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ORIGINAL RESEARCH

Molecular diversity of coral reef-associated zoanthids off Qeshm Island, northern Persian Gulf

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Abstract The Persian Gulf, a semi-enclosed sea in the subtropical northwest of the Indian Ocean, is noted for its unique biodiversity under its extreme ecological conditions. Despite high biodiversity levels, many groups of marine invertebrates in this area have remained uninvestigated. The order Zoantharia (zoanthids) is one of these taxonomically neglected groups. In this study, diversity of shallow water zoanthids off the Qeshm Island, the largest island in the Persian Gulf, was investigated for the first time. Using in situ field examination integrated with 16S rDNA sequencing and phylogenetic analysis, the presence of three zoanthid species in the inter-tidal and shallow water zone of Qeshm Island were demonstrated: *Zoanthus sansibaricus* ($n = 12$) with five morphotypes, *Palythoa* cf. *mutuki* ($n = 10$) with two morphotypes and *Palythoa tuberculosa* ($n = 4$) with just one morphotype. In addition to species identification, molecular examination determined phylogenetic relationships of specimens with other previously reported zoanthid species. While *Zoanthus sansibaricus* and *Palythoa tuberculosa* are two known zoanthid species, based on molecular data, *Palythoa* cf. *mutuki* is potentially a novel undescribed species. However, due to lack of data on zoanthid research and distribution for the entire Persian Gulf, further investigation is needed to clearly ascertain this matter.

Keywords Zoanthid · *Zoanthus* · *Palythoa* · Mitochondrial 16S rDNA · Qeshm Island

Introduction

The order Zoantharia (zoanthid) is a group of benthic colonial anthozoans which belongs to the subclass Hexacorallia. They are characterized by having two rows of tentacles and one ventral siphonoglyph (Haddon and Shackleton 1891). These cnidarians are found in many marine ecosystems and are particularly common in coral reef ecosystems worldwide (Burnett et al. 1994, 1995; Reimer et al. 2011). Although zoanthid genera,

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Zoanthus and *Palythoa* are common in shallow subtropical and tropical waters, their taxonomy and identification to species level was historically confusing due to the intraspecific variation in polyp shape, size, color, oral disk color (Burnett et al. 1997; Reimer et al. 2004) and the absence of exact morphological description of species in the past literature. In addition, most zoanthids are known for being encrusted by sand and other detritus to enhance their structure, which make their histological sectioning very difficult (Reimer et al. 2010).

Recently, taking advantage of molecular phylogenetic methods, the reorganization process of the classification of this taxonomically neglected group has begun (Reimer and Fujii 2010; Sinniger et al. 2010). The mitochondrial markers have been successfully utilized in examining zoanthid taxa (Sinniger et al. 2010). Applying mt16S rDNA as a DNA marker has aided in identification and reorganization of zoanthids species, genera and families (Reimer et al. 2006a, 2006b, 2006c, 2007; Reimer and Todd 2009; Sinniger and Haussermann 2009; Sinniger et al. 2010; Swain 2009).

Coral reefs in the Persian Gulf are part of the complex and unique inter-tidal and sub-tidal habitats. Due to isolation from the open ocean, the Persian Gulf experiences extreme environmental conditions. It is one of the warmest and the most saline waters on earth and this has imposed a harsh condition on the marine organisms (Coles and Fadlallah 1991; Reynolds 1993) and as a result, a particular and unique marine fauna has evolved in this environment. In the northern Persian Gulf, there are 17 islands off the Iranian coastline with fringing coral reefs (Sheppard and Sheppard 1991). Although coral reef communities have been described at most of the Persian Gulf Islands (Fatemi and Shokri 2001; Namin and Van Ofwegen 2009; Rezai et al. 2010; Kavousi et al. 2011; Mostafavi et al. 2007; 2013; Samiei et al. 2013), there are no data on zoanthids in this area.

The purpose of this study was morphological observation and molecular identification of shallow water zoanthids off Qeshm Island. Upon collection, zoanthid specimens were grouped based on their external appearance. Then all the grouped species were molecularly identified. In this paper chromatic variability of all the collected specimens were provided and the accuracy of initial morphological identification was discussed.

Methods

Sample collection and preliminary identification

Twenty-six colonies of zoanthids were photographed and collected between December 2011 and March 2013 by wading or SCUBA diving in the inter-tidal zones of seven locations off Qeshm Island (Fig. 1).

Depth of sampling is shown in Table 1. Specimens were stored in 100 % ethanol at −20 °C. Initial identification was carried out based on in situ photographs (Fig. 2) with the support of published literature (Burnett et al. 1997; Ryland and Lancaster 2003; Reimer 2007; Reimer et al. 2006a).

DNA extraction, PCR amplification and sequencing

Zoanthid fragments were crushed in DNAB (0.4 M NaCl, 50 mM EDTA, pH 8.0) buffer and then DNA was extracted using the CTAB-chloroform method (Baker 1999). Mitochondrial 16S rDNA was PCR amplified using zoanthid-specific primers 16Sant1a and 16SbmoH (Sinniger et al. 2005) with the following thermal cycle conditions: 2 min at 94 °C then 35 cycles: 30 s at 94 °C, 1 min at 52 °C, 90 s at 72 °C, followed by 7 min final extension at 72 °C. The amplified products were analyzed by 1.5 % agarose gel electrophoresis. Original PCR products from zoanthids specimens were sent to the Macrogen Company in South Korea and directly sequenced with ABI-3730XL analyzer by the capillary system method.

Phylogenetic analysis

Sequences obtained from this study were deposited in GenBank and their accession numbers are shown in Table 1. The nucleotide sequences obtained in this study were aligned with 16S rDNA sequences available from Genbank (accession numbers are shown in Fig. 3) using the software CLUSTALW (Thompson et al. 1994). The outgroup sequence for 16S rDNA tree was *Parazoanthus gracilis*.

The alignment dataset was analyzed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian methods. The most appropriate model selection for ML and Bayesian analyses was performed using

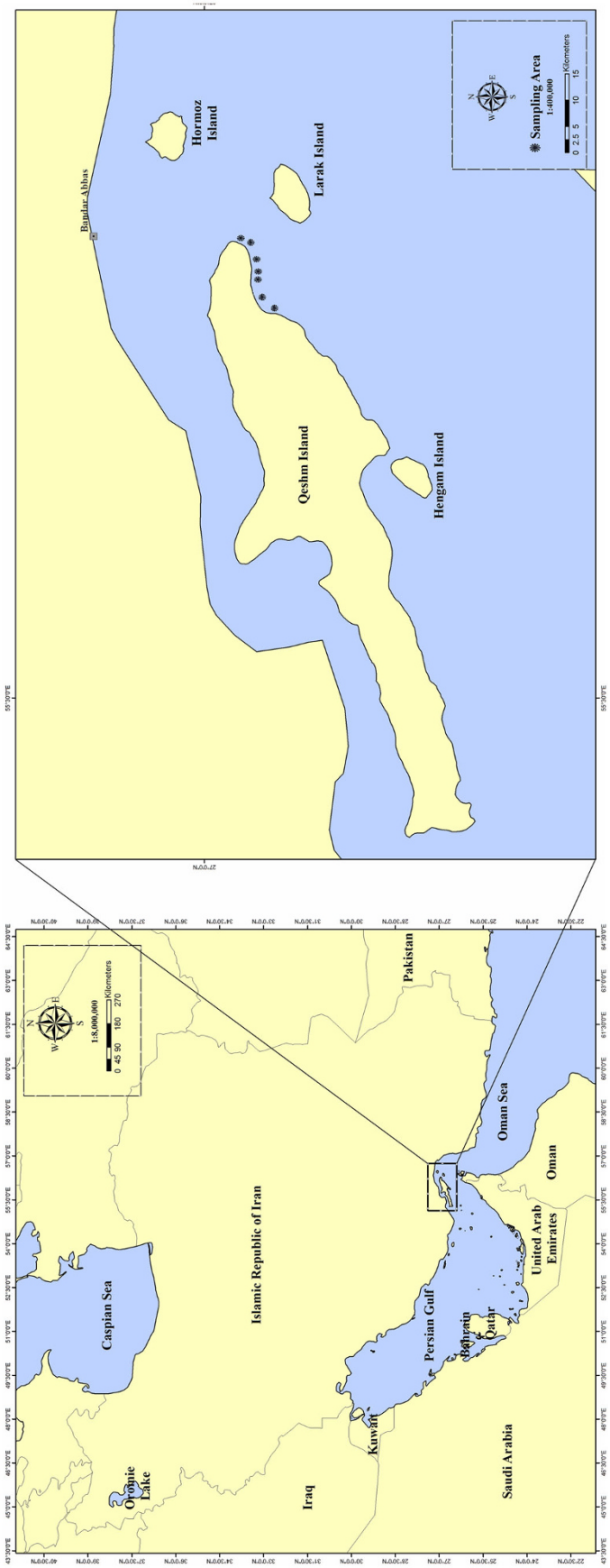

Fig. 1 Map of the Persian Gulf showing the position of the sampling locations (*dots*) in Qeshm Island

Table 1 Depth of sampling, accession numbers and final molecular identification of each specimen

Specimen name	Depth of sampling	Mt 16srDNA accession numbers	Molecular identification
QeAu1	Inter-tidal	KJ472909	*Palythoa* cf. *mutuki*
QeAu2	Inter-tidal	KJ472910	*Palythoa* cf. *mutuki*
QeAu3	Inter-tidal	KJ472921	*Palythoa tuberculosa*
QeAu4	Inter-tidal	JX845320	*Zoanthus sansibaricus*
QeAu5	Inter-tidal	KF733279	*Zoanthus sansibaricus*
QeAu6	Inter-tidal	KJ472922	*Palythoa tuberculosa*
QeAu7	Inter-tidal	JX845312	*Zoanthus sansibaricus*
QeSu1	2.5 m	KF733280	*Zoanthus sansibaricus*
QeSu2	2.5 m	KJ472919	*Palythoa tuberculosa*
QeSu3	2.5 m	KF733281	*Zoanthus sansibaricus*
QeSu4	2.5 m	KJ472911	*Palythoa* cf. *mutuki*
QeSu5	2 m	KF33282	*Zoanthus sansibaricus*
QeSu6	2 m	KJ472920	*Palythoa tuberculosa*
QeSu7	2 m	KF733283	*Zoanthus sansibaricus*
QeSu8	2 m	KJ472912	*Palythoa* cf. *mutuki*
QeWi1	Inter-tidal	KJ472913	*Palythoa* cf. *mutuki*
QeWi2	Inter-tidal	KJ472914	*Palythoa* cf. *mutuki*
QeWi3	Inter-tidal	KJ472915	*Palythoa* cf. *mutuki*
QeWi4	Inter-tidal	KJ472916	*Palythoa* cf. *mutuki*
QeWi5	Inter-tidal	KF733284	*Zoanthus sansibaricus*
QeWi6	Inter-tidal	KF733285	*Zoanthus sansibaricus*
QeWi7	Inter-tidal	KJ472917	*Palythoa* cf. *mutuki*
QeWi8	Inter-tidal	KF733286	*Zoanthus sansibaricus*
QeWi9	2 m	KJ472918	*Palythoa* cf. *mutuki*
QeWi10	2 m	KF733287	*Zoanthus sansibaricus*
QeWi11	2 m	KF733288	*Zoanthus sansibaricus*

Akaike Information Criterion (AIC) in MODELTEST 2.3 (Nylander 2004). The general time-reversible model (Rodrigues et al. 1990) with invariable sites (GTR + I) gave the best fit to the 16S rDNA data. ML and MP analyses were conducted using the PAUP beta version 4.0b10 (Swofford 2003). For ML, a heuristic search was carried out with 100 random additions of taxa, followed by tree-bisection-reconnection (TBR) branch-swapping rearrangements with a maximum of 100 optimal trees kept in each replicate. Maximum likelihood clade support was assessed by nonparametric bootstrapping with 1,000 replicates and the same heuristic-search parameters. The MP analysis, also carried out by the heuristic-search method, consisted of 100 runs of stepwise random taxon additions. MP clades were assessed with 1,000 bootstrap replicates (excluding uninformative characters), with 100 random additions of taxa for each replicate.

The Bayesian analysis was implemented in MrBayes 2.3 (Ronquist and Huelsenbeck 2003) and was based on the model selected by MODELTEST above. Starting from random trees, four Markov chains (with one cold and three heated chains) were run simultaneously to sample trees using the Markov Chain Monte Carlo (MCMC) principle which approximates the posterior probability (PP) of trees. After the burn-in phase (the first 5 million generations was discarded), every 100th tree out of 20^6 was considered. The phylogenetic trees generated in all analyses were visualized using TREEVIEW (Page 1996).

Results

Morphological preliminary observation

The 26 collected zoanthid specimens were first divided into three morphological groups. Fourteen specimens were identified as belonging to the genus *Palythoa* due to their heavy sand encrustation, external brown or

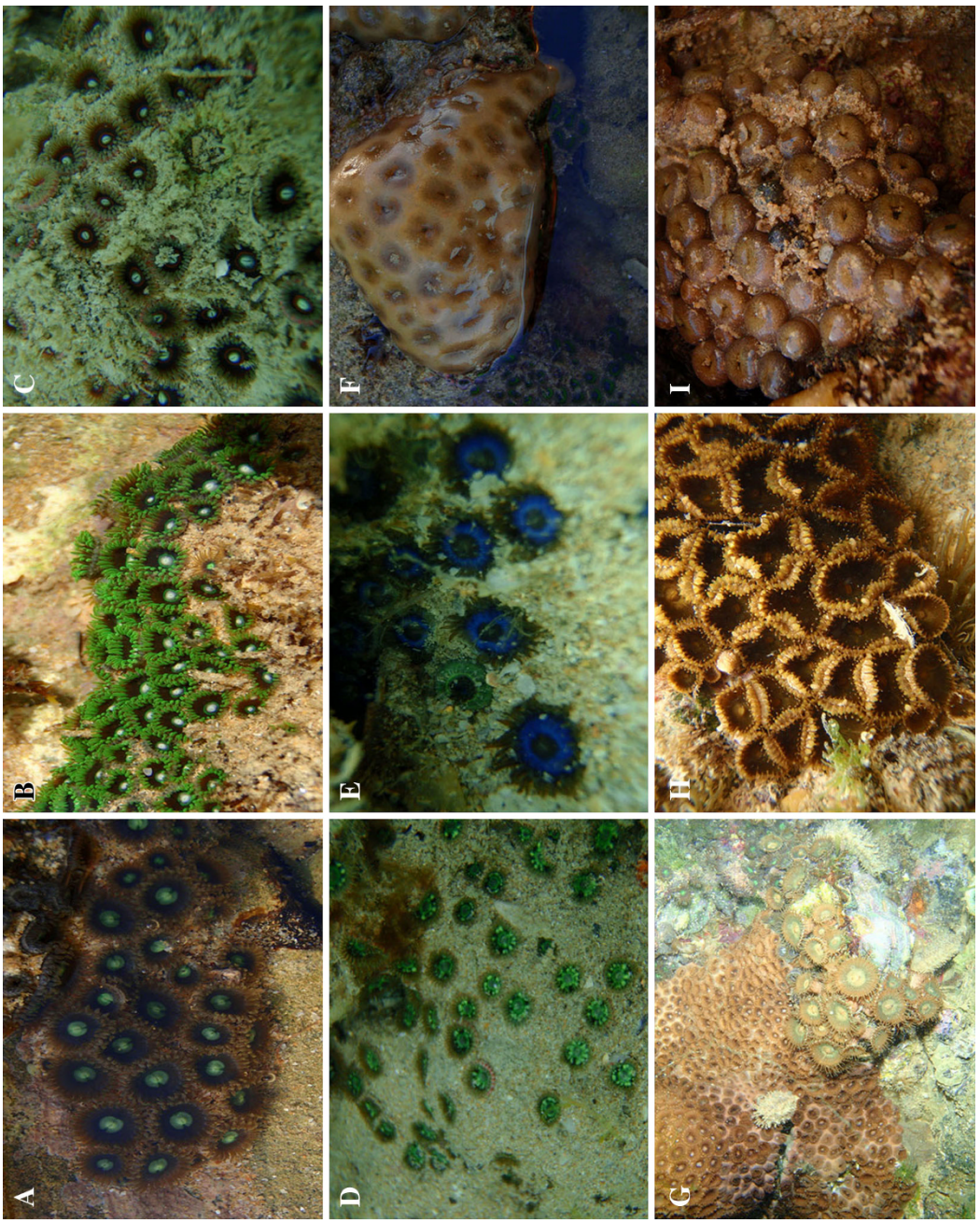

Fig. 2 In situ images of zoanthid specimens collected in this study. Each image is representative of one morphological group listed in Table 2

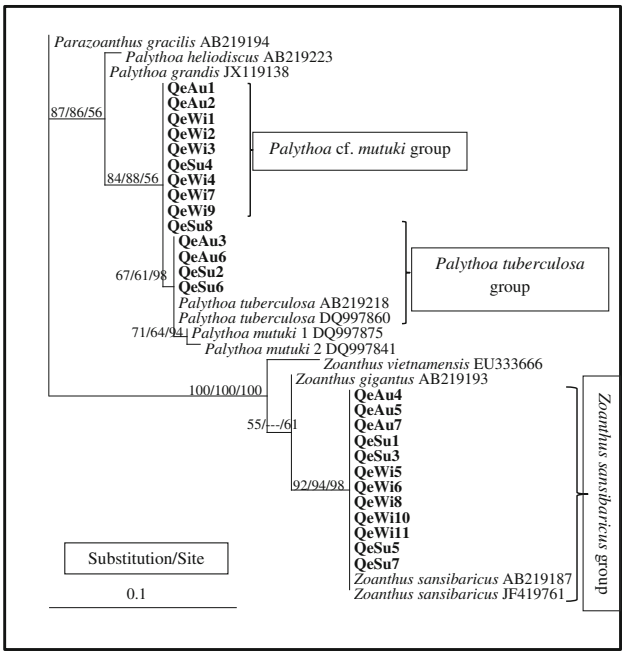

Fig. 3 Maximum likelihood tree of mitochondrial 16S ribosomal DNA sequences for zoanthid specimens. Values at branches represent maximum likelihood bootstrap percentages from 100 trees/maximum parsimony bootstrap percentages from 1,000 trees/Bayesian posterior probabilities

cream coloration (Table 2; Fig. 2) and the tentacle numbers which were almost fit with the numbers reported from *Palythoa* species of Indian and Pacific Ocean (Herberts 1972; Ryland and Lancaster 2003). Of these, four specimens (QeSu2, QeSu6, QeAu3 and QeAu6) were distinguished as *Palythoa tuberculosa* by their densely packed immersae polyps, ranging from almost light to dark brown. The other ten specimens were identified as *Palythoa mutuki* based on their elongated, cylindrical polyps with underdeveloped coenchyme (liberae polyps) and green or brown oral disk coloration. These polyps were joined basally, but their closeness and flaring columns obscured the bases (Fig. 2). The remaining morphological group (Table 2) contained 12 specimens which were clearly from the genus *Zoanthus*, as they had smooth, non-encrusted, liberae polyps. Since during the present study only chromatic variability of each specimen was monitored, species-level assignment was mostly impossible and they were just identifiable at the genus level. All morphological characters of each specimen (sand encrustation, polyp shape and color, oral groove, oral zone and oral disk colors, tentacle number and color) are summarized in Table 2.

Molecular-based Identification

Based on mitochondrial 16S ribosomal DNA, the zoanthids in this study were divided into two genus-level clades: *Zoanthus* and *Palythoa*. As shown in Fig. 3, all *Zoanthus* specimens belonged to the *Zoanthus sansibaricus* clade with a very well supported bootstrap (ML = 92 %, MP = 94 %, PP = 98 %).

The genus *Palythoa* was moderately supported (ML = 87 %, MP = 86 %, PP = 56 %). The sequences from specimens QeAu3, QeAu6, QeSu2 and QeSu6 were identical to AB219218 and DQ997860 from *Palythoa tuberculosa* and were within a moderately supported subclade (ML = 67 %, MP = 61 %, PP = 98 %). The ten sequences from specimens in the *Palythoa mutuki* morphological group differ from *Palythoa mutuki* 1 (DQ997860) and *Palythoa mutuki* 2 (DQ997841) in two and three base pairs, respectively. These sequences formed a moderately supported subclade (ML = 84 %, MP = 88 %, PP = 56 %) which was basal to *Palythoa mutuki* and *Palythoa tuberculosa* sequences.

Discussion

Utility of external morphological characteristics and molecular identification of zoanthids

In comparing morphological versus molecular identification results, we demonstrated that zoanthid identification based on the 16S ribosomal mitochondrial DNA marker is more accurate than using morphological

Table 2 Summary of morphological characteristics of collected zoanthids and final molecular identification

Morphological group	Group name	Sample name	Polyp form, color	Oral color	Oral zone color	Oral disk color	Tentacle number, color	Final molecular identification
Zoanthus sp.	A	QeSu1- QeAu5 QeWi8QeWi11	Liberae, purple	White	Fluorescent green with fluorescent green circle around it	Dark green	Greenish brown, approx. 47–52	*Zoanthus sansibaricus*
	B	QeSu7- QeAu4 QeWi6- QeWi10	Liberae, purple	White	White with fluorescent green circle around it	Dark green	Fluorescent light green, approx. 42–54	*Zoanthus sansibaricus*
	C	QeSu5- QeWi5	Liberae, purple	White	White with red circle around it	Red	Light brown, approx. 47	*Zoanthus sansibaricus*
	D	QeSu3	Liberae, purple	White	White with fluorescent green circle around it	Fluorescent light green and dark green	Light brown, not distinguishable	*Zoanthus sansibaricus*
	E	QeAu7	Liberae, purple	White	Light blue	Dark blue	Light brown, approx. 48	*Zoanthus sansibaricus*
Palythoa tuberculosa	F	QeSu2- QeSu6- QeAu3- QeAu6	Immerse, brown	Cream	Cream	Dark brown	Brown, approx. 30–34	*Palythoa tuberculosa*
Palythoa mutuki	G	QeAu1- QeWi3	Liberae, light brown	White	Light brown	Green–brown	Brown, approx. 45	*Palythoa* cf. *mutuki*
	H	QeSu4- QeAu2- QeWi1	Liberae, light brown	White	Light brown	Green	Brown, approx. 54-67	*Palythoa* cf. *mutuki*
	I (Polyps closed)	QeSu8- QeWi2- QeWi4- QeWi7- QeWi9	Liberae, light brown	Not dis.	Not distinguishable	Not distinguishable	Not distinguishable	*Palythoa* cf. *mutuki*

characteristics. This is especially so when species with polymorphic characteristics are dealt with that is not consistently reliable.

During the preliminary identification process, all specimens were identified to the generic level using only external morphological criteria, but their species-level assignment were not possible or correct in most cases. The identification of *Palythoa mutuki* specimens remained somewhat ambiguous. Although morphologically, these specimens perfectly agreed with *Palythoa mutuki*, the mt 16S rDNA data presented unexpected two or three base pair of differences with *Palythoa mutuki*. While the mitochondrial markers are very conservative in anthozoans (Shearer et al. 2002), this low level of variation has previously indicated species-level differences in *Palythoa* species. For example, *Palythoa mutuki* and *Palythoa tuberculosa* can be distinguished by one base pair of difference over mt 16S rDNA data set. Therefore, with examination of only one marker, for now we designated these specimens as *Palythoa* cf. *mutuki*.

As mt 16S rDNA are considered to be one of the mitochondrial markers which are able to correctly place zoanthid specimens, especially for Zoanthidae and Spheopidae families, within a species group (Sinniger et al. 2008; Reimer and Todd 2009; Swain 2009), applying this marker in the present study categorized these specimens into three species-level groups with a high level of confidence.

Zoanthid diversity in the Persian Gulf

All specimens during the present study have been categorized into three different species groups: *Zoanthus sansibaricus* from Zoanthidae family, *Palythoa tuberculosa* and *Palythoa* cf. *mutuki* from Sphenopidae family. Of these three species, two are identified species which have been widely distributed in the western and eastern Pacific Ocean; i.e., Great Barrier Reef (Burnett et al. 1997; Ryland and Lancaster 2003), New Caledonia (Sinniger 2006), Japan (Reimer et al. 2004, 2006a, 2011; Reimer 2007), Galapagos (Reimer et al. 2008), Singapore (Reimer and Todd 2009) and Indian Ocean (Herberts 1972), while the remaining species, *Palythoa* cf. *mutuki* did not molecularly match with any of the previously reported sequences.

As mentioned earlier in this study, there is an overall lack of data on zoanthids from the Persian Gulf. Thus, it is somewhat difficult to assess if the *Palythoa* cf. *mutuki* is an endemic species, that was previously undescribed, or range extensions of species from other regions.

The Gulf is linked to the open ocean by the narrow Strait of Hormuz which limits water exchanges (Wilson et al. 2002). The three zoanthid species, *Zoanthus sansibaricus*, *Palythoa tuberculosa* and *Palythoa mutuki* seem to be very widespread in the Indo-Pacific, and they have been found in isolated locations like Ogasawara (Reimer et al. 2011) and Galapagos (Reimer et al. 2008) Islands. It has been demonstrated that *Palythoa tuberculosa* has a larval stage of over 2 weeks (Polak et al. 2011; Hirose et al. 2011). However, there are few data on the length of time of larval stages for zoanthid species in this study aside from *Palythoa tuberculosa*. We hypothesized that many of the zoanthids that are found in the Persian Gulf may have extended larval stages which have been reached and colonized as the first species in the relatively isolated Persian Gulf from the wider Indian Ocean.

Conclusion

This study demonstrated the presence of at least two families, two genera and three species of zoanthids in the Persian Gulf. Furthermore, based on these observations, the presence of a potential new species was demonstrated. Most importantly, the results of this study indicated a need for further sampling and investigation of zoanthid from more regions to help complete the knowledge of zoanthid diversity in this area.

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Conflict of interest All authors declared that they have no conflict of interest.

Author contribution ANK carried out sampling, the experimental and phylogenetic analysis and wrote the article. PGM participated in the study design, revised the written article and submitted it. JFM and SMF made the comments and discussed on the results. All authors read and approved the final version of the manuscript.

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