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Diversity of shallow water zoantharians in Hengam and Larak Islands, in the Persian Gulf

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Zoantharians are one of the least studied orders of benthic cnidarians of the Persian Gulf. A survey and molecular analysis was conducted to determine zoantharian species diversity in the Persian Gulf. For this purpose, 63 colonies of zoantharians were collected from Hengam and Larak Islands in the Strait of Hormuz and some morphological characteristics of each specimen were recorded, i.e. sand encrustation, polyp shape and colour, oral groove, oral zone and oral disc colours as well as tentacle number and colour. After DNA extraction, mitochondrial 16S ribosomal DNA (16S rDNA) and cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR). Based on obtained 16S rDNA and COI gene sequences, five putative species-level clades were identified: Zoanthus sansibaricus *(N = 30),* Palythoa tuberculosa *(N = 12),* Palythoa mutuki *(N = 2),* Palythoa *aff.* mutuki *(N = 18) and* Neozoanthus *sp. Iran (N = 1). While the first three are known species, the last two were potentially novel undescribed species.* Palythoa *aff.* mutuki *has an external appearance similar to* Palythoa mutuki*. However, mitochondrial DNA sequences obtained from these specimens placed them in a previously undescribed species group. The* Neozoanthus *specimen was morphologically and molecularly different from other described* Neozoanthus *species. This is the first record of this genus from the Persian Gulf and neighbouring areas. Since there is not much work on zoantharians identification in the Persian Gulf, further sampling and investigation is needed to speculate on the accuracy of these potentially new species and to complete the knowledge of zoantharian diversity in this area.*

Keywords: Zoantharian, *Zoanthus*, *Palythoa*, *Neozoanthus*, 16S rDNA, COI

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INTRODUCTION

The Zoantharia, an order within the class Anthozoa, is characterized by a colonial form of life, two rows of tentacles in each polyp and one ventral siphonoglyph (Haddon & Shackleton, 1891). Each polyp distinctly protrudes from the coenenchyme (liberae or intermediae polyps) or is immersed in a fully developed coenenchyme (immersae polyps) (Pax, 1910).

The suborder Brachycnemina is distributed worldwide in marine ecosystems and found particularly on hard substrates of sub-tropical and tropical shallow coral reef environments (Burnett *et al.*, 1994, 1995). As with many other invertebrate communities on coral reefs, some zoantharian species harbour symbiotic dinoflagellates of the genus *Symbiodinium* (LaJeunesse, 2002; LaJeunesse *et al.*, 2004) that play a significant role in host nourishment (Muscatine & Porter, 1977). The need for solar energy in zooxanthellate zoantharians leads to their existence in photic zones (Swain, 2010).

Accurate identification of species is the key to our understanding of biological systems (Swain, 2009). Zoantharians are morphologically difficult to identify at the species level due to their plastic morphology, intraspecific colour variation and the lack of accurate morphological markers for proper discrimination of species (Reimer & Todd, 2009). Moreover, sand and detritus are embedded in the mesoglea of the majority of zoantharians to help strengthen their structure. Therefore, their internal morphological examination is not feasible without potentially dangerous hydrofluoric acid treatment (Reimer *et al.*, 2010).

Molecular techniques are currently being applied to determine the differences between marine invertebrate species. Genetic studies on such species resulted in the synonymization of species previously assumed as being discrete because of minor morphological differences in their characteristics (Knowlton, 2000). Over the past two decades, molecular techniques involving allozyme analyses (Burnett *et al.*, 1994, 1995, 1997) and phylogenetics of standardized DNA markers (Reimer *et al.*, 2004, Sinniger *et al.*, 2005, 2008) have been proven to be useful in zoantharian taxonomy. The results of such studies verified the morphological flexibility of certain species (Reimer *et al.*, 2004) and introduced new species (Sinniger & Haussermann, 2009), new genera (Reimer & Fujii, 2010; Sinniger *et al.*, 2010, 2013) and new families (Reimer *et al.*, 2007; Sinniger *et al.*, 2010). Those results also suggested other combinations of genera (Reimer *et al.*, 2006b) and species (Burnett *et al.*, 1997; Reimer *et al.*, 2004).

The Persian Gulf is a semi-enclosed shallow sea situated in the subtropical north-west of the Indian Ocean to which it is

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linked by just a narrow waterway, the Strait of Hormuz. Limited water exchange via this mouth of the Gulf (Pous *et al.*, 2004) in combination with high salinity, large seasonal fluctuations of sea temperature (14–34 °C) and extremely low tides generally result in extreme conditions in the Persian Gulf (Coles & Fadlallah, 1991; Sheppard & Sheppard, 1991). These factors have a profound effect on coral reefs (Wilson *et al.*, 2002), which are the most biologically diverse and economically significant ecosystems on earth, mainly found in the islands situated in the northern parts of the Persian Gulf (Rezai *et al.*, 2010). The Iranian reefs attract international scientific attention due to their development under such extreme environmental conditions (Coles & Fadlallah, 1991).

There are 17 islands off the Iranian coastline of the Persian Gulf with fringing reefs (Sheppard & Sheppard, 1991). Of these, Hengam, Larak, Qeshm and Hormuz Islands are situated in the Strait of Hormuz and greatly influenced by the less saline and nutrient-rich oceanic waters originating from the Oman Sea, especially during the summer monsoon upwelling. Subsequently, reefs around these islands are characterized by relatively high species diversity and richness and differ from those in the inner part of the Persian Gulf (Bauman *et al.*, 2013).

To date, the biodiversity of order Scleractinia (Rezai & Savari, 2004; Rezai *et al.*, 2010; Samiei *et al.*, 2013) and Alcyonaria (Namin & van Ofwegen, 2009) have been the focus of most Persian reef studies. However, despite being the third largest order of Hexacorallia, few studies have been conducted on Zoantharia so far (Noori Koupaei *et al.*, 2014).

The current study examines the diversity of shallow water zoantharians in the Hengam and Larak Islands. The application of mitochondrial markers, cytochrome oxidase subunit [COI] and 16S ribosomal DNA sequences [16S rDNA] throughout this study is an attempt to identify zoantharian species. Molecular data provided by these markers as well as some morphological characteristics and colouration notes of all collected samples can assist in the identification of zoantharians in the northern parts of the Persian Gulf.

MATERIALS AND METHODS

Sampling

Sixty-three colonies of zoantharians representing all observed morphotypes were collected between November 2011 and March 2013 from several sites in the Hengam and Larak Islands by wading in intertidal zones and snorkelling or scuba diving in subtidal zones (Figure 1, Table 1). Photographs were taken on site at the same time as sample collection (Figure 2) to assist with the collection of morphological data. Some morphological characteristics of each specimen including sand encrustation, polyp shape and colour, oral groove, oral zone and oral disc colours as well as tentacle

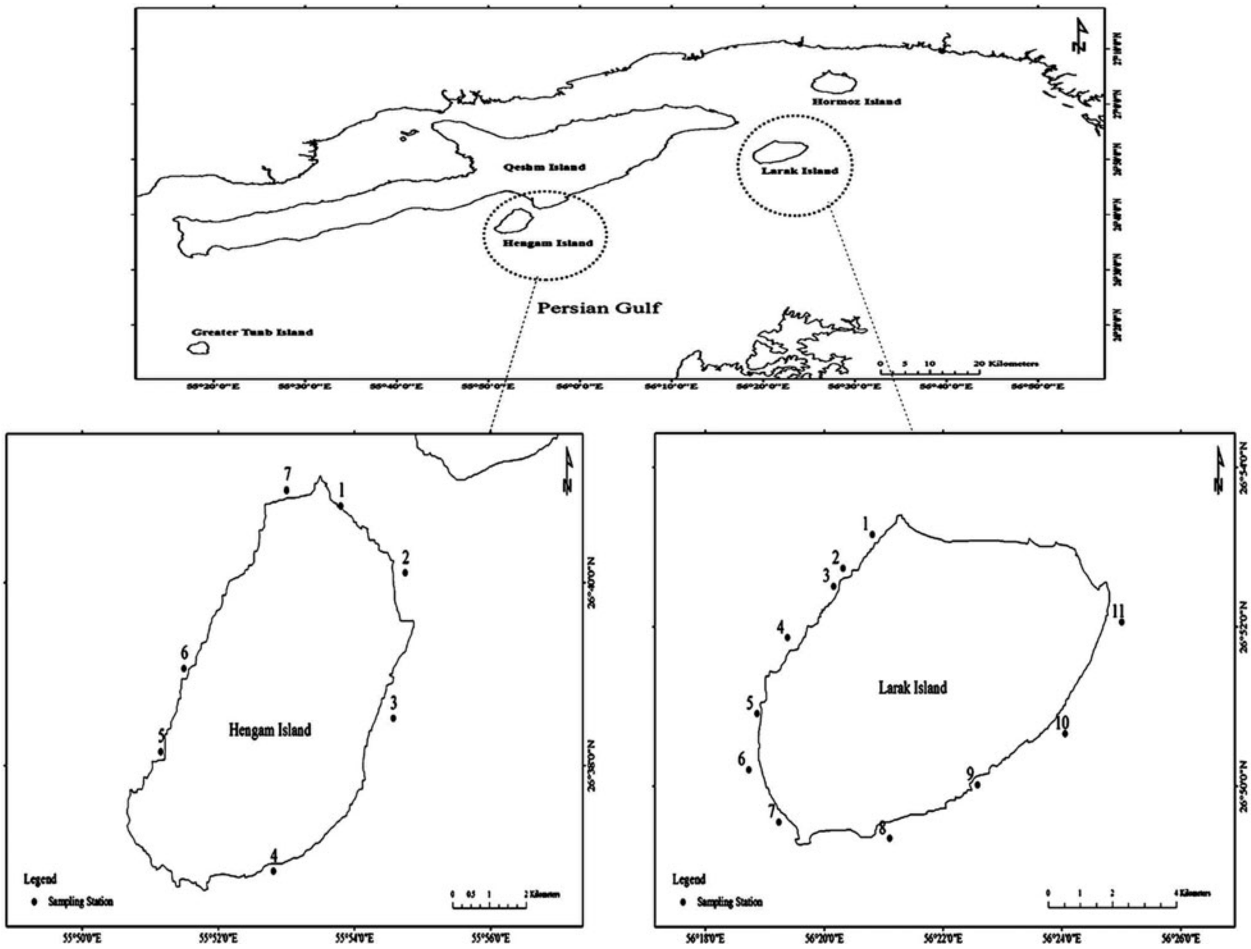

Fig. 1. Map of the Persian Gulf showing the position of the sampling locations (dots) in Hengam and Larak Islands.

Table 1. Sample sites, depth of sampling, collector, accession numbers and final molecular identification of each specimen.

Specimen name	Collection site and depth	Collector	Mt 16srDNA accession numbers	COI accession number	Molecular identification
HeSu1	Hengam Island, S1 (inter-tidal)	H. Dehghani	KJ783918	KJ776766	*Palythoa tuberculosa*
HeSu2	Hengam Island, S1 (inter-tidal)	H. Dehghani	KF733266	NA	*Zoanthus sansibaricus*
HeSu3	Hengam Island, S1 (inter-tidal)	H. Dehghani	KF733267	KJ776774	*Zoanthus sansibaricus*
HeSu4	Hengam Island, S1 (inter-tidal)	H. Dehghani	KF733268	KJ776779	*Zoanthus sansibaricus*
HeSu5	Hengam Island, S1 (inter-tidal)	H. Dehghani	KJ783901	NA	*Palythoa* aff. *mutuki*
HeWi1	Hengam Island, S1 (inter-tidal)	H. Dehghani	KF733277	KJ776780	*Zoanthus sansibaricus*
HeWi2	Hengam Island, S1 (inter-tidal)	H. Dehghani	KJ783908	KJ776765	*Palythoa* aff. *mutuki*
HeWi3	Hengam Island, S2 (2.5 m)	H. Dehghani	KJ783921	KJ776768	*Palythoa tuberculosa*
HeSp1	Hengam Island, S2 (2.5 m)	H. Dehghani	KJ783900	NA	*Palythoa* aff. *mutuki*
HeSu6	Hengam Island, S3 (2.5 m)	H. Dehghani	KJ783932	KJ776756	*Neozoanthus* sp. Iran
HeSu7	Hengam Island, S3 (2.5 m)	H. Dehghani	KF733269	NA	*Zoanthus sansibaricus*
HeWi4	Hengam Island, S3 (2.5 m)	H. Dehghani	KF733278	KJ776778	*Zoanthus sansibaricus*
HeSu8	Hengam Island, S3 (2.5 m)	H. Dehghani	KJ783902	NA	*Palythoa* aff. *mutuki*
HeSu9	Hengam Island, S4 (inter-tidal)	H. Dehghani	KF733270	NA	*Zoanthus sansibaricus*
HeSu10	Hengam Island, S4 (inter-tidal)	H. Dehghani	KJ783919	KJ776767	*Palythoa tuberculosa*
HeSu11	Hengam Island, S4 (inter-tidal)	H. Dehghani	KF733271	NA	*Zoanthus sansibaricus*
HeSu12	Hengam Island, S4 (inter-tidal)	H. Dehghani	KF733272	NA	*Zoanthus sansibaricus*
HeSu13	Hengam Island, S4 (inter-tidal)	H. Dehghani	KJ783903	KJ776762	*Palythoa* aff. *mutuki*
HeSu14	Hengam Island, S4 (inter-tidal)	H. Dehghani	KF733273	NA	*Zoanthus sansibaricus*
HeSp2	Hengam Island, S5 (inter-tidal)	H. Dehghani	KF733264	KJ776776	*Zoanthus sansibaricus*
HeSp3	Hengam Island, S5 (inter-tidal)	H. Dehghani	KF733265	NA	*Zoanthus sansibaricus*
HeSu15	Hengam Island, S5 (inter-tidal)	H. Dehghani	KJ783904	NA	*Palythoa* aff. *mutuki*
HeWi5	Hengam Island, S6 (inter-tidal)	H. Dehghani	KJ783922	KJ776769	*Palythoa tuberculosa*
HeSu16	Hengam Island, S6 (inter-tidal)	H. Dehghani	KF733274	KJ776775	*Zoanthus sansibaricus*
HeSu17	Hengam Island, S6 (inter-tidal)	H. Dehghani	KF733275	NA	*Zoanthus sansibaricus*
HeSu18	Hengam Island, S6 (inter-tidal)	H. Dehghani	KJ783905	KJ776763	*Palythoa* aff. *mutuki*
HeSu19	Hengam Island, S6 (inter-tidal)	H. Dehghani	KJ783906	KJ776764	*Palythoa* aff. *mutuki*
HeSu20	Hengam Island, S6 (inter-tidal)	H. Dehghani	KJ783907	NA	*Palythoa* aff. *mutuki*
HeSu21	Hengam Island, S7 (1.5 m)	H. Dehghani	KF733276	KJ776777	*Zoanthus sansibaricus*
HeSu22	Hengam Island, S7 (1.5 m)	H. Dehghani	KJ783920	NA	*Palythoa tuberculosa*
LaSu1	Larak Island, S1 (inter-tidal)	H. Dehghani	KJ783910	KJ776759	*Palythoa* aff. *mutuki*
LaSu2	Larak Island, S1 (inter-tidal)	H. Dehghani	KJ783923	KJ776770	*Palythoa tuberculosa*
LaSu3	Larak Island, S1 (inter-tidal)	H. Dehghani	KF733289	NA	*Zoanthus sansibaricus*
LaSu4	Larak Island, S1 (inter-tidal)	H. Dehghani	KJ783930	KJ776758	*Palythoa mutuki*
LaSu5	Larak Island, S1 (inter-tidal)	H. Dehghani	KF733290	NA	*Zoanthus sansibaricus*
LaSu6	Larak Island, S2 (inter-tidal)	H. Dehghani	KJ783924	KJ776771	*Palythoa tuberculosa*
LaSu7	Larak Island, S2 (inter-tidal)	H. Dehghani	KJ783911	KJ776760	*Palythoa* aff. *mutuki*
LaSu8	Larak Island, S2 (inter-tidal)	H. Dehghani	KF733291	NA	*Zoanthus sansibaricus*
LaSu9	Larak Island, S2 (inter-tidal)	H. Dehghani	KF733292	NA	*Zoanthus sansibaricus*
LaSu10	Larak Island, S2 (inter-tidal)	H. Dehghani	KJ783912	NA	*Palythoa* aff. *mutuki*
LaSu11	Larak Island, S3 (inter-tidal)	H. Dehghani	KJ783913	NA	*Palythoa* aff. *mutuki*
LaSu12	Larak Island, S3 (inter-tidal)	H. Dehghani	KJ783914	NA	*Palythoa* aff. *mutuki*
LaSu13	Larak Island, S3 (inter-tidal)	H. Dehghani	KF733293	KJ776781	*Zoanthus sansibaricus*
LaWi1	Larak Island, S4 (3 m)	H. Dehghani	KJ783925	KJ776772	*Palythoa tuberculosa*
LaWi2	Larak Island, S4 (3 m)	H. Dehghani	KJ783926	NA	*Palythoa tuberculosa*
LaAu1	Larak Island, S4 (3 m)	H. Dehghani	KF733294	NA	*Zoanthus sansibaricus*
LaAu2	Larak Island, S4 (3 m)	H. Dehghani	JX845311	KJ776782	*Zoanthus sansibaricus*
LaWi3	Larak Island, S4 (3 m)	H. Dehghani	KF733295	KJ776786	*Zoanthus sansibaricus*
LaWi4	Larak Island, S4 (3 m)	H. Dehghani	KJ783916	NA	*Palythoa* aff. *mutuki*
LaAu3	Larak Island, S5 (1 m)	H. Dehghani	JX845313	KJ776783	*Zoanthus sansibaricus*
LaWi5	Larak Island, S5 (1 m)	H. Dehghani	KF733296	NA	*Zoanthus sansibaricus*
LaWi6	Larak Island, S6 (3 m)	H. Dehghani	KJ783927	NA	*Palythoa tuberculosa*
LaAu4	Larak Island, S6 (3 m)	H. Dehghani	KJ783909	NA	*Palythoa* aff. *mutuki*
LaWi7	Larak Island, S6 (3 m)	H. Dehghani	KF733297	NA	*Zoanthus sansibaricus*
LaWi8	Larak Island, S7 (3 m)	H. Dehghani	KJ783917	NA	*Palythoa* aff. *mutuki*
LaAu5	Larak Island, S7 (3 m)	H. Dehghani	JX845314	KJ776784	*Zoanthus sansibaricus*
LaWi9	Larak Island, S8 (3 m)	H. Dehghani	KJ783928	NA	*Palythoa tuberculosa*
LaSu14	Larak Island, S9 (inter-tidal)	H. Dehghani	KJ783915	KJ776761	*Palythoa* aff. *mutuki*
LaWi10	Larak Island, S10 (3 m)	H. Dehghani	KJ783929	KJ776773	*Palythoa tuberculosa*
LaWi11	Larak Island, S10 (3 m)	H. Dehghani	KF733298	NA	*Zoanthus sansibaricus*
LaWi12	Larak Island, S11 (3 m)	H. Dehghani	KJ783931	KJ776757	*Palythoa mutuki*
LaAu6	Larak Island, S11 (3 m)	H. Dehghani	JX845315	KJ776785	*Zoanthus sansibaricus*
LaWi13	Larak Island, S11 (3 m)	H. Dehghani	KF733299	NA	*Zoanthus sansibaricus*

NA = not available.

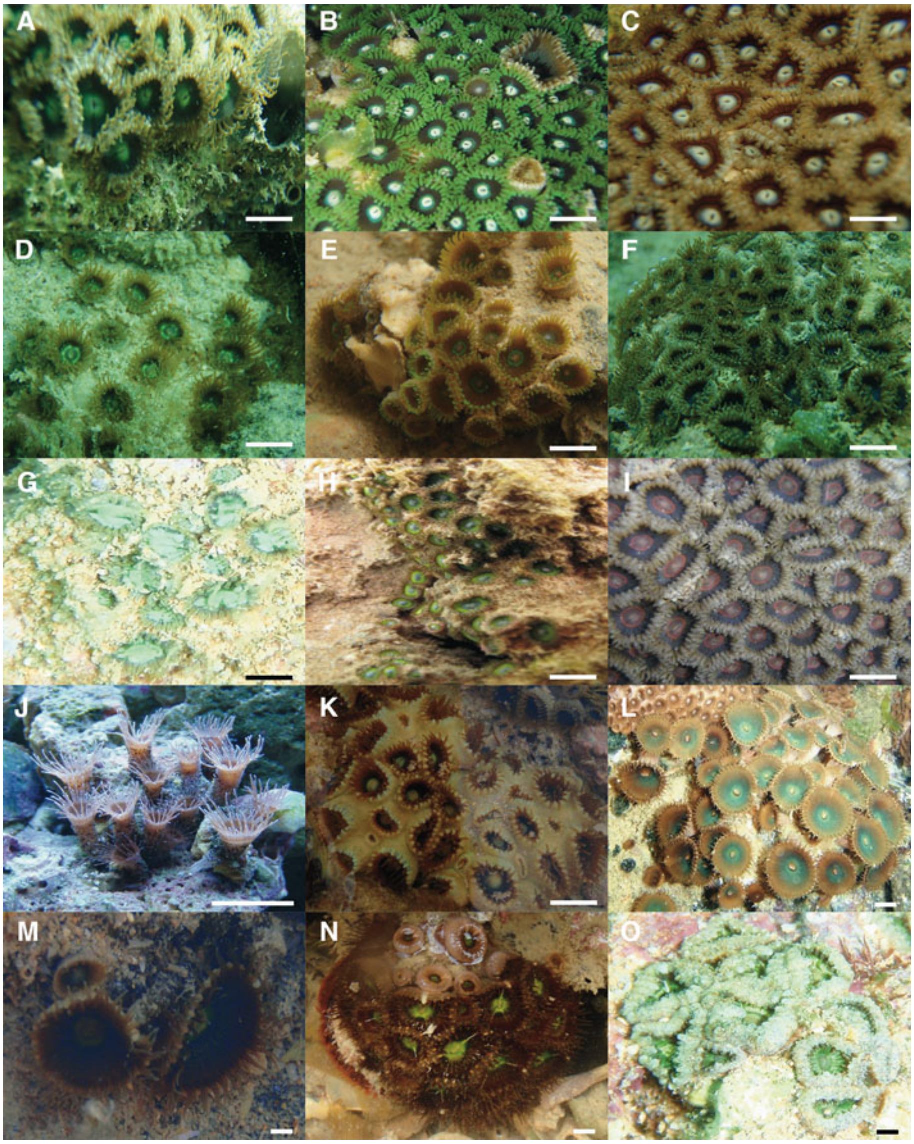

Fig. 2. *In situ* images of zoantharian specimens collected in this study. Each image is representative of one morphological group listed in Table 2. Scale bar = 1 cm.

number and colour were recorded and initial identification was carried out by comparison with published literature (Burnett *et al.*, 1997; Ryland & Lancaster, 2003; Reimer *et al.*, 2006a; Reimer, 2007). Specimens were then stored in absolute ethanol at −20°C for further analyses.

All specimens were finally deposited in the marine biology laboratory of Islamic Azad University, Science and Research branch.

DNA extraction, PCR amplification and sequencing

Parts of the specimens weighing 5–10 mg were crushed in DNAB buffer (0.4 M NaCl, 50 mM EDTA, pH 8.0). DNA was extracted from the obtained slurries using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Baker (1999).

Mitochondrial 16S ribosomal DNA (mt 16S rDNA) was amplified using a set of zoantharian-specific primers provided by Sinniger *et al.* (2005). PCR amplification was performed under the following thermal profile: 2 min at 94°C; 30 s at 94°C, 1 min at 52°C, 90 s at 72°C (35 cycles); and 7 min final extension at 72°C.

The mitochondrial cytochrome oxidase c subunit I (COI) was amplified using the primers described by Folmer *et al.* (1994). The thermal cycle conditions included an initial denaturing step at 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 43°C for 1 min, 72°C for 90 s and a final extension at 72°C for 5 min.

mt 16S rDNA and COI amplification products were visualized by 1.5% agarose gel electrophoresis. Original PCR products from zoantharians specimens were sent to the Macrogen Company in South Korea and directly sequenced with an ABI- 3730XL analyser 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

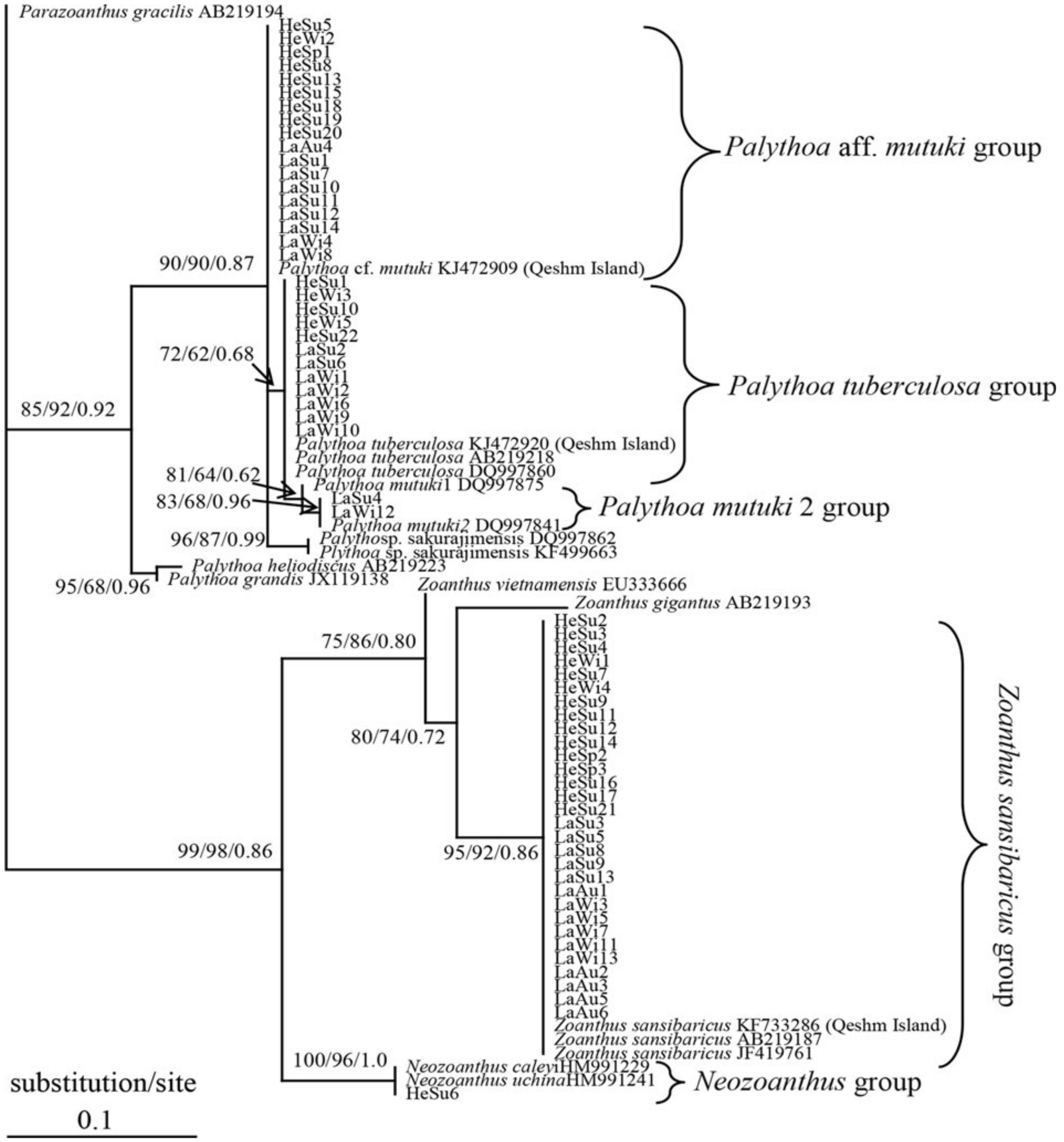

Fig. 3. Maximum likelihood tree of 16S rDNA sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees/Bayesian posterior probabilities.

were aligned with 16S rDNA and COI sequences available from GenBank (accession numbers shown in Figures 3 & 4) using the software CLUSTALW (Thompson *et al.*, 1994). The outgroup sequence for both 16S rDNA and COI tree belonged to the genus *Parazoanthus*. Consequently, two alignment datasets were generated: (1) 965 sites of 81 sequences (mt 16S rDNA) and (2) 655 sites of 44 sequences (COI). The alignment data are available on request from the first author (atoosa.noori.koupaei@gmail.com).

The alignment dataset was analysed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian methods. Maximum-likelihood inference was implemented in RAxML HPC-2 (Stamatakis, 2006; Stamatakis *et al.*, 2008) on the CIPRES Science Gateway V. 3.3 (Miller *et al.*, 2010) by means of the general time-reversible model of nucleotide substitution that is fixed in RAxML and gamma distributed rates across sites. The rapid bootstrapping algorithm was used to obtain clade support values in a maximum likelihood framework performing 1000 replicates. Maximum parsimony analysis was conducted using the PAUP beta version 4.0b10 (Swofford, 2002). For MP, 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. MP clade support was assessed by non-parametric bootstrapping with 1000 replicates and the same heuristic-search parameters. The Bayesian analysis was implemented in MrBayes 2.3 (Ronquist & Huelsenbeck, 2003) and was based on the model

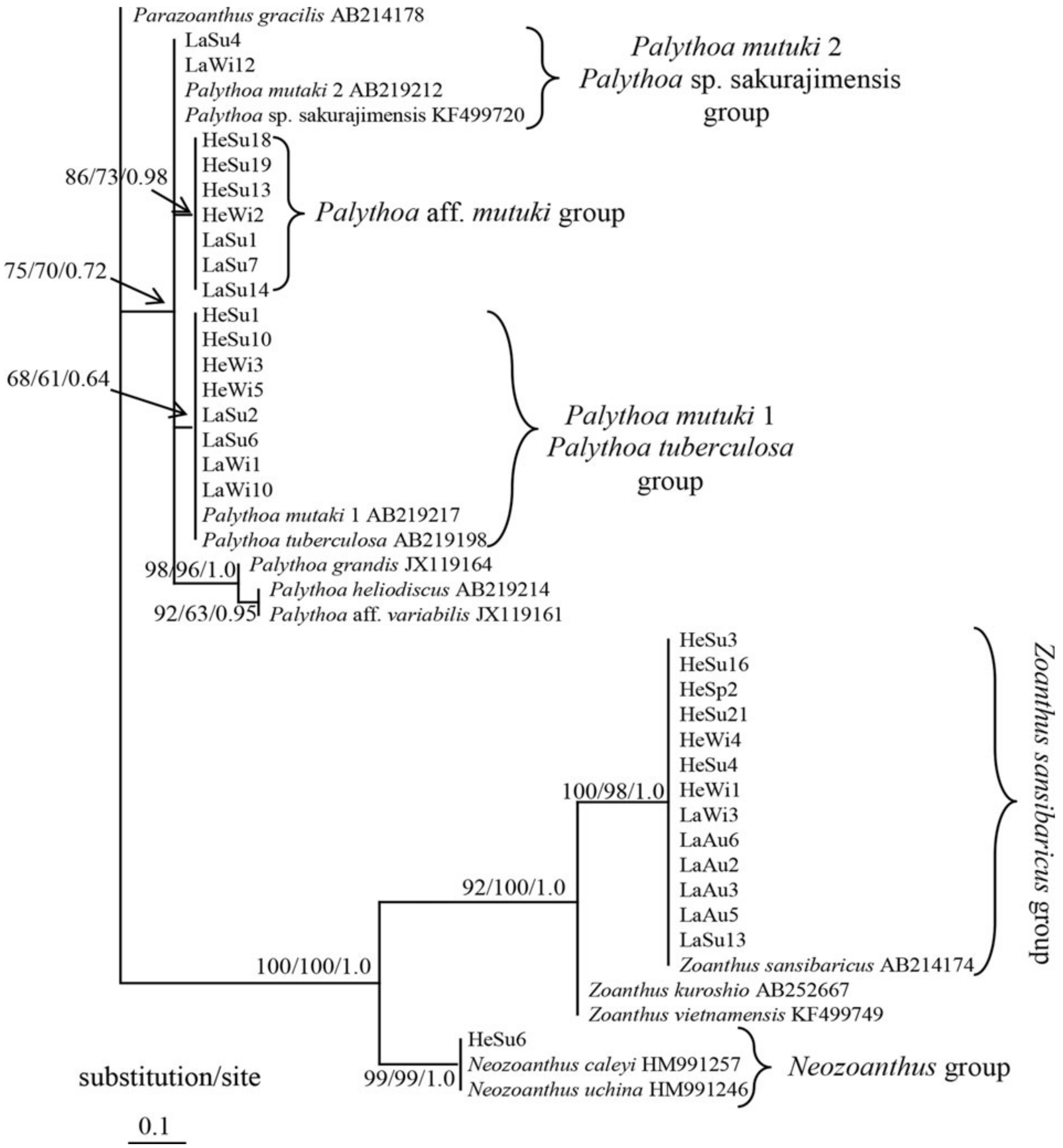

Fig. 4. Maximum likelihood tree of COI sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees/Bayesian posterior probabilities.

selected by MODELTEST. The most appropriate model selection for Bayesian analyses were performed using Akaike Information Criterion (AIC) in MODELTEST 2.3 (Nylander, 2004). The general time-reversible model (Rodriguez *et al.*, 1990) with invariable sites and gamma parameter (GTR + I) gave the best fit to the 16S rDNA data. The Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985) with gamma parameter (HKY) gave the best fit to the COI data. 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.

RESULTS

In situ external appearance observation

All morphological characters are summarized in Table 1. Figure 2 shows images of some specimens, one sample of each morphotype.

From images taken *in situ* (Figure 2), specimens were categorized in four morphological groups. Two groups were identified as belonging to the genus *Palythoa* due to their heavy sand encrustation and light or dark brown exterior. Of these two groups, one is *Palythoa tuberculosa* Esper, 1805 that is easily recognized by its densely packed embedded polyps (immersae) while the *Palythoa mutuki* (Haddon &

Shackleton, 1891) group, is characterized by its elongated, free-standing polyps extending above the underdeveloped coenenchyme (Liberae form), green or brown oral disc and visible white radii. These polyps were joined basally but their closeness and flaring columns obscure the bases (Figure 2, Table 2).

Another group with their smooth, non-encrusted polyps attached to rocks or dead corals, clearly belonged to the genus *Zoanthus*. Since during the present study only chromatic variability of each specimen was recorded, species level assignment was very difficult and they were just identifiable at the genus level. Consequently, the present study placed all *Zoanthus* samples (N = 30) in one group, recording their chromatically morphological characters in Table 2.

The last group, *Neozoanthus* genus, was observed in one sample; only HeSu6. The specimen which was itself quite small in size was encrusted with relatively large particles. Unlike the rest of the column, however, the oral end of the closed polyp was not encrusted. With cone-shaped short tentacles, the specimen was a light pink except for its oral opening which was white (Figure 2, Table 2).

PCR amplification

Amplification of 16S rDNA and COI yielded single compact bands of ~1000 and 700 bp nucleotides in length, respectively.

DNA sequence and phylogenetic analysis

MT 16S RDNA

The resulting phylogenetic tree for the aligned mitochondrial 16S ribosomal DNA sequences is shown in Figure 3. The current study placed mt 16S rDNA sequences from specimens in three large genus-level clades: *Zoanthus, Palythoa* and *Neozoanthus*. All *Zoanthus* spp. sequences were identical to JF419761, DQ997871 and also to a sequence from Qeshm Island (KF733286), pertaining to *Zoanthus sansibaricus* Carlgren, 1900, very well-supported by this clade (ML = 95%, MP = 92%, PP = 0.86). Sequence from putative *Neozoanthus* specimen were found to be closely linked to the *Neozoanthus uchina* Reimer *et al.*, 2012 from Japan and *Neozoanthus caleyi* Reimer *et al.*, 2012 from Australia (ML = 100%, MP = 96%, PP = 1.0). Sequences used in the phylogenetic tree together with the two large clades formed one well-supported large *Zoanthus–Neozoanthus* monophyly (ML = 99%, MP = 98%, PP = 0.86). An insertion of 25 base pairs (at position 531–555 in the mt 16S rDNA alignment) distinguishes the *Neozoanthus* mt 16 S rDNA sequences from the *Zoanthus* and *Palythoa* which do not pose such characteristics.

The genus *Palythoa* was a well-supported clade (ML = 85%, MP = 92%, PP = 0.92). Despite not being highly supported (ML = 72%, MP = 62%, PP = 0.68), putative *Palythoa tuberculosa* specimens all formed a monophyletic clade with reference sequences (DQ997860-AB219218) and previously reported *Palythoa tuberculosa* sequence (KJ472920) from Qeshm Island. The sequences from LaSu4 and LaWi12, two green polyps from *Palythoa mutuki* morphological group, matched exactly with *Palythoa mutuki* 2 (DQ997841) and formed together a well-supported monophyletic clade (ML = 83%, MP = 68%, PP = 0.96). The remaining *Palythoa* sequences had two and three base pair differences from *Palythoa mutuki* 1 and *Palythoa mutuki* 2, respectively. These sequences were identical to sequence KJ472909 from *Palythoa* cf. *mutuki* from Qeshm Island and clustered into strongly supported (ML = 90%, MP = 90%, PP = 0.87) monophyly which was seen to be basal to the *Palythoa mutuki-Palythoa tuberculosa* clade.

COI

Similar to the mt 16S rDNA phylogenetic tree, the resulting tree from the COI alignment (Figure 4) showed that *Zoanthus* spp. clustered with high support (ML = 100%, MP = 98%, PP = 1.0) with *Zoanthus sansibaricus* species.

Sequence from HeSu6 specimen formed a very well-supported clade (ML = 99%, MP = 99%, PP = 1.0) with *Neozoanthus uchina* and *Neozoanthus caleyi* sequences. The *Neozoanthus* clade was again sister to the *Zoanthus* clade, and these two clades formed a highly supported monophyly (ML = 100%, MP = 100%, PP = 1.0), obviously distinct from *Palythoa* sequences.

The genus *Palythoa* was moderately supported (ML = 75%, MP = 70%, PP = 0.72). Putative *Palythoa tuberculosa* specimens formed a moderately supported monophyletic clade (ML = 68%, MP = 61%, PP = 0.64). The sequences from these specimens, HeSu1, HeSu10, HeWi3, HeWi5, LaSu2, Lasu6, LaWi1 and LaWi10, were identical to AB219198 from *Palythoa tuberculosa* and AB219217 from *Palythoa mutuki* 1. Similar to mt 16S rDNA results, the two acquired COI sequences from specimens LaSu4 and LaWi12 were identical to *Palythoa mutuki* 2 sequence (AB219212). However, a COI sequence from *Palythoa* sp. *sakurajimensis* (KF499720) was also seen within this group.

The seven sequences from remaining specimens in the *Palythoa mutuki* morphological group, HeSu18, HeSu19, HeSu13, HeWi2, LaSu1, LaSu7 and LaSu14, had one or two base pairs differences from *Palythoa mutuki* 1 and *Palythoa mutuki* 2, respectively and these formed together a distinct well-supported monophyletic clade (ML = 86%, MP = 73%, PP = 0.98). Therefore, we designated these specimens as *Palythoa* aff. *mutuki* (Figure 4).

DISCUSSION

Previously three species of zoantharians were reported from Qeshm Island based on one mitochondrial marker, 16S rDNA (Noori Koupaei *et al.*, 2014). The present study clearly demonstrated the presence of five species groups in the islands located in the vicinity of the Strait of Hormuz, Persian Gulf. The zoantharians found in this study were *Zoanthus sansibaricus, Palythoa tuberculosa, Palythoa* aff. *mutuki* (also reported in Noori Koupaei *et al.*, 2014), *Palythoa mutuki* and *Neozoanthus* sp. Iran. Of these five species, *Palythoa tuberculosa* was the only one with genetic data that matched its morphology. Despite the lack of ambiguity in identifying *Palythoa tuberculosa*, all samples within *Palythoa* aff. *mutuki* group were initially misidentified as *Palythoa mutuki* due to their morphological appearance. However, molecular examination of two mitochondrial markers (16S rDNA and COI) showed that they were not conspecific with this known species. These specimens were seen to be closely related to a specimen previously reported from

Table 2. Summary of morphological characteristics of collected zoantharians and final molecular identification.

Morphological group	Group name	Sample name	Polyp form, colour	Oral colour	Oral zone colour	Oral disc colour	Tentacle number, colour	Final molecular identification
Zoanthus sp.	a	HeSu2-HeSu3-HeSu9-HeSu11-HeSu16-LaSu3-LaSu5-LaSu8-LaSu13-LaAu1-LaWi11-LaWi13	Liberae, purple	White	Fluorescent green with fluorescent green circle around it	Dark green	Greenish brown, sim 47–52	*Zoanthus sansibaricus*
	b	HeSu4-HeSu12-HeSu17-LaSu9-LaAu5-LaWi5	Liberae, purple	White	White with fluorescent green circle around it	Dark green	Fluorescent light green, sim 42–54	*Zoanthus sansibaricus*
	c	HeSu14-HeWi1-LaAu3-LaWi7	Liberae, purple	White	White with red circle around it	Red	Light brown, sim 47	*Zoanthus sansibaricus*
	d	HeSp2-HeSp3-HeSu7	Liberae, purple	White	Fluorescent green with fluorescent green circle around it	Green and white	Light brown, sim 45–48	*Zoanthus sansibaricus*
	e	HeWi4	Liberae, purple	White	Light brown with fluorescent green circle around it	Brown	Fluorescent light green, sim 47	*Zoanthus sansibaricus*
	f	HeSu21	Liberae, purple	White	Cream with cream circle around it	Dark blue	Brownish green, sim 48	*Zoanthus sansibaricus*
	g	LaWi3	Liberae, fluorescent green	Fluorescent green	Fluorescent green	Fluorescent green	Fluorescent green, sim 36	*Zoanthus sansibaricus*
	h	LaAu6	Liberae, purple	White	White	Fluorescent green	Light brown, not distinguishable	*Zoanthus sansibaricus*
	i	LaAu2	Liberae, purple	White	Pink with pink circle around it	Gray	Light brown, sim 45	*Zoanthus sansibaricus*
Neozoanthus sp.	j	HeSu6	Liberae, light pink	White	Light pink	Light pink	Light pink, sim 38–40	*Neozoanthus* sp. Iran
Palythoa tuberculosa	k	HeSu1-HeSu22-HeSu10-HeWi3-HeWi5-LaSu2-LaSu6-LaWi1-LaWi2-LaWi6-LaWi9-LaWi10	Immerse, brown	Cream	Cream	Dark brown	Brown, sim 30–34	*Palythoa tuberculosa*
Palythoa mutuki	l	HeSu8-HeSu18-HeSu19-HeSu20-LaSu7-LaSu12-LaSu14-LaAu4-LaWi4	Liberae, light brown	White	Light brown	Green-brown	Brown, sim 45	*Palythoa* aff. *mutuki*
	m	HeSp1-HeSu5-HeSu13-HeSu15-LaSu1-LaSu10-LaSu11-LaWi8	Liberae, light brown	White	Light brown	Green	Brown, sim 54–67	*Palythoa* aff. *mutuki*
	n	HeWi2	Liberae, light brown	White	Fluorescent green	Dark brown	Dark brown, sim 50	*Palythoa* aff. *mutuki*
	o	LaSu2-LaWi12	Liberae, light green	White	Light green	Light green	Light brown, not distinguishable	*Palythoa mutuki*

Qeshm Island which was identified as *Palythoa* cf. *mutuki* based on mt 16S rDNA phylogeny.

While the mitochondrial markers are very conservative in anthozoans (Shearer *et al*., 2002), a low level of variation indicates species level differences in *Palythoa* species. For example, *Palythoa mutuki* and *Palythoa tuberculosa* can be distinguished by one or two base pair of differences over the mt 16S rDNA dataset. (Reimer *et al*., 2006b; Sinniger *et al*., 2008). Although COI sequences are unable to distinguish between *Palythoa tuberculosa* and *Palythoa mutuki* 1 (Reimer *et al*., 2006b), this marker made a clear distinction between *Palythoa* aff. *mutuki* and *Palythoa tuberculosa*.

Results obtained from the sequencing of mitochondrial markers indicated that all *Zoanthus* morphotypes were entirely conspecific despite variation in colour. The previous published data (Burnett *et al*., 1997; Reimer *et al*., 2004, 2006a, 2011b) demonstrate extreme difficulty or inability to identify proper species of the genus *Zoanthus* based on morphological characters. The *Zoanthus sansibaricus* species, in particular, comes in a wide variety of colourful types.

Based on external appearance, the HeSu6 specimen, a unique specimen collected in this study, corresponds morphologically to the genus *Neozoanthus*. The *Neozoanthus* in this study is morphologically characterized by partially encrusted particles of various sizes (i.e. lacking encrustation on the oral part of closed polyps) and 38–40 relatively short and conical tentacles which match the typical characteristics of *Neozoanthus* (Herberts, 1972). Colour was the only morphological characteristic that did not fit with the three previously defined species of this genus (Herberts, 1972; Reimer *et al*., 2012), which has been shown to be highly variable in many zoantharian species (Burnett *et al*., 1997; Reimer *et al*., 2004).

Zoantharians do not have accurate morphological markers to find out inter-species differentiation. However, the two mitochondrial molecular markers (i.e. mt16S rDNA and COI) are successfully used to identify most zoantharian species (Reimer *et al*., 2006a, 2007, 2011a, 2011b; Sinniger *et al*., 2008; Swain, 2009). Similar to previous studies (Burnett *et al*., 1997; Reimer *et al*., 2004), data presented in this study indicated that while morphological markers were not adequate to properly clarify the status of species, the genetic method was a useful tool for correctly placing all specimens within a species group and discerning phylogenetic relationships.

Similar to Qeshm Island (Noori Koupaei *et al*., 2014), the most commonly seen species in this study were *Zoanthus sansibaricus, Palythoa tuberculosa* and *Palythoa* aff. *mutuki*. Since these three studied islands are located in the Strait of Hormuz, the similarity in zoantharian diversity is not surprising.

As mentioned earlier, there is an overall lack of data on the Persian Gulf zoantharians. However, based on the worldwide distribution patterns of zoantharians, the Persian Gulf zoantharians diversity observed in this and the recent study followed the same patterns of zoantharians observed in shallow subtropical and tropical waters (Herberts, 1972; Burnett *et al*., 1997; Ryland & Lancaster, 2003; Reimer & Todd, 2009). Zooxanthellate zoantharians of the genera *Palythoa* (family Sphenopidae) and *Zoanthus* (family Zoanthidae) are common in infra-littoral and shallow subtropical and tropical waters, particularly coral reef ecosystems. *Zoanthus sansibaricus, Palythoa tuberculosa* and *Palythoa mutuki*, three reported species here, are previously described as widely distributed in the western and eastern Pacific Ocean; i.e. Great Barrier Reef (Burnett *et al*., 1997; Ryland & Lancaster, 2003), New Caledonia (Sinniger, 2006), Japan (Reimer *et al*., 2004, 2006a, b; Reimer, 2007), Galapagos (Reimer *et al*., 2008), Singapore (Reimer & Todd, 2009) and the Indian Ocean (Herberts, 1972). Conversely, *Palythoa* aff. *mutuki* was shown to have significant phylogenetic differences with *Palythoa mutuki* and other described *Palythoa* species indicating that it might be an undescribed species.

During the present study, one specimen of an unknown zoantharian fitting the morphology of *Neozoanthus* was collected from Hengam Island. The monotypic genus *Neozoanthus*, a single genus in the third shallow water zoantharian family, Neozoanthidae, represents a unique evolutionary step in the zoantharian phylogeny as the only partially encrusted group of zoantharians (Reimer *et al*., 2011b). The *Neozoanthus* specimen from this study showed unique oral disc colouration and patterns. Additionally, its mt 16S rDNA sequences did not match with published sequences (HM991243 and HM991257) and phylogenetic analysis placed this specimen in a different clade. However, species identification based on COI marker could not distinguish between *Neozoanthus* species. COI sequences of specimen HeSu6 were identical to *Neozoanthus uchina* from Okinawa Island, Japan and *Neozoanthus caleyi* from GBR, Australia. Overall, it is concluded that the specimen HeSu6 represents an undescribed species within *Neozoanthus*, preliminarily designated as *Neozoanthus* sp. The genus *Neozoanthus* was described in 1972 from specimens found in Madagascar (Herberts, 1972), and was reported again nearly 40 years later from Japan and Australia (Reimer *et al*., 2011a, 2012, 2013). Finding this genus for the first time in the Persian Gulf, which is located between Madagascar, the Great Barrier Reef and Japan with thousands of kilometres distances, demonstrates the wide distributional range of this genus.

In this study, three families, three genera and five species of zoantharians, including potentially two new species, were found in the Persian Gulf. Molecular identification techniques combined with morphological observations have been proved to be functional in identifying collected zoantharian specimens.

Concerning the existence of at least two undescribed species in the waters of the studied islands, it appears that more research may result in finding more species in the region.

Undoubtedly, future research should be conducted on zoantharians in other parts of the Persian Gulf and also the surrounding area. Since little information exists on zoantharians from this region, it is not clear whether these two potentially new species are endemic to this area or originated in the Oman Sea and Indian Ocean.

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