Ascension Island shallow-water Zoantharia (Hexacorallia: Cnidaria) and their zooxanthellae (Symbiodinium)

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This is the first report on the Zoantharia fauna (Cnidaria: Hexacorallia) of the isolated oceanic island of Ascension, southern Atlantic, where zoantharians are a dominant component of the shallow-water benthos. Specimens from two expeditions (1983, 2012) were examined regarding external morphology and molecular phylogeny using three DNA markers, which confirmed the presence of four zoantharian species: Palythoa caribaeorum, Palythoa aff. clavata, Parazoanthus swiftii; and Zoanthus sp. Two of these, Palythoa aff. clavata and Parazoanthus swiftii, were previously only known from the western Atlantic and Caribbean. Molecular examination of the zooxanthellae (=Symbiodinium spp.) of Palythoa specimens showed that they were in association with only one type, subclade C1.

Keywords: Ascension Island, Atlantic, benthos, isolation, Palythoa, Parazoanthus, Symbiodinium, Zoantharia, zoogeography

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INTRODUCTION

Zoantharians are an order of Hexacorallia (Cnidaria: Anthozoa: Zoantharia) found in many marine environments, and possibly one of the most basal cnidian groups (Kayal et al., 2013). In particular, the suborder Brachycnemina is common in shallow subtropical and tropical waters (Carlson, 1980; Swain, 2009; Irie et al., 2011), and most species in this group are zooxanthellate (i.e. contain symbiotic unicellular algae of the genus Symbiodinium). Zoantharian taxonomy has been hindered by a combination of various factors, including but not limited to: a lack of clear diagnostic morphological characteristics (Burnett et al., 1997); high levels of intraspecific variation (Burnett et al., 1997; Reimer et al., 2004; Ong et al., 2013); and difficulties in conducting histological examinations due to the uptake of sand/detritus in the body wall of most species (Reimer et al., 2010b). Consequently, there are undoubtedly many synonyms in the literature, and the actual diversity of shallow water zoantharians, particularly in the zooxanthellate genera Palythoa and Zoanthus, may be considerably lower than previously thought (Burnett et al., 1997; Reimer et al., 2004, 2012a).

Over the last two decades, the utilization of molecular techniques has allowed researchers to re-examine zoantharian taxa, and while our understanding of the group is far from complete, the taxonomy of supraspecific levels probably reflects the evolutionary phylogeny of this order (Sinniger et al., 2010). Undoubtedly there are still many more species to be described (e.g. Reimer et al., 2012b; Sinniger et al., 2013), and some of a dubious nature to be combined (e.g. Hibino et al., 2014), but as progress is made on the understanding of zoantharian species diversity, research can begin to focus on answering ecological and biogeographical questions for this group.

Although there are many regions with little information, it appears that some brachycnemic zoantharian species are very widely distributed (e.g. Reimer et al., 2008; Hibino et al., 2014), and this may be due to their long planktonic larval stage (up to 190 d; Polak et al., 2011). All subtropical and tropical islands of the world specifically investigated thus far have zoantharians present. Similar results are found from plankton net trawls; zoantharian larvae were present in all trawls in waters above 20°C, regardless of the location or relative isolation of the trawl (Ryland et al., 2000). These data suggest that some zoantharian species are highly dispersive and widespread.

One of the most isolated islands is Ascension Island in the southern equatorial eastern Atlantic. It lies at 07°57′S 14°22′ W. The nearest land, St Helena Island, is about 1200 km south-east of it. The distance to Brazil is about 2300 km and the distance to West Africa about 1500 km.

Zoantharians have previously been reported from Ascension Island (Irving, 1989, 2013; Manning & Chace, 1990; Den Hartog & Türky, 1991), although the identifications are often only as ‘zoanthid’ or to genus level. Furthermore, ‘Palythoa sp.’ has been reported to be the dominant benthos in many areas on Ascension Island (Irving, 1989, 2013), which has been suggested to be one of the reasons why this island has a depauperate scleractinian coral fauna (Irving, 2013). When Den Hartog (1989a) visited
Ascension on his way to St Helena in 1983, he specifically reported on actinarians that he collected in the rock pools of Shelly Beach but not on his collection of zoantharians, and neither on the corals that he sampled from here (see Hoeksema, 2012).

Research has shown that Ascension Island’s shore-fish fauna is of eastern and western Atlantic origin, but is most closely related to that of Brazil (Lubbock, 1980; Briggs & Bowen, 2012). However, as the species identifications of the dominant zoantharians of Ascension have not yet been clearly ascertained, the relationship between Ascension Island’s benthos and other Atlantic regions remains less clear. Thus, in this study, we use previously reported DNA markers to ascertain the species identity of the zoantharians of Ascension Island by examining specimens collected during the SMSG/South Atlantic Environmental Research Institute (SAERI) expedition funded by the Darwin Initiative, supplemented with specimens from the Naturalis Biodiversity Center in Leiden, The Netherlands collected in 1983. We also examined endosymbiotic Symbiodinium spp. dinoflagellates of the zooxanthellate specimens by phylogenetic methods, as it has been demonstrated that there are often some levels of specificity between zoantharian hosts and their Symbiodinium type (Reimer et al., 2007b), and such data could also help infer the source of Ascension Island zoantharians.

MATERIALS AND METHODS

Specimens

Specimens from Ascension Island were collected from tide pools and by SCUBA diving in a depth range down to 25 m. They were photographed in the field and preserved in 96% ethanol. New specimens have been deposited in the Coelenterata collection of the Naturalis Biodiversity Center (Leiden, The Netherlands) collection under specimen numbers RMNH Coel. RMNH 41606–RMNH 41611.

During research in 2012 at Naturalis Biodiversity Center by the first author, uncatalogued zoantharian specimens collected by J.C. den Hartog from Ascension Island in 1983 were examined and are included in this study.

DNA extraction and PCR amplification

DNA extraction was performed on specimens either utilizing a guanidine method as described in Sinniger et al. (2010) or by using a spin column Dneasy Blood & Tissue Extraction kit following the manufacturer’s instructions (Qiagen, Tokyo). PCR amplification using extracted genomic DNA was done using HotStarTaq Plus Master Mix Kit (Qiagen, Tokyo) following the manufacturer’s instructions. Three zoantharian DNA markers were amplified in this study: mitochondrial 16S ribosomal DNA (mt 16S rDNA), cytochrome oxidase subunit I (COI), and the nuclear internal transcribed spacer region of ribosomal DNA (ITS-rDNA), with primers and amplification conditions as reported by Reimer et al. (2007a, c) and Sinniger et al. (2010), respectively. The nuclear internal transcribed spacer region of ribosomal DNA (ITS-rDNA) of Symbiodinium spp. was additionally amplified in this study, using primers previously reported (White et al., 1990; Rowan & Powers, 1992; Hunter et al., 1997) and amplification conditions reported by Reimer et al. (2007b).

All amplified products were visualized by 1.0% agarose gel electrophoresis and positive products were treated with Shrimp Alkaline Phosphatase (SAP; Takara) and Exonuclease I. Sequencing was performed by Fasmac (Kanagawa) on an ABI sequencer.

Phylogenetic analyses

Novel sequences obtained in this study were deposited in GenBank (Accession Numbers KF794166–KF794186). For mitochondrial COI and 16S-rDNA alignments, sequences of other zoantharian genera were incorporated into the alignment, while for zoantharian ITS-rDNA, two alignments of (1) only Palythoa spp. sequences, and (2) only Parazoanthidae sequences were generated due to high levels of divergence between sequences in other taxa. For all zoantharian alignments, sequences were aligned with previously reported DNA sequences from Atlantic zoantharians (Swain & Wulff, 2007; Reimer et al., 2010a, 2012a). Novel Symbiodinium ITS-rDNA sequences (Table 1) were aligned with previously obtained clade C Symbiodinium sequences (see resulting phylogenetic tree), with a Symbiodinium clade F sequence as the outgroup.

The three zoantharian alignments (mt 16S rDNA + COI, Palythoa ITS-rDNA, Parazoanthidae ITS-rDNA) were constructed as mentioned in Reimer et al. (2012a), following previous alignments as guides. All alignments were inspected by eye and ambiguous sites (ambiguous or double peaks, <2 sites/alignment) were removed prior to analyses. Three zoantharian alignment datasets were generated: (1) a concatenated mt 16S-rDNA + COI alignment with 44 sequences and 1060 sites; (2) a Palythoa ITS-rDNA alignment with 21 sequences and 909 sites; and (3) a Parazoanthidae ITS-rDNA alignment with 17 sequences and 955 sites. A Symbiodinium alignment of 318 sites of 20 sequences was additionally generated. All four alignments are available from the corresponding author upon request, and at the homepage www.miseryyukyu.com.

The best-fitting model of nucleotide substitution was selected for each dataset using the corrected Akaike information criterion (AICc) in MrModeltest v.2 (Nylander, 2004) and Paup v4.0b10 (Swofford, 2000). Gene trees were inferred using the Bayesian method implemented in BEAST v.1.7.5 (Drummond & Rambaut, 2007). A Yule speciation model was used as a tree prior and rate variation among lineages was modelled using an uncorrelated lognormal relaxed clock (Drummond et al., 2006), with the mean substitution rate fixed to 1 to get branch lengths in units of substitution per site. Posterior distributions of trees and parameters were estimated using Markov chain Monte Carlo (MCMC) sampling. Samples were drawn every 1000 steps over a total of 10 million MCMC steps. Each analysis was run four times, with mixing and convergence assessed using Tracer v.1.5. After discarding 10% of the samples as burn-in, samples from the four runs were thinned (sampling every 4000 steps) and pooled together. The maximum-clade-credibility tree was drawn from the pooled samples. Maximum likelihood (ML) trees were inferred using Treefinder (Jobb et al., 2004) for each dataset and their robustness was evaluated through bootstrapping (1000 replications).
### RESULTS

#### Field collection

During the SMSG/South Atlantic Environmental Research Institute (SAERI) expedition in 2012, six zoantharian specimens were collected from Ascension Island, belonging to three morphospecies (Table 1; Figure 1).

Two of these were zooxanthellate, heavily sand-encrusted *Palythoa* spp. One of them (N = 3 specimens) was 'immersae' (Pax, 1910), with polyps embedded within a very well developed coenenchyme (Figure 1A). This morphospecies matched closely with *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860), and was preliminarily designated as *Palythoa cf. caribaeorum*. This morphospecies locally dominated the benthos around the island, from shallow water down to at least 15 m depth, forming colonies that could reach a size of more than 1 m². Colony colour varied from light grey to dark brown.

The second *Palythoa* morphospecies was much more 'liberae' (Pax, 1910), with large polyps free and clear of the coenenchyme, and was preliminarily designated as *Palythoa* sp. (Figure 1B). *Palythoa* sp. was found in a tide pool at Soudan Bay.

The third species was designated as *Parazoanthus* sp. (Figure 1C). It was common on overhanging walls and at cave entrances from about 5 m to at least 15 m depth, and was bright yellow in colour.

In the Naturalis Biodiversity Center coelenterate collection (RMNH Coel.), an additional three zoantharian specimens belonging to three morphospecies were found (Table 1). Two of the specimens were collected from the intertidal regions of Shelly Beach (precise date unknown, 1983) by J.C. den Hartog (see also Van der Land, 2003), and matched with *Palythoa cf. caribaeorum* and *Palythoa* sp., respectively (Table 1). Den Hartog visited Ascension Island for two days (31 May–1 June 1983) on the way from the United Kingdom to Brazil, and collected *Palythoa aff. clavata* (specimen AS5) and *Zoanthus* sp. (specimen AS6). Further specimens from Ascension Island were collected by the RMNH Coel. expedition in 2012, but none were zooxanthellate.

#### Table 1. Zoantharian specimens from Ascension Island examined in this study.

<table>
<thead>
<tr>
<th>Specimen number</th>
<th>Preliminary identification</th>
<th>Location</th>
<th>Collector, date</th>
<th>mt 16S</th>
<th>mtsDNA</th>
<th>COI</th>
<th>ITS-rDNA</th>
<th>ZX</th>
<th>Species identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS1 (RMNH Coel.</td>
<td><em>Palythoa cf. caribaeorum</em></td>
<td>Southeast Bay</td>
<td>P. Wirtz, 1 Sept. 2012</td>
<td>12 m</td>
<td>2012</td>
<td>2012</td>
<td>KJ794167</td>
<td>NA</td>
<td><em>Palythoa caribaeorum</em></td>
</tr>
<tr>
<td>AS2 (RMNH Coel.</td>
<td><em>Palythoa cf. caribaeorum</em></td>
<td>Two Hooks</td>
<td>P. Wirtz, 1 Sept. 2012</td>
<td>12 m</td>
<td>2012</td>
<td>2012</td>
<td>KJ794167</td>
<td>NA</td>
<td><em>Palythoa caribaeorum</em></td>
</tr>
<tr>
<td>AS3 (RMNH Coel.</td>
<td><em>Parazoanthus sp.</em></td>
<td>Boatswain Bird Island</td>
<td>P. Wirtz, 1 Sept. 2012</td>
<td>8 m</td>
<td>2012</td>
<td>2012</td>
<td>KJ794167</td>
<td>NA</td>
<td><em>Parazoanthus swiftii</em></td>
</tr>
<tr>
<td>AS6 (RMNH Coel.</td>
<td><em>Parazoanthus sp.</em></td>
<td>Boatswain Bird Island</td>
<td>P. Wirtz, 1 Sept. 2012</td>
<td>8 m</td>
<td>2012</td>
<td>2012</td>
<td>KJ794167</td>
<td>NA</td>
<td><em>Parazoanthus aff. clavata</em></td>
</tr>
</tbody>
</table>

* Based on external morphological characters only; ZX, zooxanthellae; NA, not available.
Kingdom to St Helena onboard the MV 'Centaur' (Den Hartog, 1989a). The zoantharians could have been collected on 1 June 1983, because on this date Den Hartog sampled corals from Shelly Beach (Hoeksema, 2012). The third specimen was from an exposed tide pool at Mars Bay (31 May 1983, J.C. den Hartog), and had been determined as Zoanthus sp. due to being zoonanthallate, with smooth polyps, and no sand encrustation (Figure 1D). In this study, we kept the Zoanthus sp. designation for this specimen.

**Phylogenetic analyses**

DNA was successfully amplified from all six newly collected specimens for all markers, but could not be amplified from the older Naturalis specimens, which were initially preserved in formalin.

**Zoantharian mt 16S-rDNA + COI tree**

In the mt 16S-rDNA + COI tree created from zoantharian sequences, sequences from specimens AS1, AS2 and AS5 fell within a well-supported clade (PP: 0.98) consisting of previously reported *Palythoa tuberculosa* (Pacific), *P. caribaeorum* (Atlantic) and *P. mutuki* (Pacific) sequences (Figure 2). The concatenated sequences for AS4 fell within a grouping that included previously reported *Palythoa aff. clavata sensu Reimer et al. (2012a)* (Florida) sequences and *Palythoa sp. 'sakurajimensis' (Japan) sequences. Sequences from a specimen from the Cape Verde Islands (*Palythoa sp. 265 sensu Reimer et al. 2010a*) were basal to this group.

Within Parazoanthidae, sequences from specimens AS3 and AS6 were within a well supported clade (PP: 0.93) that also contained previously reported sequences from *Parazoanthus swiftii* Haddon & Shackleton, 1891 from the Caribbean and *Parazoanthus sp. 1401* from the Cape Verde Islands.

**Zoantharian ITS-rDNA trees**

In the *Palythoa* ITS-rDNA tree, sequences from AS1, AS2 and AS5 were within a well supported *P. caribaeorum/P. tuberculosa* clade (PP: 0.99), closely related to but distinct from a
P. mutuki/P. grandiflora clade (Figure 3A). The sequence from AS4 was within a Palythoa aff. clavata clade (from Florida), distinct from Palythoa sp. ‘sakurajimensis’.

In the Parazoanthus ITS-rDNA tree, sequences from AS3 and AS6 were within a well supported P. swiftii clade (PP: 0.83), distinct from other species’ sequences (Figure 3B).

**Symbiodinium ITS-rDNA tree**

Symbiodinium ITS-rDNA sequences were successfully amplified for three of four specimens (AS2, AS4 and AS5). All three sequences were identical over the ITS2 region with previously reported Symbiodinium clade C1 (GenBank Accession Number EU074885) sensu LaJeunesse (2002) as well as Symbiodinium reported previously from some other zoantharian hosts in Japan (e.g. from Isaurus tuberculatus Gray, 1828 and Palythoa tuberculosa (Esper, 1791)) (Figure 4), and slightly different from previously reported Symbiodinium within zoantharians from the Cape Verde Islands.

**Specimen identities**

Based on the molecular results combined with external morphology, the Ascension specimens were identified as: Palythoa aff. clavata (AS4); Palythoa caribaeorum (AS1, AS2 and AS5); and Parazoanthus swiftii (AS3 and AS6). The
Geographical locations of host zoantharian specimens are given. Novel sequences from this study in with specimen number (see Table 1) from Boatswain Bird Rock and Spire Rock. Additionally, one specimen from the Naturalis Biodiversity Center were identified as: *Palythoa aff. clavata* (RMNH Coel. 40617); *P. caribaeorum* (RMNH Coel. 40618); and *Zoanthus* sp. (RMNH Coel. 40625) (Table 1).

**DISCUSSION**

Previously, very few records of zoantharians existed for Ascension Island. Irving (1989) reported *Parazoanthus swiftii* from many sites at 'shaded areas under overhangs and in cliffs', and 'Palythoa sp.' on shallow upward facing surfaces around Boatswain Bird Island and Boatswain Bird Rock. The subsequent report by Irving (2013) again mentions *P. swiftii* as present at numerous locations, and *Palythoa sp.* from Boatswain Bird Rock and Spire Rock. Additionally, one image of a massive *Palythoa* sp. colony appears, and this 'immersa' colony corresponds well with *P. caribaeorum* as identified in our study. Manning & Chase (1990) reported on the presence of the crab *Platypothis indica* (A. Milne Edwards, 1869) on Ascension Island. They mention that this crab lives in association with zoantharians as reported by Den Hartog & Holthuis (1984) but did not mention that the latter refer to the Cape Verdes, thus only indirectly reporting the presence of zoantharians on Ascension Island.

The findings from field collection and subsequent molecular phylogenetic examination show that there are at least four zoantharian species present in the shallow waters of Ascension Island. *Palythoa caribaeorum* (corresponding to ?*Palythoa* sp. in Irving (1989), *Palythoa* sp. in Irving (2013), and *Palythoa cf. caribaeorum* as collected by den Hartog) is found extensively in sheets (Irving 2013) around the whole of Ascension Island. Surprisingly, *Palythoa caribaeorum* was not mentioned in an earlier report on Ascension Island's benthic communities (Price & John, 1980). *Palythoa aff. clavata* (=*Palythoa* sp. as collected by den Hartog) was found so far only at Soudan Bay and at Shelly Beach. *Parazoanthus swiftii* (correctly identified by Irving (1989, 2013) was commonly found in shaded areas or caves. Finally, an unidentifiable *Zoanthus* sp. was collected from Mars Bay by den Hartog in 1983, and was not found in 2012 surveys associated with this study. It may be that *Zoanthus* sp. is rare on Ascension Island. All in all, these results confirm the importance of historical museum collections as complementary and baseline material in faunistic studies based on recent surveys (Hoeksema et al., 2011; Hoeksema & Wirtz, 2013).

The results of the phylogenetic analyses indicate that the zoantharian fauna of Ascension Island has affinities with the fauna of the western Atlantic and Caribbean. In particular, the finding of both *Palythoa aff. clavata* and *Parazoanthus swiftii*, species hitherto only reported from the Caribbean and western Atlantic, demonstrates this. Not only slower evolving mitochondrial DNA (Shearer et al., 2002) but also ITS-rDNA sequences from Ascension specimens unequivocally identify both these species. Furthermore, in a recent examination of zoantharians from the Cape Verde Islands in the north-eastern Atlantic (Reimer et al., 2010a), species with similar morphology (*Palythoa* sp. 265 = perhaps *P. canariensis* Haddon & Duerden, 1896; and *Parazoanthus* sp. 1401) had different COI or mt 16S rDNA sequences than *Palythoa aff. clavata* and *Parazoanthus swiftii* (see Figures 2, 3), respectively. Thus, given slow mtdNA rates of change in zoantharians, such results indicate that the north-eastern Atlantic may harbour species different from both Ascension Island and the western Atlantic. However, before any final conclusions on the origins of zoantharians at Ascension can be made, more data are needed from the subtropical and tropical West African coastline. Regardless, our data show that molecular identification of zoantharians is perhaps the most accurate way to identify to species or species-group levels (Sinninger et al., 2008), particularly in genera such as *Palythoa* and *Zoanthus* in which there exists high levels of intraspecific variation (Burnett et al., 1997; Reimer et al., 2004).

The presence of four zoantharian species at such an extremely isolated island such as Ascension Island demonstrates how widely some zoantharian species are distributed. While this is not unexpected for brachycnemic *Palythoa*...
and Zoanthus based on past research showing such species with potentially long planktonic larval stages (Ryland et al., 2000; Hirose et al., 2011; Polak et al., 2011), the length of the larval stages of macroenigmatic zoantharians such as Parazoanthus swiftii are relatively unknown (but see Ryland & Westphalen, 2004), and our results here suggest that they too may have long-lived planktonic larval stages. Long-distance dispersal by rafting may be another explanation for widespread distributions in shallow-water benthic species, as demonstrated in a brooding scleractinian reef coral species (Hoeksema et al., 2012), but free-floating material appears to be almost entirely absent around Ascension Island (Price & John, 1980).

If the zoantharians at Ascension Island are affiliated with the western Atlantic (e.g. Brazilian) fauna, it is open to speculation how these species reached Ascension Island, particularly given the presence of the South Equatorial Current flowing from east to west between 2°N and 2°S. Den Hartog (1989b) remarked on similarities between the marine fauna of Ascension and St Helena with both the Mediterranean–north-west African marine fauna and that of the Caribbean, which he also linked to the South Equatorial Current. There are two possible alternative explanations. One is that the Brazilian coast, despite being further away from Ascension Island, is much more speciose than the African coast, and therefore more species from the western Atlantic arrive at Ascension Island (e.g. Floeter et al., 2008). The other contributing factor is the Atlantic Equatorial Undercurrent, which flows from 3°S to 5°S, runs from west to east at a depth of less than 100 m, and has temperatures potentially warm enough (>20°C, Ryland et al., 2000) to transport brachycnemimic larvae to Ascension Island. This current has been suggested to be a factor in Ascension Island’s fish fauna being more related to the Brazilian fauna as opposed to the African fauna (Briggs & Bowen, 2012).

Brachycnemimic zoantharians are known to thrive in disturbed environments (Sebens, 1982), and it may be that dominant Palythoa caribaeorum was among the first benthic species to arrive to the shallow Ascension waters. It is interesting to note that zoantharians have been reported from all tropical and subtropical areas where they have been looked for, indicating that they are effective shallow-water benthic colonizers. Future research into the diversity of the zoantharian fauna of the West African coast, as well as from other isolated oceanic island regions, should help to expand the findings of this study.

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