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Molecular Systematics of Zoantharian species (Cnidaria) and their Endosymbionts in the Bostaneh Bay, the Persian Gulf

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Abstract
Zoantharians have an important ecological role in marine ecosystems. Despite their worldwide distribution, studies have been conducted in some areas of the Persian Gulf, these investigations do not cover all regions. For this purpose, 18 samples of zoantharian colonies in three transects of shallow waters of Bostaneh port were collected by snorkeling and scuba diving. 4 extra colonies also were sampled in the intertidal zone of Khark Island for comparison with the sampling areas of BP. All zoantharian specimens were photographed in situ and morphological characteristics of each specimen were recorded, i. e. polyp shape, polyp color, tentacle number and color, external coloration, oral plate color and oral region color. 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, three species were identified: *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki* and *Palythoa tuberculosa*. We developed quantitative PCR (qPCR) assays to distinguish each of the three genera (*Symbiodinium*, *Cladocopium* and *Durusdinium*) of dinoflagellate endosymbionts through three clade-specific primer sets aiming at ITS1-5.8S-ITS2 (for *Symbiodinium*), partial ITS-1 (for *Cladocopium*), and LSU (for *Durusdinium*) of the nuclear rDNA of Symbiodiniaceae from which, (genus *Symbiodinium*) commonly found in Zoantharia. In this study, the Zoantharia of Bostaneh Port were identified for the first time.

Keywords: Zoantharian, COI, 16S rDNA, Persian Gulf, Bostaneh, *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, *Palythoa tuberculosa*, Symbiodiniaceae, *Symbiodinium*, *Cladocopium*, *Durusdinium*

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INTRODUCTION

The order Zoantharia, which belongs to class Anthozoa, comprises 27 genera allocated to 9 families in 2 suborders: Brachycnemina and Macrocnemina (Belford, 2023; Reimer & Sinniger, 2018). This order is one of the hexacorallian groups that has been categorized by morphological and molecular methods (Bazzaz *et al*., 2024). They are characterized by two rows of tentacles in each polyp and one ventral siphonoglyph, and they form a colony (Haddon & Shackleton, 1891). Despite worldwide distribution in

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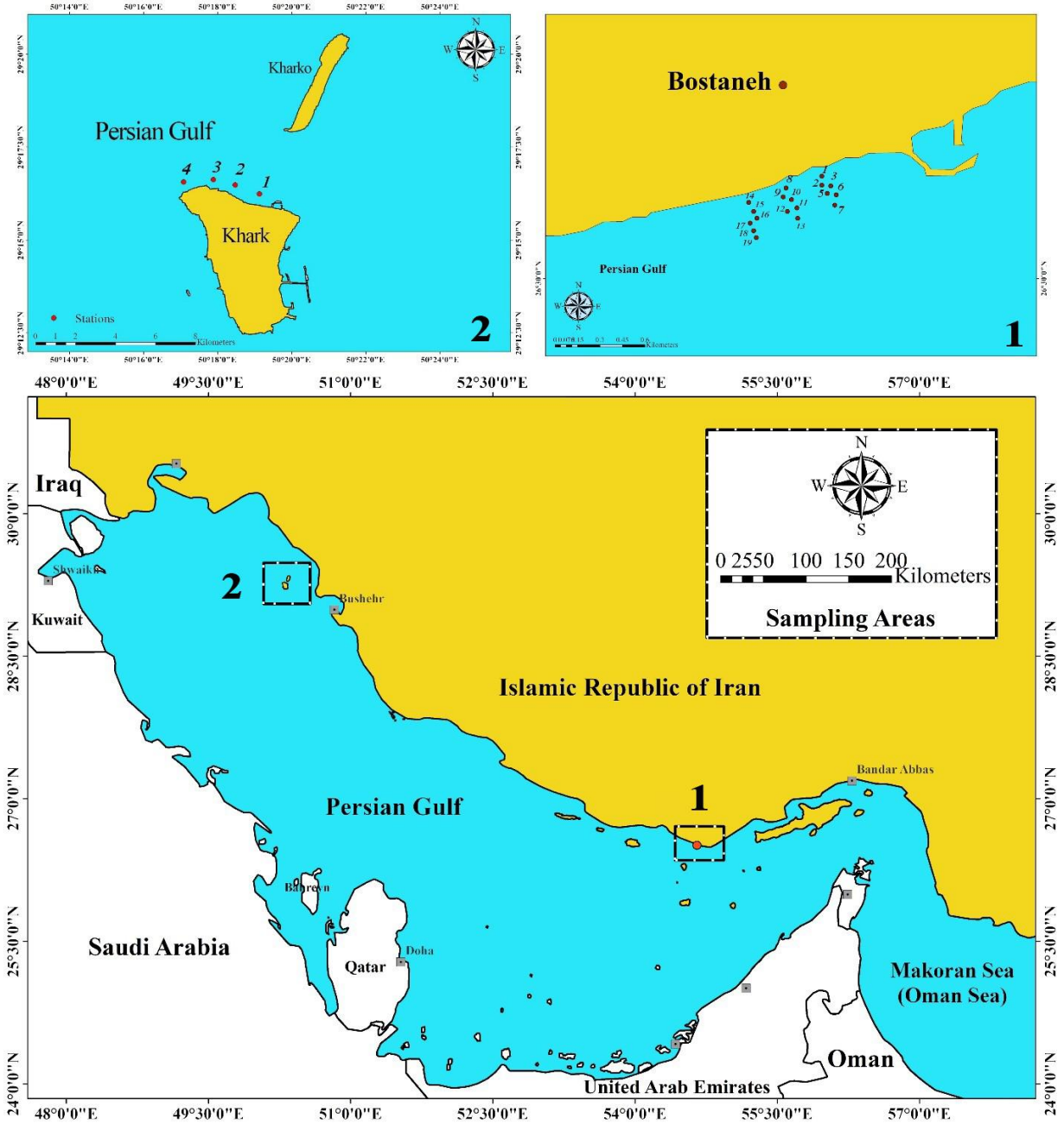

FIGURE 1. Map of the Persian Gulf showing the position of the sampling locations in the (1) Bostaneh Bay and (2) Khark Island.

tropical and subtropical waters (Reimer *et al.*, 2004; Reimer *et al.*, 2012), zoantharians and symbiodiniaceae genera have yet to be identified across all regions of the Persian Gulf.

Zoantharian is the ecologically significant group that is connected to reef-building corals (Reimer *et al.*, 2022). They make mutualistic symbioses with unicellular algae of the Symbiodiniaceae family (Fujiwara *et al.*, 2021). A genus Symbiodinium is a large group of dinoflagellates living as endosymbionts in the endoderm of tropical and subtropical cnidarians, where the products of their photosynthetic processing are exchanged in the host for the inorganic molecules (Baker, 2003; Muscatine & Cernichiari, 1969; Noda *et al.*, 2017). The ability of symbiotic micro-algae not only to carry out

photosynthesis but also to translocate the photosynthetic products to their host species is believed to be the foundation of the success of coral reef-dwelling species such as corals and mollusks (Garcia-Ramos & Banaszak, 2007).

The Persian Gulf is a large, shallow, semi-enclosed marginal sea located in the subtropical northwest of the Indian Ocean. This region is characterized by extreme thermal variability and high salinity, and as a result, many organisms in this area are living near the margins of their physiological limits. Bostaneh port is in the central part of Bandar Langeh city in Hormozgan province, situated on the northern coast of the Persian Gulf. Bostaneh Fishing Port is situated at the geographic coordinates of 26.511°N and 54.653°E and features a seabed characterized by rocky and coral textures. These beds have a diverse range of benthic species due to their distance from urban communities. From the high diversity qualities of marine invertebrate creatures displayed in this ecoregion, the beginning taxonomical endeavors had devoted towards the zoantharian due to their wide substrate cover and taxonomical differing qualities. Comparatively few investigations have been done on the diversity of zoantharian taxonomy in the Persian Gulf: Hormoz, Qeshm, Kish, Hengam, and Larak islands (Koupaei *et al*., 2016a; Koupaei *et al*., 2014; Koupaei *et al*., 2016b; Koupaei *et al*., 2018), Farur, Bani Farur, Hormoz and Chabahar Bay (Panahi Bazzaz *et al.*, 2024). Also, through these investigations, the following species had been recorded: *Zoanthus sansibaricus*, *Z.* sp., *Palythoa* aff. *mutuki*, *Palythoa tuberculosa*, *Palythoa* cf. *mutuki*, and *Neozoanthus* sp.

Due to the lack of existing information on zoantharians in the coastal waters of Bostaneh, we undertook a comprehensive assessment of these organisms using both morphological and molecular approaches. Zoantharian species were identified through mitochondrial markers, including cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (16S rDNA), and the associated Symbiodiniaceae genera were also characterized. This study represents the first documentation of molecular data for these benthic organisms along the northern coast of the Persian Gulf. Furthermore, because no prior research has investigated zoantharians within the islands of Bushehr Province, additional sampling was carried out in the intertidal zone of Khark Island to enable comparison with the sampling sites in Bostaneh Port.

MATERIAL AND METHODS

Specimen collection

In mid-November 2023, 18 samples of zoantharian colonies in three transects of shallow waters of Bandar Bostaneh port (26° 30′ 37″ N 54° 39′ 20″ E), Hormozgan Province, and 4 zoantharian colonies in the intertidal zone of Khark island have been collected by snorkeling in lower intertidal (1-1.5 m depth) and by scuba diving in subtidal zones (2-3.5 m depth) during 3 days (Fig.1 & Table 1). The line Intercept Transect (LIT) method was adopted to estimate intertidal and subtidal zoanthid distribution pattern and abundance. Water temperature and salinity were measured simultaneously using Horiba U10 probe. Specimens were stored in a 20% dimethyl sulfoxide (DMSO) saturated by NaCl buffer and were transferred to the Razi laboratory in Science and Research Branch, IAU for further analysis.

Morphological analyses

All zontharian were photographed in situ and identified following valid identification keys (López *et al*., 2019; Mizuyama *et al*., 2018; Reimer & Sinniger, 2018) based on external morphological features: polyp shape, polyp color, tentacle number and color, external coloration, oral plate color, and oral region color (Fig.2).

DNA extraction, PCR amplification and sequencing

DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Baker, 1999). Amplification of mitochondrial cytochrome oxidase c subunit I (COI) has been done using a set of primers (For: 5'-GGTCAACAAATCATAAAGATATTGG-3' and Rev: 5'-TAAACTTCAGGGTGACCA

TABLE 1. Sample sites, depth of sampling, collector, geographical positions and temperature- salinity results

Specimen name	Collection site and depth	Collector	Longitude	Latitude	Temperature (°c)	Salinity (psu)
Bost 1	Bostaneh bay, (1m)	M.Q.Ashrafi	54°39′21.6″	26°30′21.6″	22.71± 0.18	37.20± 0.21
Bost 2	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′20.99″	26°30′20.122″	22.31± 0.11	37.22± 0.18
Bost 3	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′22.931″	26°30′19.973″	22.12± 0.21	37.45± 0.10
Bost 5	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′22.187″	26°30′18.335″	21.45± 0.20	36.94± 0.11
Bost 6	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′24.123″	26°30′18.037″	21.32± 0.17	36.55± 0.22
Bost 7	Bostaneh bay, (3.5 m)	M.Q.Ashrafi	54°39′23.825″	26°30′15.804″	21.54± 0.10	36.90± 0.23
Bost 8	Bostaneh bay, (1m)	M.Q.Ashrafi	54°39′13.252″	26°30′19.526″	21.45± 0.12	36.76± 0.32
Bost 9	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′12.656″	26°30′17.59″	22.40 ± 0.12	36.29± 0.31
Bost 10	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′14.443″	26°30′16.995″	22.40± 0.16	36.47± 0.21
Bost 11	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′15.635″	26°30′15.208″	22.23± 0.18	37.14± 0.19
Bost 12	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′13.55″	26°30′14.463″	22.15± 0.11	37.54± 0.20
Bost 13	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′15.784″	26°30′12.974″	22.38± 0.19	37.43± 0.33
Bost 14	Bostaneh bay, (1m)	M.Q.Ashrafi	54°39′5.36″	26°30′16.25″	22.41± 0.23	37.45± 0.16
Bost 15	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′6.253″	26°30′14.463″	23.15± 0.27	37.11± 0.27
Bost 16	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′6.998″	26°30′12.974″	23.18± 0.35	37.17± 0.11
Bost 17	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′5.509″	26°30′11.932″	23.23± 0.15	37.61± 0.35
Bost 18	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′6.253″	26°30′10.294″	21.88± 0.24	37.20± 0.12
Bost 19	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′6.849″	26°30′8.805″	21.46± 0.19	37.30± 0.25
Kharg 1	Kharg island, (3.5m)	M.Q.Ashrafi	50°16′58.8″	29°16′30″	23.18± 0.12	38.15± 0.22
Kharg 2	Kharg island, (3.5m)	M.Q.Ashrafi	50°17′42″	29°16′40.8″	24.11± 0.20	37.84± 0.21
Kharg 3	Kharg island, (3.5m)	M.Q.Ashrafi	50°18′32.4″	29°16′37.2″	24.16± 0.14	38.10 ± 0.10
Kharg 4	Kharg island, (3.5m)	M.Q.Ashrafi	50°19′26.4″	29°16′19.2″	23.97± 0.17	38.19± 0.35

AAAAATCA-3') (Folmer *et al*, 1994). Cytochrome oxidase 1 (COI) gene amplification with a length of 760 base pairs was carried out under the following thermal cycle condition: 30 cycles including 30 seconds at 95°C for denaturation, 30 seconds at 47°C for annealing, and 90 seconds at 72°C for extension. For additional precision, mitochondrial 16S ribosomal DNA (mt 16S rDNA) with a length of 1000 base pairs were amplified using specific primers of zoantharian (For: 5'- GCCATGAGTATAGACGCACA -3'

and Rev: 5'- CGAACAGCCAACCCTTGG -3') provided by Sinniger et al, 2005. PCR amplification was performed under the following thermal condition: 30 cycles, including 30 (s) at 94°C for denaturation, 1 minute at 54°C for annealing, and 90 (s) at 72°C for elongation. Also, three clade-specific primers had been utilized to acquire the replications of Symbiodiniaceae genus genes. These primer sets aiming ITS 1-5.8 S-ITS 2 regions (for *Symbiodinium*, F: 5'-CCTCTTGGACCTTCCACAAC-3' and R: 5'-GCATGCAGCAACACTGCTC-3') (Correa *et al.*, 2009), LSU (for *Cladocopium*, F: AAGGAGAAGTCGTAACAAGGTTTCC and R: AAGCATCCCTCACAGCCAAA) (Ulstrup & Van Oppen, 2003), and domain 2 of the LSU (for *Durusdinium*, F: 5'-GCCGTGTACGGTGCTC GCTCTCAA-3' and R: 5'-GGCCACTCGCAA ATGGACAGC-3') (Correa et al., 2009).

Quantitative PCR

Three clade-specific primer sets aiming at ITS1-5.8S-ITS2 (for *Symbiodinium*) and LSU (for *Cladocopium* and *Durusdinium*) of the nuclear rDNA of *Symbiodinium* were approved in terms of their specificity and efficiency. The thermal condition of qPCR includes 35 cycles of 30 (s) at 94°C for denaturation, 30 (s) at 60°C for annealing, and 30 (s) at 72°C for extension. Reaction voluminosity was achieved using 8 µL ddH_2O, 10 µL SYBR Green Master Mix (2x), 0.5 µL each of forward and reverse (10 µm), and 1 µL template DNA (430 ng/ml). To compare the threshold cycle results for all three genera, equal concentrations of the genomes were used during qPCR (Correa et al., 2009).

Phylogenetic Analysis

The accession numbers that are acquired from this investigation have been submitted to GenBank and have been shown in the table 3. The aligned sequences were analyzed by way of Maximum Likelihood, Maximum Parsimony and Bayesian procedures.

Zoantharia

The genomic sequences obtained from this study, were aligned with COI and 16S-rDNA sequences from NCBI's nucleotide database utilizing the BLAST online software and therefore aligned with earlier reported zoantharian sequences on GenBank, applying the CluscalW algorithm (Thompson *et al.*, 1994). For analyzing the alignment databases, maximum likelihood (ML), maximum parsimony (MP), and Bayesian methods were used.

Maximum likelihood conclusion was accomplished in MEGA 7 software (Kumar *et al.*, 2016) by setting 1000 bootstrap replications. In order to analyze Maximum Parsimony, the PAUP 4.0 beta win version was used (Swofford, 2000). The Bayesian analysis was conducted in MrBayes 2.3 (Ronquist & Huelsenbeck, 2003) and was based on the model selected through MODELTEST 2.3 (Nylander, 2004). For selecting the best model for both ML and Bayesian, the Akaike Information Criterion was used. The Hasegawa–Kishino–Yano model (HKY) was the best fit for both ML and MrBayes of COI data (Hasegawa *et al*., 1985). For the data of 16S-rDNA, the general time-reversible (GTR) model (Rodriguez *et al*., 1990) with invariable sites (GTR + I) gave the best-fit model.

Symbiodiniaceae

The sequences obtained from our study were aligned by the ITS1-5.8S-ITS2 (for *Symbiodinium*), partial ITS-1 (for *Cladocopium*), and LSU (for *Durusdinium*) with the recorded sequences of GenBank. Sequences obtained from this study were deposited in GenBank, and their accession numbers are provided in the table 3. The new nucleotide sequences acquired during the present study were aligned with sequences available from GenBank utilizing the software CLUSTALW (Thompson *et al.*, 1994). Three alignment datasets for the following genus (*Symbiodinium*, *Cladocopium*, and *Durusdinium*) were generated and analyzed through utilizing maximum likelihood (ML), maximum parsimony (MP), and Bayesian methods. The most qualified model selection for ML and Bayesian analyses was carried out using the Akaike Information Criterion (AIC) in MODELTEST 2.3 (Nylander, 2004). The general time-

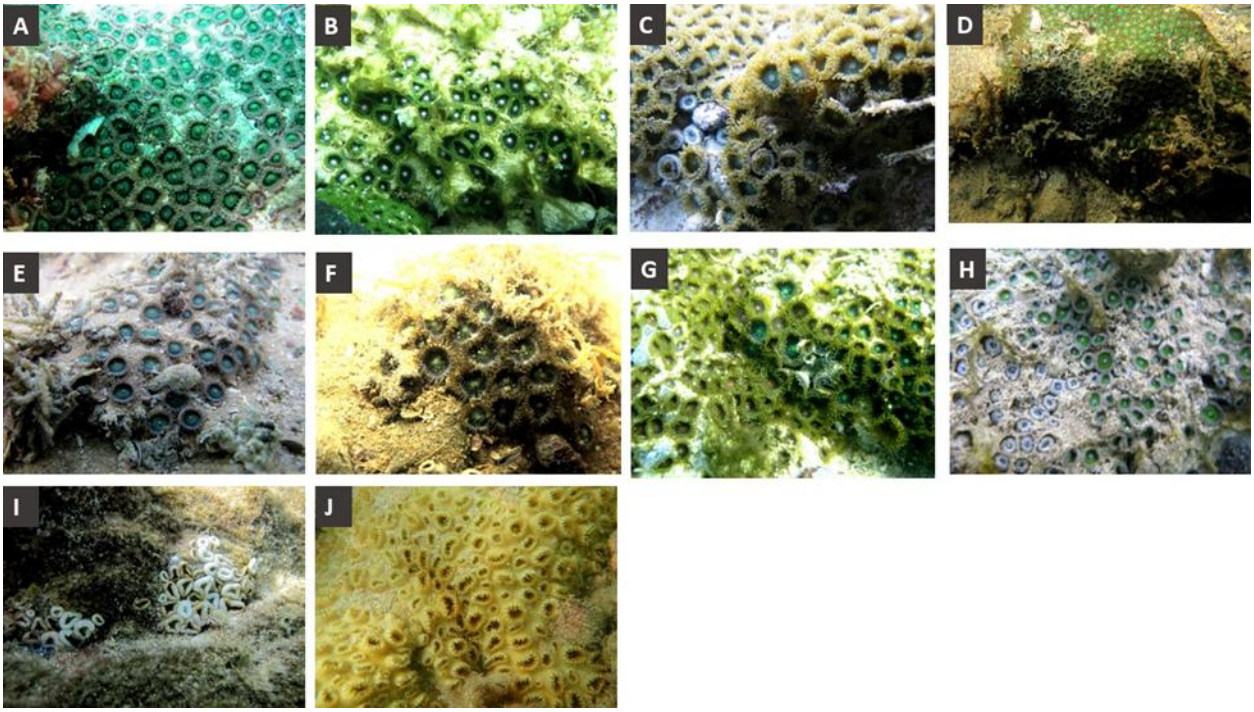

FIGURE 2. In situ images of zoanthid specimens collected in this study. Each image is representative of one morphological group listed in Table1.

reversible model with a gamma parameter (GTR+G+I) presents the best selection for the data (*Symbiodinium*, *Cladocopium*, and *Durusdinium*). Maximum likelihood and maximum parsimony analyses were accomplished by MEGA7 software and PAUP beta version 4.0b10 individually. ML and MP trees were evaluated with 1,000 bootstrap replicates.

RESULTS

In situ external appearance observation

The morphological identification recognized three species: *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, and *Palythoa tuberculosa*, which belong to two families, Zoanthidae and Sphenopidae. Morphological features are placed in a table based on verified identification keys (Koupaei *et al.*, 2016; Koupaei *et al.*, 2018; López *et al*., 2019; Mizuyama *et al*., 2018; Reimer & Sinniger, 2018). Also, the images that have been taken from specimens in situ have been shown in Figure 2.

DNA sequence and phylogenetic analysis

COI

COI gene fragment sequences were obtained from 18 samples of Bostaneh port and 4 samples of Kharg island. The outgroup sequence for both the COI and 16S-rDNA trees was *Parazoanthus gracilis*. The results from the COI alignment tree presented that the sequences of these specimens (Bost: 15/16/9/2/17/14/11/10/8/3/6/13/7/1/12 and Kharg: 1/2/3) were identical to *Zoanthus sansibaricus*. with high support (ML=96%, MP=99%, PP=1). The *Zoanthus kuroshio* and *Zoanthus gigantus* were sister to the *Zoanthus sansibaricus* cluster with great support (ML=99%, MP=100%, PP=1), apparently distinct from *Palythoa* spp. The *Palythoa* genus was supported moderately (ML=96%, MP=74%, PP=0.9). *Palythoa tuberculosa* species create a moderately supported cluster (ML=61%, MP=64%, PP=0.99). Two sequences, specimens Kharg4 and Bost5, had one or two base pair differences from *Palythoa mutuki* and formed a distinct moderate cluster (ML=63%, MP=65%, PP=0.95). Consequently, we define them as *Palythoa* aff. *mutuki* (Fig. 3).

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 sansibaricus.	A	Kharg 1- Kharg 2	Liberae, Light Brown	Fluorescent green	Dark grey with fluorescent green circle around it	Dark green	Light brown, approx. 56-61	*Zoanthus sansibaricus*
	B	Bost 10- Bost 7- Bost 9	Liberae, Brown	White	White, with gray circle and red circle around it	Purple	Fluorescent green, approx. 49-58	*Zoanthus sansibaricus*
	C	Bost 3- Bost 2	Liberae, Light Brown	White	Brown with fluorescent green circle around it	Purple with white lines	Light brown, approx. 48	*Zoanthus sansibaricus*
	D	Bost 1- Kharg 3	Liberae, Light Brown	White	Dark green with white lines	Light green	Light brown, approx. 60	*Zoanthus sansibaricus*
	E	Bost 12- Bost 14- Bost 16	Liberae, Light Brown	Light grey	Light grey with fluorescent green circle	Purple with white lines	Light brown, approx. 58-65	*Zoanthus sansibaricus*
	F	Bost 6- Bost 15- Bost 17	Liberae, Light Brown	Light grey	Dark grey with light green circle	Dark green with white lines	Light brown, approx. 54-70	*Zoanthus sansibaricus*
	G	Bost 13- Bost 11	Liberae, Brown	Some light green, Some white	Some red, Some green	Purple with white lines	Light brown, approx. 52	*Zoanthus sansibaricus*
	H	Bost 8	Liberae, Light cream	White	Purple	Fluorescent green	Cream, approx. 50	*Zoanthus sansibaricus*
Palythoa aff. *mutuki*	I	Kharg 4- Bost 5	Liberae, cream	White	Cream	Green to brown with white lines	Brown, approx. 62	*Palythoa* aff. *mutuki*
Palythoa tuberculosa	J	Bost 19- Bost 18	Immersae, Brown	Cream	Cream	Brown	Brown, approx. 30	*Palythoa tuberculosa*

Mt 16s- rDNA

The sequences of the mt 16S-rDNA gene fragment were obtained from 18 samples collected in the Bostaneh area and 4 samples collected from Kharg Island. In the phylogenetic trees obtained by matching mt 16S-rDNA sequences, the genera *Zoanthus* and *Palythoa* each formed a large clade. Bootstrap values and posterior probability for *Zoanthus* and *Palythoa* clades are as follows, respectively: *Zoanthus* (ML=96%, MP=100%, and PP=0.1) and *Palythoa* (ML=99%, MP=91%, and PP=0.57) (Fig 4).

SYMBIODINIACEAE FAMILY

The results of phylogenetic trees showed that there are three genera of the Symbiodiniaceae family from Bostaneh port: *Symbiodinium*, *Cladocopium*, and *Durusdinium*. Sequences belonging to *Symbiodinium* (ITS1-5.8S-ITS2) were identified with high support (ML=100%) from *Palythoa* aff. *mutuki*, *Zoanthus sansibaricus*, and *Palythoa tuberculosa*; *Cladocopium* was well supported (ML=92%) from *P.* aff. *mutuki*, Z. sansibaricus, and *P. tuberculosa*; and *Durusdinium* was well supported (ML=99%) from *P.* aff. *mutuki*, *Z. sansibaricus*, and *P. tuberculosa*. Figs. 4, 5, and 6 show the ML tree for the aligned sequences. Because of the similarity in the tree topologies in all of the analyses, the bootstrap values of Mr. Bayes and MP are shown on the ML tree.

REAL-TIME PCR

Detection of *Symbiodinium*, *Cladocopium* and *Durusdinium* DNA

The average threshold cycle (Ct) was obtained from quantitative PCR of each of the studied groups. Examining the Ct of the samples showed that the Ct obtained from the PCR related to the genus *Symbiodinium* was lower compared to other genera, which means that this genus was more abundant in the studied zoantharian samples. Also, the information obtained from quantitative PCR showed that the Ct related to the *Durusdinium* genus was higher than other genera, which means less frequency of this

genus in the studied samples. The results of the quantitative comparison of three species of symbiotic micro algae in the hosts of *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, and *Palythoa tuberculosa* are given in Table 2. The ratio of species to each other was obtained from the following formula, where Ct was the threshold cycle associated with each extracted DNA sample (Correa *et al*., 2009). *Clade x (DNA) / Clade y (DNA)= 2* $^{(Ct\ Clade\ y-\ Ct\ Clade\ x)}$

DISCUSSION

The aim of this investigation was identifying the zoantharian species of Bostaneh bay through morphological features and the mitochondrial molecular markers include COI and 16s-rDNA, which were applied successfully to distinguish the most species of them (Kise *et al*., 2021; Koupaei *et al*., 2014; Koupaei *et al*., 2018; López *et al*., 2019; Bazzaz *et al*., 2024).

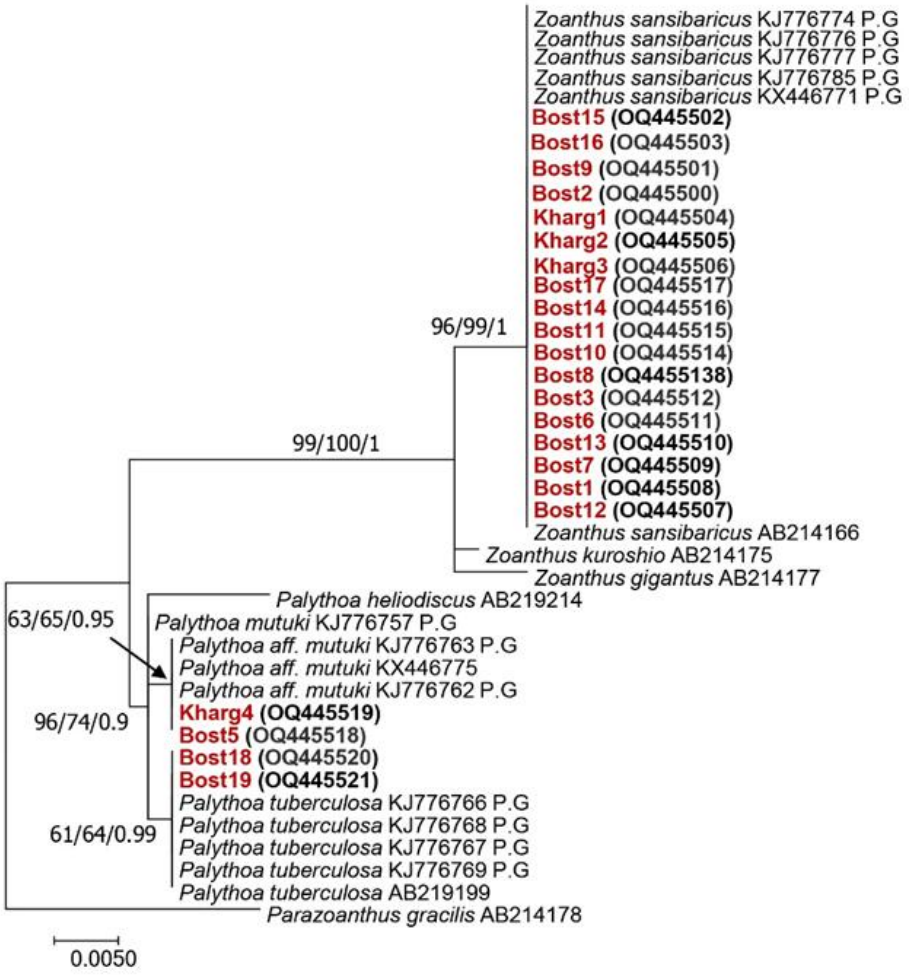

FIGURE 3. Maximum likelihood tree of COI sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees/Bayesian posterior probabilities.

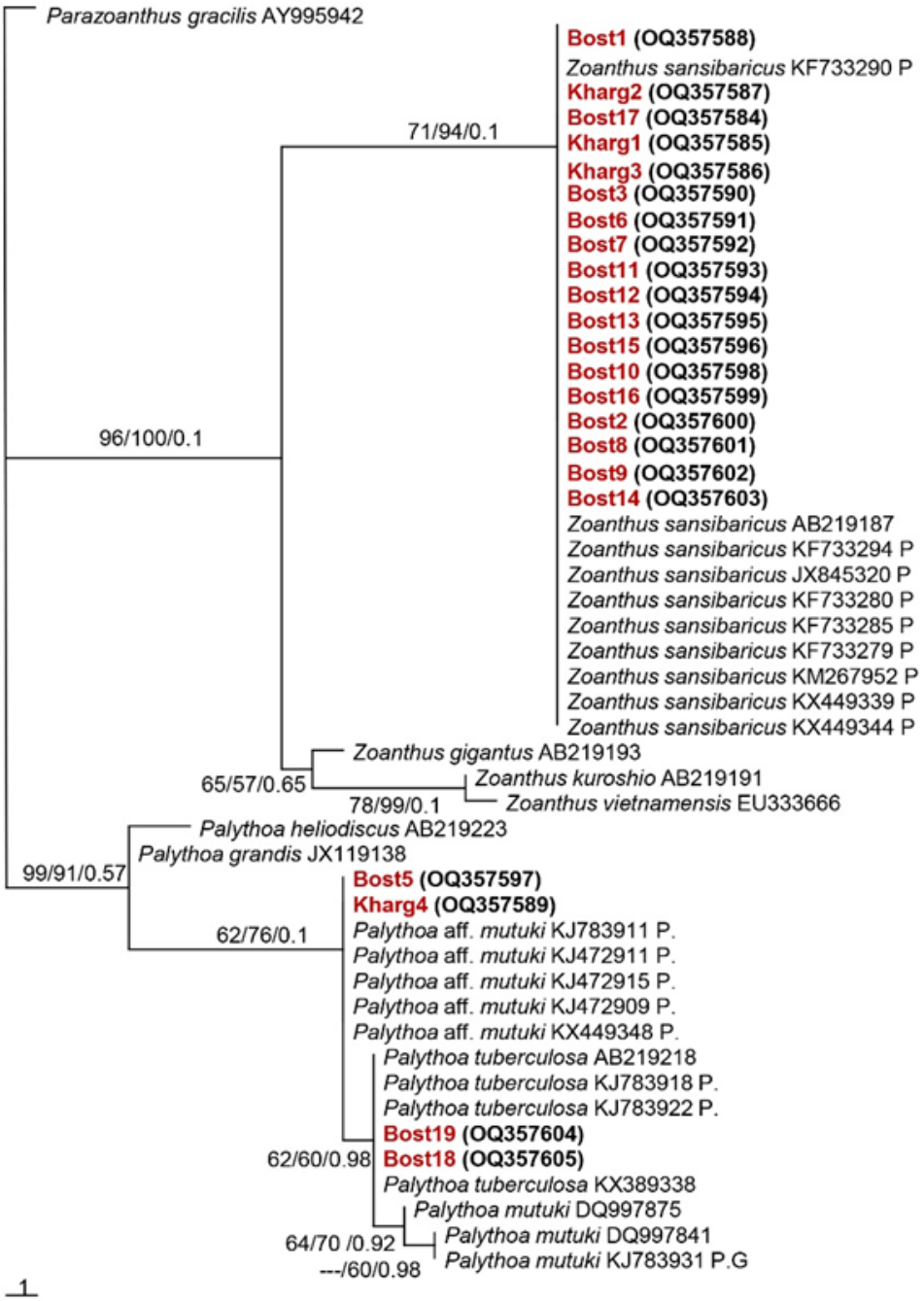

FIGURE 4. Maximum likelihood tree of 16s-rDNA sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees/Bayesian posterior probabilities.

TABLE 3. accession numbers and final molecular identification of each specimen**.**

Specimen name	Mt 16srDNA accession numbers	COI accession numbers	Molecular identification
Bost 1	OQ445508	OQ357588	*Zoanthus sansibaricus*
Bost 2	OQ445500	OQ357600	*Zoanthus sansibaricus*
Bost 3	OQ445512	OQ357590	*Zoanthus sansibaricus*
Bost 5	OQ445518	OQ357597	*Palythoa aff. mutuki*
Bost 6	OQ445511	OQ357591	*Zoanthus sansibaricus*
Bost 7	OQ445509	OQ357592	*Zoanthus sansibaricus*
Bost 8	OQ445513	OQ357601	*Zoanthus sansibaricus*
Bost 9	OQ445501	OQ357602	*Zoanthus sansibaricus*
Bost 10	OQ445514	OQ357598	*Zoanthus sansibaricus*
Bost 11	OQ445515	OQ357593	*Zoanthus sansibaricus*
Bost 12	OQ445507	OQ357594	*Zoanthus sansibaricus*
Bost 13	OQ445510	OQ357595	*Zoanthus sansibaricus*
Bost 14	OQ445516	OQ357603	*Zoanthus sansibaricus*
Bost 15	OQ445502	OQ357596	*Zoanthus sansibaricus*
Bost 16	OQ445503	OQ357599	*Zoanthus sansibaricus*
Bost 17	OQ445517	OQ357584	*Zoanthus sansibaricus*
Bost 18	OQ445520	OQ357605	*Palythoa tuberculosa*
Bost 19	OQ445521	OQ357604	*Palythoa tuberculosa*
Kharg 1	OQ445504	OQ357585	*Zoanthus sansibaricus*
Kharg 2	OQ445505	OQ357587	*Zoanthus sansibaricus*
Kharg 3	OQ445506	OQ357586	*Zoanthus sansibaricus*
Kharg 4	OQ445519	OQ357589	*Palythoa* aff. *mutuki*

Formerly, five species of zoantharian were reported from the Persian Gulf through the mitochondrial markers (COI and 16S-rDNA) (i.e., Z.*sansibaricus*, *Palythoa* aff. *mutuki*, *Palythoa tuberculosa*, *Palythoa* mutuki, *Neozoanthus* sp.) which were collected from Kish, Hormoz, Lark, Hengam, Qeshm, Farur, BaniFarur islands and Chabahar Bay (Koupaei *et al.*, 2014; Koupaei *et al.*, 2016a,b ; Koupaei *et al.*, 2018; Bazzaz *et al.*, 2024). The present investigation vividly demonstrated the presence of three species groups in Bostaneh port, which included *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, and *Palythoa tuberculosa*, also reported in Koupaei et al. (2014), Koupaei et al. (2016a,b), Koupaei et al. (2018) and Bazzaz et al. (2024). Mitochondrial marker sequencing revealed that all Zoanthus morphotypes, despite their wide variation in coloration, belonged to the same species. In particular, every specimen analyzed was identified as *Zoanthus sansibaricus*, a species known for its extensive range of color forms.As can be seen in Fig 3, all eighteen *Zoanthus* specimens of our investigation formed a well-supported sub-cladal monophyly (ML=96%, MP=99%, PP=1) with *Zoanthus sansibaricus* reported from the Persian Gulf, Hengam (KJ776774, KJ776776, KJ776777), Larak (KJ776785), Kish (KX446771), and Pacific Ocean, Japan (AB214166). Within the *Palythoa* genus, sequences Bost18 and Bost19 were identical to each other and also to *Palythoa tuberculosa* sequences from the Persian Gulf, Hengam (KJ776766, KJ776768, KJ776767, KJ776769), and Japan (AB219199) (ML=61%, MP=64%, PP=0.99). Nevertheless, Kharg4 and Bost5, being somewhat distant from *Palythoa mutuki*, resolved with formerly reported *Palythoa* aff. *mutuki* from the Persian Gulf, Hengam (KJ776763, KJ776762), and Kish (KX446775) (ML=63%, MP=65%, PP=0.95)(Koupaei *et al*, 2016; Koupaei *et al.*, 2018). Although the anthozoans' mitochondrial markers are really conservative (Shearer *et al*, 2002), a low level of variation displays species-level differences in *Palythoa* species. The position of these specimens in the phylogeny trees was comparable to that of the *Palythoa* sp. collected from Sakurajima and Ogasawara Island, which was introduced as *Palythoa* aff.*mutuki* in a study by (Koupaei *et al.*, 2016a).

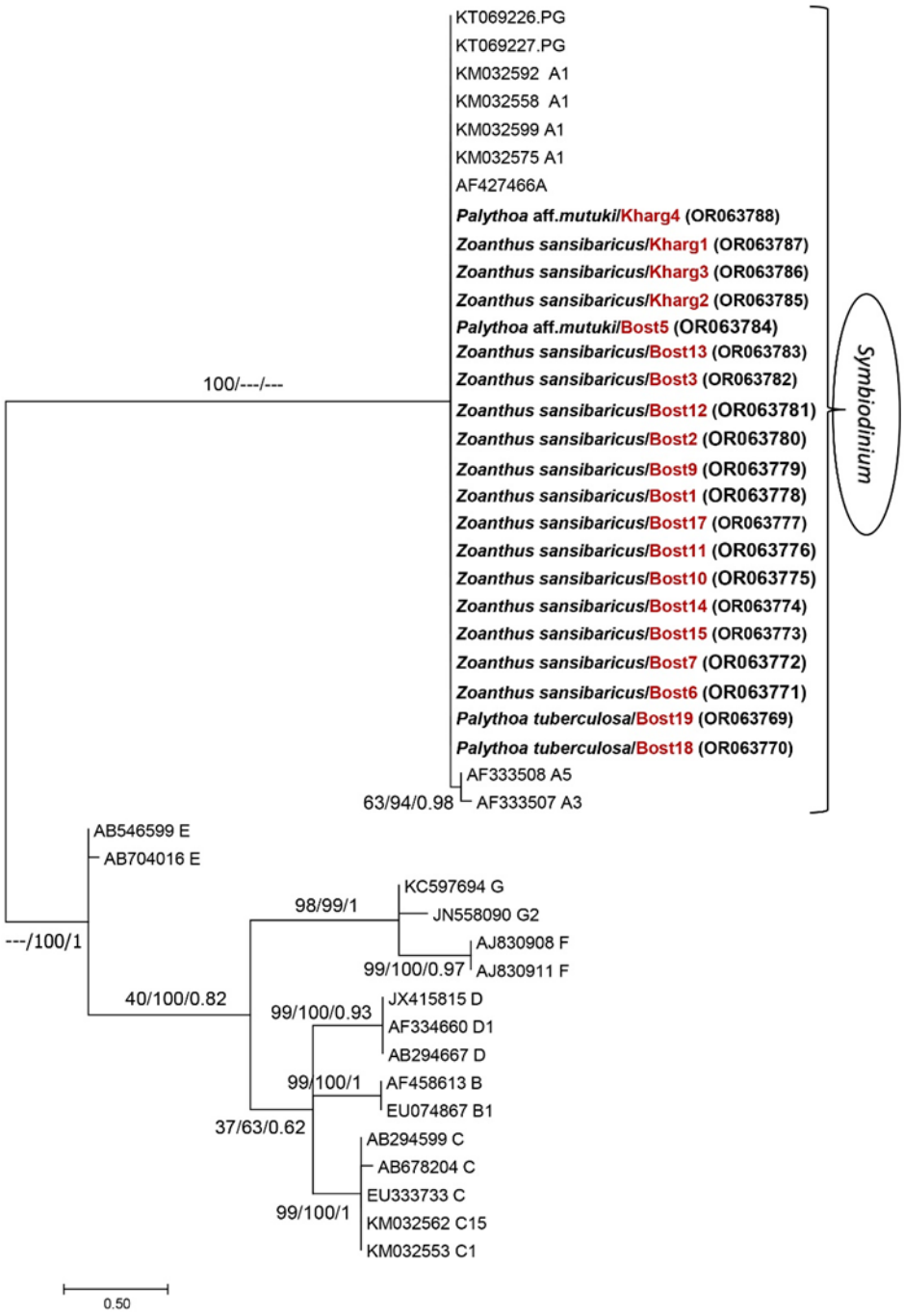

FIGURE 5. Maximum likelihood tree of ITS1-5.8S-ITS2 sequences for *Symbiodinium*. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/ maximum parsimony bootstrap percentages from 1000 trees /Bayesian posterior probabilities.

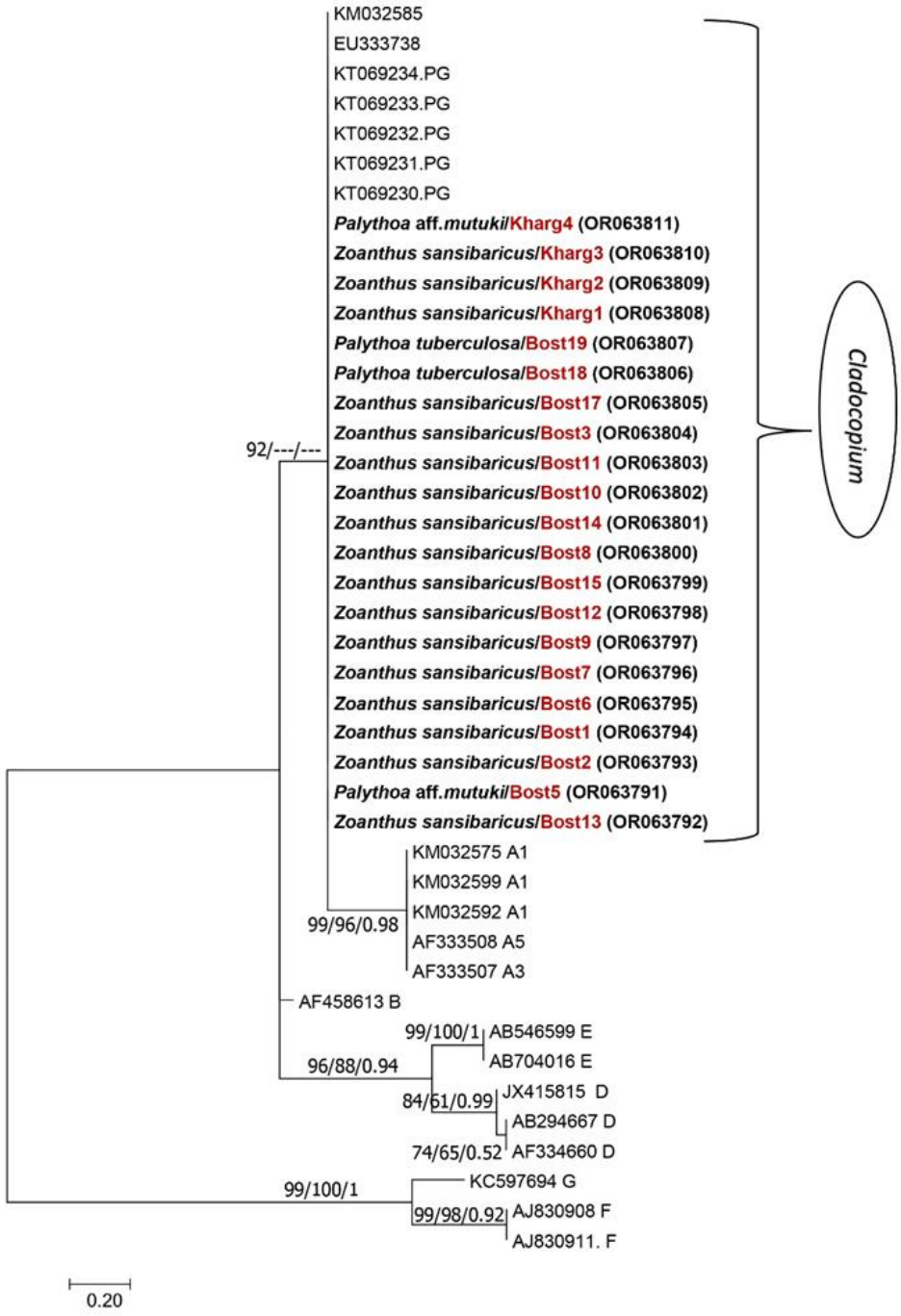

FIGURE 6. Maximum likelihood tree of partial ITS 1 for *Cladocopium*. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/ maximum parsimony bootstrap percentages from 1000 trees /Bayesian posterior probabilities.

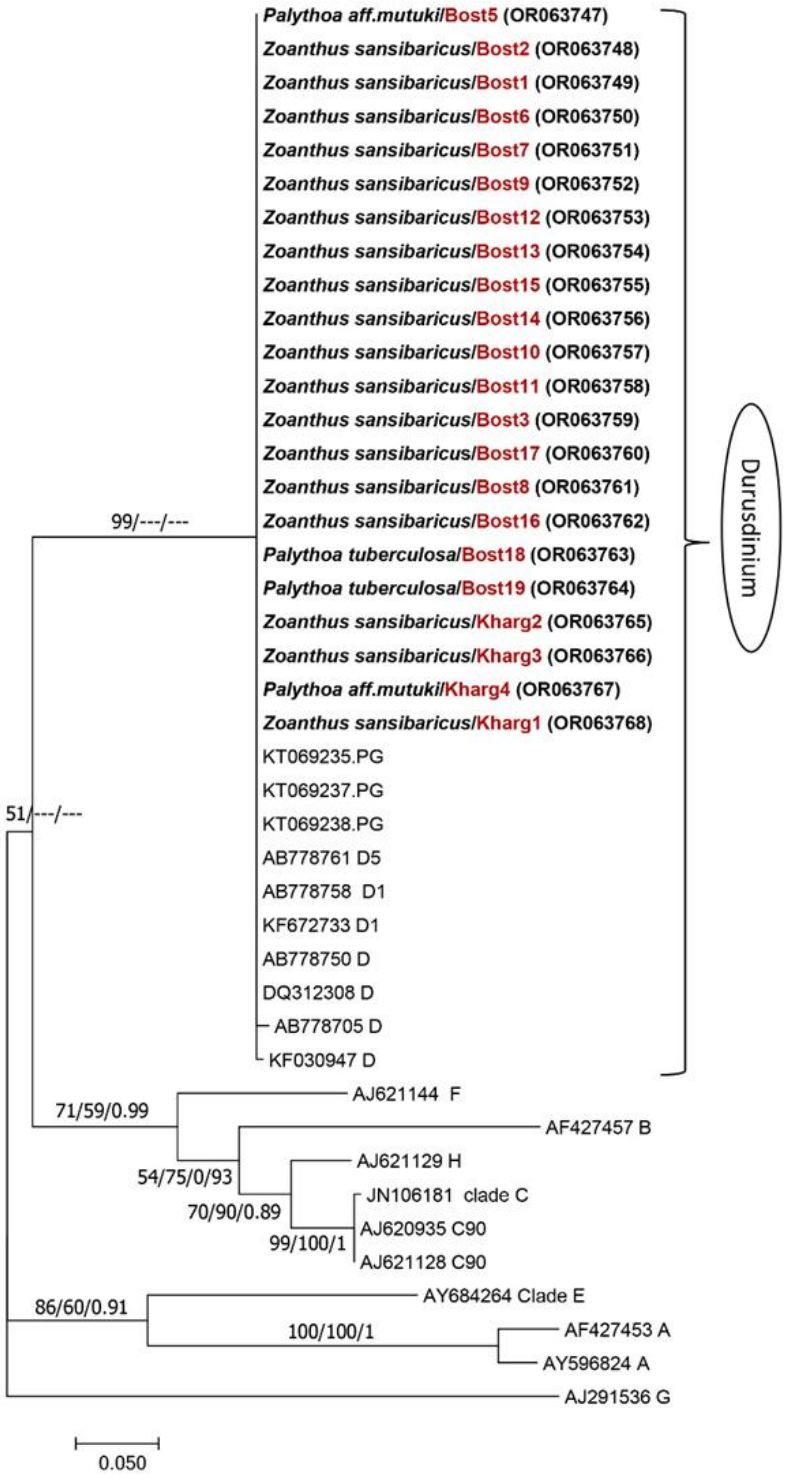

FIGURE 7. Maximum likelihood tree of LSU for *Durusdinium*. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/ maximum parsimony bootstrap percentages from 1000 trees /Bayesian posterior probabilities

TABLE 4. Zooxanthellae species abundance ratio based on quantitative comparison

Host Zoantharian		*Symbiodinium*/*Cladocopium*	*Cladocopium*/*Durusdinium*	*Durusdinium*/*Symbiodinium*
Zoanthus sansibaricus	Bost 1	75.58	107.63	8135.41
	Bost 2	427.57	4.35	1858.60
	Bost 3	1234.75	7.46	9216.48
	Bost 4	1209.34	2.58	3125.78
	Bost 6	9089.59	0.40	3615.55
	Bost 7	537.45	82.71	44453.21
	Bost 8	*Cladocopium* only	0.81	*Durusdinium* only
	Bost 9	0.19	0.37	0.07
	Bost 10	14562.80	0.52	7590.61
	Bost 11	6746.86	0.62	4153.18
	Bost 12	8135.41	1.59	12944.04
	Bost 13	0.01	30.70	0.38
	Bost 14	16728.26	1.02	17079.76
	Bost 15	7.62	5.86	44.63
	Bost 16	*Durusdinium* only	*Durusdinium* only	*Durusdinium* only
	Bost 17	891.44	14.12	12590.08
	Kharg 1	942.27	4.06	3821.70
	Kharg 2	3848.29	1.54	5914.33
	Kharg 3	1200.98	5.94	7131.55
Palythoa aff. *mutuki*	Bost 5	0.00	230.72	0.20
	Kharg 4	1.27	237.21	302.33
	Bost 19	278.20	6.36	1770.57

The present study results were compared with historical data in the literature and recent results from the Indian ocean, the Red Sea and the Persian Gulf. Results show a relatively limited number of zoantharian species documented, despite being an early area of study within the Indo-Pacific (Reimer et al., 2017). The largest number of zoantharian species identified in the Red Sea belongs to the study by Reimer et al (2017) with 13 species in 2017. Kumari et al, (2015) and Risi and Macdonald (2016) each identified seven species of zoantharian in the Indian Ocean and Kise (2019) identified three new species from the Indopacific Ocean. This is while three species have been identified in the present study, which is similar to studies conducted in Qeshm, Hormuz and Kish Islands by Koupaei et al., (2014, 2015, 2018) with three species, Darvishi et al., (2017) on the coast of Bushehr with four species and Koupaei et al., (2016a,b) in Larak Island with five species.

Comparing the obtained sequences by mt 16s-rDNA with the previously reported sequences showed that all samples of the *Zoanthus* morphological group are consistent with the recorded sequences of the *Zoanthus sansibaricus* species from the Persian Gulf, Larak (KF733290, KF733294), Qeshm (JX845320, KF733280, KF733285, KF733279), Hormuz (KM267952), Kish (KX449339, KX449344) (Koupaei et al. 2014, 2016), and Japan (Reimer *et al*. 2006a) (AB219187) with high support (ML=71%, MP=94%, and PP=0.1). The sequences of the morphological group (Bost18 and Bost19) of *Palythoa tuberculosa* also completely matched the recorded sequences of this species from the Persian Gulf, Hengam (KJ783918, KJ783922), and southwestern Japan (AB219218), which is supported moderately (ML=62%, MP=60%, and PP=0.98) (Koupaei et al. 2014, 2016; Reimer *et al*. 2006b). However, the sequences obtained from the samples of the morphological group of *Palythoa mutuki* (Bost5 and Kharg4),

having two nucleotide differences with the samples from Japan and Persian Gulf (DQ997841, DQ997875) and Larak (KJ783931) and supported moderately (ML=62%, MP=76%, and PP=0.1) with the recorded sequences of *Palythoa* aff. *mutuki*, which are introduced from the Persian Gulf, Larak (KJ783911), Qeshm (KJ472911, KJ472915, KJ472909), and Kish (KX449348) (Koupaei et al. 2014, 2016) (Fig 4).

Symbiotic Micro Algae in Zoantharia

The phylogenetic tree of *Symbiudinium* (ITS1-5.8S-ITS2 sequence) from three species strongly clustered (ML=100%) with subclade A1 that is hosted by *Dipsastrea pallida* (KT069226), *Leptastrea transversa* (KT069227) of the Persian Gulf, Larak Island (Dehghani *et al*. 2018), *Zoanthus sansibaricus* (KM032592), *Zoanthus natalensis* (KM032558, KM032599, KM032575) of South Africa (Risi, 2014), and *Zoanthus sociatus* (AF427466) of New York (Santos *et al*., 2002) (Fig 5). The sequences of *Cladocopium* (partial ITS-1) from three zoantharian species with previously reported sequences, including some sequences from *Zoanthus durbanensis* (KM032585) of South Africa (Risi, 2014), *Zoanthus sansibaricus* (EU333738) of Singapore (Reimer and Todd, 2009), *Dipsastrea pallida*, *Leptastrea transversa*, *Psammocora contigua*, *Stylophora pistillata*, and *Pocillopora damicornis* (KT069230-KT069234) of the Larak island (Persian Gulf) (Dehghani et al. 2018), supported highly with *Cladocopium* radiation (ML=92%). According to the phylogenetic tree of the *Durusdinium* genus, sequences belonging to LSU showed more similarity with the sequences of *Durusdinium* includes KT069235, KT069237, and KT069238 (Dehghani *et al*., 2018) from hard corals (the Persian Gulf), AB778761, AB778705, AB778750, and AB778758 from nudibranchs (Yorifuji *et al*., 2015), and, DQ312308 (Ghavam Mostafavi *et al*., 2007), and KF030947 (Fard Yazdani *et al*., 2014) from hard corals.

qPCR

Since several types of symbionts can be present on the zoanthid at the same time, the qPCR method can show us the amount of these symbionts. The qPCR investigation and the primers have been used for detecting the presence of Symbiodiniaceae genuses, establishing a very sensitive and well-optimized method. In the present study, *Symbiodinium* was the dominant symbiont with zoantharians in Bostaneh bay, the previous studies in the Persian Gulf by Koupaei et al 2016 also showed that the most dominant symbionts in zoantarians are genus *Symbiodinium*. This type of symbiotic micro algae appears to be adapted to stressful environmental conditions by producing substantial amounts of mycosporine-like amino acids (MAAs) (Banaszak *et al*., 2000), compounds which provided strong protection against UV radiation (Neale *et al*., 1998).

CONCLUSION

This study demonstrated the presence of two families, two genera and three species of zoanthids including *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki* and *Palythoa tuberculosa* and their endosymbionts namely *Symbiodinium*, *Cladocopium* and *Durusdinium* in the Bostaneh bay- Hormozgan province in the Persian Gulf. Zoantharians are valuable genetic and biological resources that have been neglected in the Persian Gulf until now. Identifying these species is the first step towards protecting these genetic resources in the region. Based on the phylogenetic results obtained in the present study, the markers COI and 16S ribosomal mitochondrial DNA are considered appropriate for similar genetic studies in the Persian Gulf.

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