported sequence AF427465 from *Aiptasia* anemones in Florida. These sequences, together with three novel sequences from *Symbiodinium* of two *Z. sociatus* colonies from Caribbean Costa Rica, formed a well-supported monophyly (ML=98%, NJ=90%).

The remaining *Z. aff. pulchellus* colony *Symbiodinium* ITS2 sequence was from specimen 277 and grouped with two other ITS2 sequences that belonged to clade B, EU074874 from culture and AF195152 from *Aiptasia* anemones in Bermuda in a well-supported monophyly (ML=95%, NJ=94%) within the clade B lineage.

Symbiodinium ITS2 sequences from *Palythoa* (specimens 265, 267, and 273) and *Isaurus* (278) were unambiguously placed within clade C (ML=72%, NJ=73%), within a large group that included many subclades of C previously observed in a variety of hosts, including isolates from *Zoanthus*, *Isaurus*, and *Palythoa* spp. from Japan.

Discussion

Suborder Brachycnemina

The results of this report are similar to recent studies conducted in the Galapagos Islands, another relatively isolated oceanic region, demonstrating the widespread distribution of some brachycnemic species (families Sphenopidae, Zoanthidae) (Reimer and Hickman, 2009), and the presence of potentially undescribed species within the families Parazoanthidae and Hydrozoanthidae in the suborder Macrocnemina (Reimer *et al.*, 2008c; Reimer and Fujii, 2010).

In our study, most specimens of both *Palythoa* and *Zoanthus* appear to belong to species commonly found in the Caribbean. Specimens 264, 267, and 273 are assigned to *Palythoa caribaeorum*, as they are identical in ecology, morphology, and phylogenetics to previously examined specimens. It has previously been theorized that much of the Cape Verde Islands’ coral fauna arrived from the Caribbean during the mid-Pleistocene (Boekschoten and Best, 1988), and our results support this. These zoanthid specimens (along with specimen 265) also contained ‘generalist’ clade C *Symbiodinium*, as previously seen in *Palythoa* spp. in

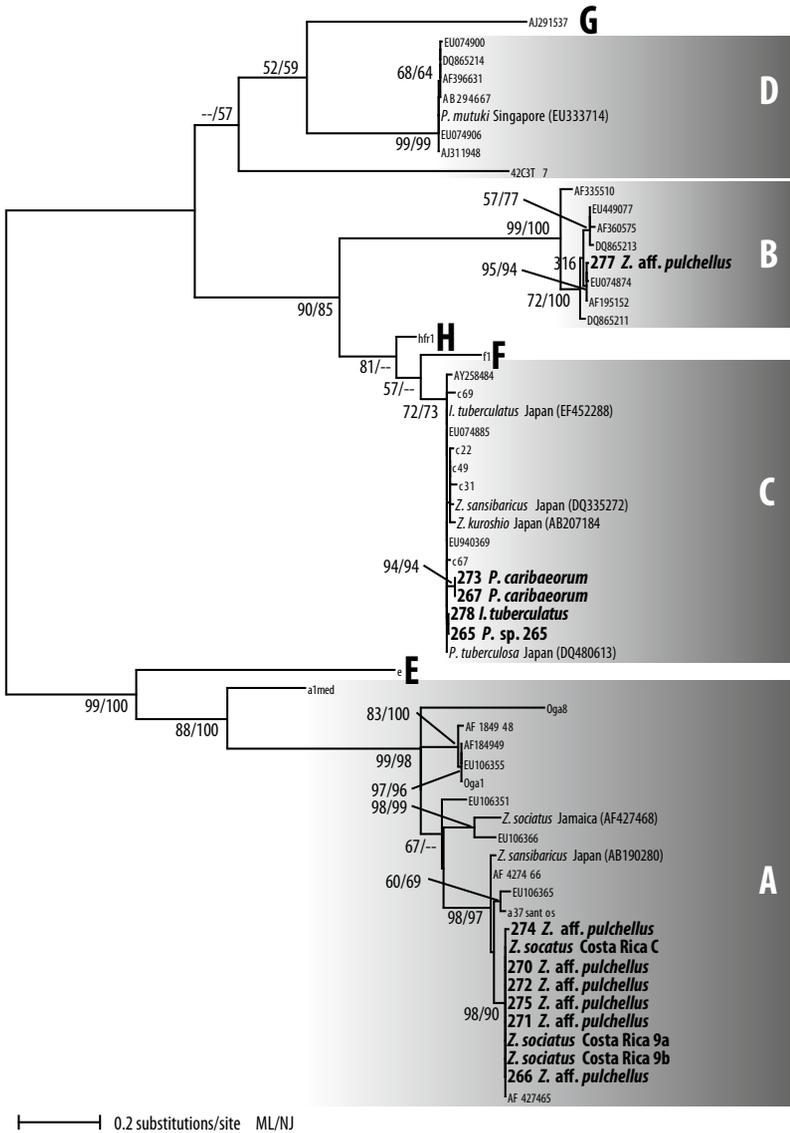


Fig. 4. Maximum likelihood tree of the internal transcribed spacer of ribosomal DNA (ITS-rDNA) sequences for *Symbiodinium* including specimens from this study. Values at branches represent ML and NJ bootstrap probabilities, respectively. New isolates and sequences from this study in **bold**, with host species and specimen number. Sequences/species names from previous studies in regular font with GenBank Accession Numbers, zoanthid-associated isolates from previous studies with species name and location, as well as GenBank Accession Number. *Symbiodinium* clades confirmed to be in symbiosis with zoanthid species (this study and previous studies) designated with shaded boxes. For specimen information see Table 1.

the northwestern Pacific (Reimer et al., 2006b).

Carlgren (1939) described a single *Palythoa* specimen from Sao Vicente Island as *P. vincentina* but given the large amount of morphological variation observed within *Palythoa* species (Burnett et al., 1997; Reimer et al., 2007c) we agree with the other previous reports of *Palythoa* in the Cape Verde Islands (Morri and Bianchi, 1995; Morri et al., 2000; Monteiro et al., 2008) that this is most likely *P. caribaeorum*. Similarly, although both *P. dartevillei* and *P. senegalensis* have been described from West Africa (Pax, 1952; Pax and Müller, 1956, respectively), these species, for the same reasons as above, are likely to be *P. caribaeorum*.

Preliminary phylogenetic analyses of *Palythoa* from Senegal indicate specimens from this region are monophyletic with *P. caribaeorum*, and will be presented elsewhere (Reimer and Wirtz, unpublished data).

While it is clear specimen 265 belongs to the genus *Palythoa*, it is different in both morphology and DNA sequences from known *Palythoa* species, and may be an undescribed species. Although in outward appearance (color, tentacle length) it appears similar to an undescribed ‘brown zoanthid’ from the Caribbean in Humann (2002), specimen 265 was found in both shallow tidepools and at deeper (9 m) locations, unlike the ‘brown zoanthid’ found only at 6 to 18 meters.

Additionally, DNA analyses did not match previously investigated *Palythoa* species, although there is a lack of sequences from Atlantic species. For now, the species identity of this single specimen remains elusive and we preliminarily designate this species as *Palythoa* sp. 265.

We did not observe *P. variabilis* in this study, which is known to be amphi-atlantic (Pax and Müller, 1956), and we expected to locate this species in the Cape Verde Islands during the course of this study. Further examinations may yet confirm *P. variabilis* in the Cape Verde Islands.

Zoanthus specimens 266, 270, 271, 272, 274, 275, and 277 all grouped closely with previously examined *Z. aff. vietnamensis* and *Z. kuroshio* specimens from Japan, and it is very likely all the Cape Verde Islands *Zoanthus* specimens belong to one Atlantic species closely related to the Pacific *Z. aff. vietnamensis*/*Z. kuroshio* group. While *Zoanthus* specimens here have been preliminarily assigned to the species group *Z. aff. pulchellus*, the phylogenetic results demonstrate problems with this identification, as specimens previously identified by zoanthid researchers as '*Z. sociatus*' fall into both of the major *Zoanthus* clades (Fig. 3, additional data not shown). However, the *Zoanthus* sequences from the Cape Verde Islands very clearly did not match with *Z. sociatus* sequences from specimens collected by the first author from the Caribbean coast of Costa Rica (Fig. 3b). We tentatively identify these specimens as *Z. aff. pulchellus*, as they could also perhaps be *Z. solanderi* Lesueur, 1817 *sensu* Karlson (1980), although sketches of both species in Duerden (1898) show the Cape Verde Islands specimens fit most closely with *Z. pulchellus*. *Zoanthus* specimens from the Cape Verde Islands were found primarily in the intertidal zone, while both *Z. pulchellus* and *Z. solanderi* have been generally found in the subtidal zone (Karlson, 1980; Humann, 2002). Similar to previous studies (Burnett *et al.*, 1997; Reimer *et al.*, 2004), these results demonstrate the difficulty of proper species identification in the genus *Zoanthus*. These are the first *Zoanthus* spp. records documented from the Cape Verde Islands.

The majority of the Cape Verde Islands *Zoanthus* specimens here (six of seven) were shown to contain *Symbiodinium* from clade A, as were two *Z. sociatus* samples from Caribbean Costa Rica. Clade A has previously been found in zoanthids from Jamaica in the Caribbean, and from intertidal *Z. sansibaricus* specimens in southern Japan (Reimer *et al.*, 2006c), and is speculated to be adapted to high levels of UV light (LaJeunesse, 2002). This theory fits well with the distribution of the specimens from the Cape Verde Is-

lands in the intertidal zone. Additionally, *Zoanthus* species may offer less protection from UV light than both *I. tuberculatus* (thicker body walls) and the sand-encrusted *Palythoa* species, which were not observed to contain clade A in the Cape Verde Islands.

Surprisingly, *Zoanthus* specimen 277 contained *Symbiodinium* most closely related to clade B, which has not previously been found in association with brachyzoanthids (T. LaJeunesse, pers. comm.), despite numerous papers specifically examining the symbionts of *Zoanthus* and *Palythoa* spp. (Burnett, 2002; Reimer *et al.*, 2006b, 2006c, 2007b; Reimer and Todd, 2009). As only a single colony containing clade B was found in this study, future *Symbiodinium* research in the Cape Verde Islands should examine *Zoanthus* spp. in more detail to more clearly understand how widespread clade B is within the zoanthid community.

The identification of *Isaurus tuberculatus* was relatively easy given the unique polyp shape of this genus, and the previous reference to this species in the Cape Verde Islands (Wirtz and Debelius, 2003). However, as Pacific and Atlantic zooxanthellate zoanthids have probably been separated since the closing of the Isthmus of Panama (Reimer *et al.*, 2008a), this species may be split into Atlantic and Pacific sibling species in the future. Further research utilizing ITS-rDNA or other relatively fast-evolving DNA markers is needed to test this hypothesis. Similar to previous results from the Pacific (Reimer *et al.*, 2008b), *I. tuberculatus* was seen to contain 'generalist' *Symbiodinium* belonging to clade C.

Suborder Macrocnemina

In this study we found only four species from the suborder Macrocnemina in the Cape Verde Islands, including *Antipathozoanthus macaronensis*, which has already been well documented (Ocaña and Brito, 2003; Ocaña *et al.*, 2007; Sinniger *et al.*, 2010; as 'undescribed zoanthid from Cape Verde' in Sinniger *et al.*, 2005). It is likely that more macrocnemic species exist in the waters around the Cape Verde Islands given the rich marine environment.

The discovery of a species within the genus *Terrazoanthus* in the Cape Verde Islands (specimen 276) is notable in that this is the first record from the Atlantic for this newly created genus, making this genus circumtropical in distribution - both previous species were known from the Indo-Pacific. *Terrazoanthus* sp. 276 was found under rocks, similar to *T. sinnigeri* from the Galapagos. Although the polyps of this species are not particularly small for zoanthids, it may

have a cryptic ecology similar to *T. sinnigeri*. In this study, only one colony was found at Murdeira Bay on Sal Island, and more specimens are needed to properly discuss this species and to formally describe it.

Morphology and DNA sequences from specimen 269 did not fit with any known zoanthid, although due to its association with sponges and phylogenetic placement, this specimen appears to be an undescribed *Parazoanthus* species, which here is called *Parazoanthus* sp. 269. Morphologically, this species appears divergent from most known *Parazoanthus* species, with relatively long and thin tentacles, and a very large oral disc diameter compared to the diameter at the base of the polyps. Similar to the *Terrazoanthus* species above, more specimens and in situ observation along with morphological analyses are needed to properly describe this species.

The fourth species recorded in this study was another *Parazoanthus* species, preliminarily designated *Parazoanthus* sp. 1401. This species was phylogenetically very closely related to both *Parazoanthus swiftii* and *Parazoanthus darwini*. However, morphologically it is very different from *P. darwini*, with polyps quite clear from the coenenchyme, unlike *P. darwini*. Additionally, in coloration (pale yellow opposed to pink or orange for *P. darwini*) tentacle number (approximately 24 tentacles, opposed to 24-30 for *P. darwini*), and distribution (Cape Verde Islands as opposed to the Galapagos Islands) these specimens are different and clearly not *P. darwini*. Morphologically, these specimens do resemble *P. swiftii* with regards to tentacle number (*sensu* Swain, 2009) and polyp shape, but *P. swiftii* is currently known only from the Caribbean, and as COI sequences are also different between these two species, it is likely *Parazoanthus* sp. 1401 is an undescribed species (Sinniger et al., 2008). One possible future method to further ascertain this would be to examine the faster-evolving ITS-rDNA, as performed in Reimer and Fujii (2010).

Historically, there is a single zoanthid record, of a species designated *Thoracactis topsenti* Gravier, 1918, commensal on a sponge found at 800-1100 off the Cape Verde Islands. This species, the only representative of its genus, is currently placed within the family Epizoanthidae. Gravier (1918) thought that this species was a 'zoanthid-like actinian', although its presence on sponges, two rows of tentacles, bright yellow color, and encrustation all strongly suggest this is a zoanthid, not of the family Epizoanthidae but more likely from the family Parazoanthidae.

Despite the low number of brachycnemic zoanthid specimens in this study, it is clear the Cape Verde Is-

lands harbor undescribed, possibly endemic species. Future investigations should help understand the true levels of brachycnemic zoanthid diversity in these islands, and will likely lead to the discovery of more undescribed species.

Conclusions

The Cape Verde Islands are home to at least eight species of zoanthids, four of them zooxanthellate brachycnemic zoanthids. These zooxanthellate zoanthids are for the large part in symbioses with clades of *Symbiodinium* that have previously been recorded in association with zoanthids (Clades A and C). However, one colony of *Z. aff. pulchellus* was found to contain clade B *Symbiodinium*, a first for zoanthids. These results indicate a need for further, more in-depth investigations into *Symbiodinium* diversity in the Cape Verde Islands and surrounding areas. Furthermore, it appears that there may be undiscovered zoanthid diversity, as currently at least two undescribed species exist in the waters of the Cape Verde Islands, *Terrazoanthus* sp. 276 and *Parazoanthus* sp. 269, and likely two additional species, *Palythoa* sp. 265 and *Parazoanthus* sp. 1401.

Acknowledgements

Emanuel d'Oliveira and Georg Bachschmid of the King Bay Diving Centre at Tarragal/Santiago Island and Nuno Marques da Silva of the Manta Diving Centre at Santa Maria/Sal Island were of immeasurable help during field work. Mr. d'Oliveira also collected the *Isaurus* specimen in this study. Drs. H. Zibrowius and D. Fautin helped in the search for historical records of zoanthids from the Cape Verde Islands. The Centro de Ciências do Mar (CCMAR) of the University of the Algarve partly financed three trips to the Cape Verde Islands by P.W. Dr. Todd LaJeunesse (Pennsylvania State University) is thanked for his advice regarding *Symbiodinium*. J.D.R. was supported in part by the Rising Star Program at the University of the Ryukyus, and a grant-in-aid ('Wakate B') from the Japan Society for the Promotion of Science. Two anonymous reviewers comments greatly improved this manuscript.

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Received: 12 April 2010

Revised and accepted: 17 September 2010

Published online: 15 November 2010

Editor: R.W.M. van Soest

Appendix

Zoanthids of the Cape Verde Islands

Family Zoanthidae

This is the only family of zoanthids that is not sand-encrusted, and includes three zooxanthellate genera; *Zoanthus*, *Isaurus*, and *Acrozoanthus*. Found in shallow tropical and subtropical environments. All species have brachycaemic mesentery arrangement, with the 5th mesentery from the dorsal directive imperfect.

1. *Zoanthus* aff. *pulchellus* Duchassaing de Fombressin and Michelotti, 1864; Fig. 2c

In the Cape Verde Islands, this species was found primarily in tidepools in the lower intertidal zone at both Santiago and Sal Islands. Colonies can be partly out of the water during low tide. Colonies can be quite large (e.g. up to hundreds or thousands of polyps), and completely cover the hard substrate (rock, dead coral) to which they are attached. Colonies edges can be ‘tongue-like’ in appearance. Polyps closely packed together, often crowding one another and giving each polyp a ‘hexagonal’ shape. Polyps are ‘immersae’ or ‘intermediae’ (Pax, 1910) in form, not totally extending free and clear of the well-developed coenenchyme. Oral disk colors include pale salmon pink, dark green, light green, and fluorescent green, with approximately 50 tentacles that are usually olive green or reddish-brown in color. Tentacle length usually less than oral disk diameter; oral disk diameters up to 10 mm when expanded, contracted polyps 3–6 mm in diameter. This species appears to be closely related to the Pacific *Z. vietnamensis*/*Z. kuroshio* species group.

2. *Isaurus tuberculatus* Gray, 1828; Fig. 2d

This species has so far only been found around Tarrafal, Santiago Island in the Cape Verde Islands, on rocky substrate, in deeper (at least 18 m) waters. Colony color varied from light brown to green to bright red. The few colonies observed consisted of < 20 polyps. Polyps have tubercles (bumps) on their outer surfaces, and this species is rarely observed with its oral disks open. Polyps up to 20–30 mm in length, connected at their base to reduced coenenchyme.

Family Sphenopidae

Similar to Zoanthidae this family is common in tropical and subtropical waters worldwide. Unlike Zoan-

thidae, species of this family is generally very heavily encrusted with sand. This family consists of two genera, the zooxanthellate *Palythoa* and azooxanthellate, solitary *Sphenopus*, which has only a few species known from the Indo-Pacific. All species have brachycaemic mesentery arrangement, with the 5th mesentery from the dorsal directive imperfect.

3. *Palythoa caribaeorum* (Duchassaing and Michelotti, 1864); Fig. 2a

This species is widespread throughout the Atlantic, from the Caribbean and Florida to southern Brazil, and along the west coast of Africa. In the Cape Verde Islands it has been found at both Santiago and Sal Islands (this study) and Sao Vicente Island (Carlgrén, 1939). *P. caribaeorum* can be distinguished by its ‘immersae’ form, with tightly packed polyps embedded with a very well developed coenenchyme. This species is found from the intertidal to depths of approximately 20 m (Morri and Bianchi, 1995; Morri *et al.*, 2000; Monteiro *et al.*, 2008; this study) in the Cape Verde Islands, and similar to the closely related Pacific *P. tuberculosa*/*P. caesia* Dana, 1846, can often dominate the substrate, covering rock and dead coral with a large mat. Intertidal colonies may be completely out of the water during low tide. Edges of colonies often ‘tongue-like’ in appearance. Colonies may be very large (>1m²). Although colony color depends much on the color of encrusted detritus, most colonies are usually brown, with some light fluorescent yellow and darker brown colonies present. Polyps up to 15 mm in diameter although usually smaller, with oral disk usually brown, tan, or white. Tentacle number may vary, but usually approximately 34–48. Tentacles usually quite short (e.g. < half oral disk diameter).

4. *Palythoa* sp. 265; Fig. 2b

This potentially undescribed species was observed only at Tarrafal, Santiago Island in the Cape Verde Islands. Although similar in appearance to the Pacific *P. mutuki*, this species is clearly molecularly a different species. Colonies are found in cracks of rocks in the low intertidal. One colony was found in 9 m depth in a shaded depression in a horizontal rock platform. Polyps are larger than *Zoanthus* species (contracted polyp diameter up to approximately 10 mm), and extend free and clear of the well-developed basal coenenchyme (‘liberae’). Oral disks up to 20 mm in diameter, usually smaller, and somewhat regularly

spaced, with a less crowded appearance than both *P. caribaeorum* and *Z. aff. pulchellus* above. Observed colonies were small, with <50 polyps. As with *P. caribaeorum* above, encrustation color likely affects colony color; most colonies are dark brown in color, with oral disks dark brown with some lighter brown or gold patterns (flecks or small stripes), lighter towards the oral opening, with septae slightly visible. Capitular ridges prominent on closed polyps. Up to 64-68 short tentacles, which are brown or olive brown-gray.

Family Parazoanthidae

This family has been recently redefined (Sinniger et al., 2010). Many species associated with hydrozoans, or present directly on rock or dead coral substrate (Reimer and Fujii, 2010) have been moved to the family Hydrozoanthidae. The redefined Parazoanthidae includes mostly zoanthids that are commensal on other marine invertebrates, including antipatharians, precious corals, and sponges. As currently defined, most species in this family can be assigned to a genus based on their substrates. All species have macrocnemic mesentery arrangement, with the 5th mesentery from the dorsal directive perfect.

5. *Antipathozoanthus macaronesicus* (Ocaña and Brito, 2003); Fig. 2e

Originally described as *Savalia macaronesica*, this species has recently been re-assigned to a genus of zoanthids found exclusively on antipatharians (black corals), and is the type species of this new genus; *Antipathozoanthus* (Sinniger et al., 2010). Described from the Canary Islands and the Cape Verde Islands, in the Cape Verde Islands *A. macaronesicus* is usually found growing on antipatharians of the genus *Tanacetipathes*, and thus can be encountered only at deeper SCUBA depths or inside caves, where the antipatharian is present. In this study, *A. macaronesicus* was only found at Tarrafal, Santiago Island, but has previously been recorded from Sal Island as well (Ocaña and Brito, 2003). Polyps are spaced regularly, attached to a well-developed coenenchyme, and 3-6 mm in diameter, 4-15 mm in height. Expanded oral disks up to approximately 8-10 mm in diameter. Coenenchyme and the outer surface of polyps generally light pink, with oral disks brighter orange-pink. Light sand encrustations often clearly visible on the outside of polyps. Tentacles approximately 42 in number, and can be very long (up to twice oral disk diameter).

6. *Parazoanthus* sp. 269; Fig. 2f

This species is currently known from only one area (Tarrafal, Santiago Island) in the Cape Verde Islands, where it was found on the rear walls of deep depressions in the rock face (i.e. in dim light) in about 20 m depth, occasionally associated with an encrusting sponge but often on rock or shell surfaces. Phylogenetically, *Parazoanthus* sp. 269 fits well within a *Parazoanthus* clade, although it does not match with any known *Parazoanthus* species. Polyps are long and more slender at the base than at the oral end (contracted polyp diameter approximately 1 mm), and bright orange-yellow in color, both on the outside of polyps and the oral disk. Polyps are much smaller than *Parazoanthus* sp. 1401. Polyps are connected by a reduced, often almost stoloniferous coenenchyme. There are small white dots on the capitular ridges just below the edge of the oral disk and tentacles. Tentacles are long (>oral disk diameter) and slender, and number approximately 24-28 in total.

7. *Parazoanthus* sp. 1401; Fig. 2g

This species is currently only known from one area, Tarrafal Bay on Santiago Island. It was encountered on overhanging rock faces in 10-15 m depths and in a dark cave in 20 m depth. Polyps are cream-yellow often with a pink or orange hue, with a bright orange oral disk and a yellow mouth. The outer surfaces of polyps are encrusted with large amounts of coarse detritus of many different colors (black, white, gray), but the encrustations are not numerous enough to mask the yellow tissue color. Polyps extend free from a well-developed coenenchyme that is often stoloniferous. Tentacles number approximately 24, and are slightly longer than the oral disk diameter (approx. 4-8 mm). Contracted polyp diameter is approximately 1-4 mm, much larger than *Parazoanthus* sp. 269. Additionally, tentacles appear to be thicker at the base compared to other macrocnemic zoanthid species in the Cape Verde Islands, and taper to a slender ending. This species is often but not always associated with a yellow or orangish-red encrusting sponge.

Family Hydrozoanthidae

Like Parazoanthidae, all species in the family Hydrozoanthidae have a macrocnemic mesentery arrangement, with the 5th mesentery from the dorsal directive perfect. However, molecular examinations demonstrated this group to be a monophyly very divergent from Parazoanthidae, and perhaps more closely related to

Sphenopidae. Additionally, the two genera in this family either associate uniquely with hydrozoans (*Hydrozoanthus* spp.), or are found on rock substrate (*Terrazoanthus* spp), similar to many Zoanthidae and Sphenopidae species. Thus, based on both molecular and ecological data, this new family was recently erected (Siniger *et al.*, 2010).

8. *Terrazoanthus* sp. 276; Fig. 2h

This genus was recently erected (Reimer and Fujii, 2010) by grouping together hydrozoanths that are found attached to rock or dead coral substrate, and not to hydrozoans as in the other genus of the family,

Hydrozoanthus. The Cape Verde Islands species was found only at one location (Murdeira Bay, Sal Island), on the underside of a large rock, suggesting a somewhat cryptic lifestyle similar to *Terrazoanthus sinnigeri* from the Galapagos Islands.

Polyps are relatively small (contracted polyps' approximate diameter = 3-4 mm) and translucent pink in color, with large-sized encrustations peppering the outer surface of polyps. The coenenchyme appears to be somewhat reduced. Unfortunately, no in situ images of open polyps exist, and therefore the numbers of tentacles and oral disk coloration remain unknown for now. More specimens are needed to describe this species formally.

