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Research Article

Do the reef-building corals around non-residential Shidvar Island tend to be symbionts with heat-resistant Clade D?

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Abstract

Coral reefs are considered among the most important marine ecosystems and host a wide range of species, but in most habitats, including the Persian Gulf, they are under serious threat due to several factors such as human activities and climate change. The formation of coral reefs is due to the symbiosis of a type of symbiotic microalgae that plays an important role in providing organic matter and calcium carbonate sediments for corals. This study investigates and molecularly identifies symbiotic microalgae associated with 17 species of reef-building corals collected from Shidvar Island: *Porites lobata, Dipsatrea pallida, Porites compressa, Dipsatrea matthaii, Acropora downingi, Platygyra daedalea, Plesiastrea versipora, Cyphastrea serailia, Cyphastrea microphthalma, Leptastrea transversa, Platygyra sinensis, Porites* sp., *Pavona decussata, Psammocora stellata, Favites pentagona, Anomastrea irregularis*, and *Acropora arabensis*. DNA was extracted using the Chloroform-CTAB method and the target gene ITS2 was amplified with a zooxanthellae-specific primer. Then, the PCR product was sequenced and a Bayesian phylogenetic tree and maximum likelihood were constructed using MrBayes and Mega5 software. Phylogenetic results showed the dominance of the heat-resistant clade D, especially subclade D1a, followed by clade C1, and the presence of clade A in *Leptastrea transversa*. The dominance of clade D, which seems natural due to its high resistance to thermal stresses, highlights the adaptation and resistance of the Persian Gulf corals to high temperatures, salinity fluctuations, and other stressors.

Introduction

Hermatypic corals, classified within the phylum Cnidaria and the order Scleractinia, as one of the most important and diverse marine ecosystems, cover less than 1% of the World's seas and ocean floors (Spalding and Grenfell, 1977). However, they provide the livelihoods of more than 275 million people (Huang *et al.,* 2021). Coral reefs are also known as "rainforests of the seas" (Nama *et al.,* 2023). In addition, 25% of all known marine species rely on coral reefs for feeding and habitat (Plaisance *et al.,* 2011).

The symbiotic relationship between a type of unicellular algae with hermatypic corals, which provides the organic materials needed by the host coral with its photosynthesis and helps to deposit more calcium carbonate, causes the creation of coral reefs (Ghavam Mostafavi *et al.,* 2007;2013; Dehghani *et al.,* 2018; Kaveh *et al.,* 2025). This dinoflagellate was previously classified under the genus *Symbiodinium* and included clades A-I (Rowan and Powers, 1991; Pochon *et al.,* 2006; Koupaei *et al.,* 2016a,b), which later studies classified it in the genera *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Fugacium*, *Gerakladium*, *Halluxium* and "*Symbiodinium*" Clade I (LaJeunesse *et al.,* 2018; Arabeyyat *et al.,* 2024).

The Persian Gulf is a semi-enclosed sea that connects to the Oman Sea through the Strait of Hormuz (Sheppard *et al.,* 1992). As one of the habitats of Reef Building Corals, the Persian Gulf, due to its location in the subtropical latitudes, has created some limitations for coral communities. Due to the hot summers and cold winters of the adjacent land masses, the Persian Gulf also has the largest annual fluctuations in water temperature (16 to 36°C) among areas with coral reefs. High salinity and turbidity in the Persian Gulf have reduced the diversity of coral reefs in this region, and the coral reefs of the Persian Gulf are always under stress due to special environmental conditions (Baker *et al.,* 2004; Sheppard *et al.,* 2010; Ghavam Mostafavi *et al.,* 2013; Mashini *et al.,* 2015; Koupaei *et al.,* 2016a).

Shidvar Island is a small, ecologically significant island located in the Persian Gulf, near the southern coast of Iran. It is part of the Hormozgan Province and lies close to Lavan Island, one of the country's key oil export terminals. Shidvar is uninhabited by people but holds great importance for wildlife conservation, especially birds and marine species (Rastgoo *et al*., 2022).

Coral bleaching is a process in which corals lose their symbiotic algae due to stressors such as high sea temperatures. This incident has affected coral ecosystems in recent decades and it has caused frequent and more intense bleaching events that have led to widespread coral mortality and the decline of this ecosystem. Over-fishing, pollution, and anthropogenic climate change have seriously affected the world's coral reefs (Hoegh-Guldberg *et al.,* 2007; Hughes *et al.,* 2018; 2023). Coral bleaching is a critical threat to marine biodiversity (Eakin *et al.,* 2019). In the Persian Gulf, the bleaching of corals has also intensified due to the increase in sea temperature, which has led to significant destruction of coral communities in recent years in this unique region (Riegl and Purkis, 2012).

This research has the objectives to identify hard corals and phylogeny of their symbionts in coral reefs off Shidvar Island, Persian Gulf.

Material and methods

Sampling area

Sampling was conducted from Shidvar Island, located on the northern coasts of the Persian Gulf (Fig. 1) at 53° 25′ longitude and 26° 47′ latitude, in May 2020 by scuba diving. The Manthatow survey was carried out with the purpose of finding the distribution of coral reefs around Shidvar Island. Coral samples were collected from five transects (Fig. 2), whose coordinates are listed in Table 1. Collected samples were preserved in 20% dimethyl sulfoxide (DMSO) buffer saturated with NaCl (pH=8) and were transferred to the marine biology laboratory in the Razi Laboratory Complex, Islamic Azad University, Science and Research Branch, and stored in freezer-20. The samples were classified in the laboratory using the Veron identification key (Veron, 2000).

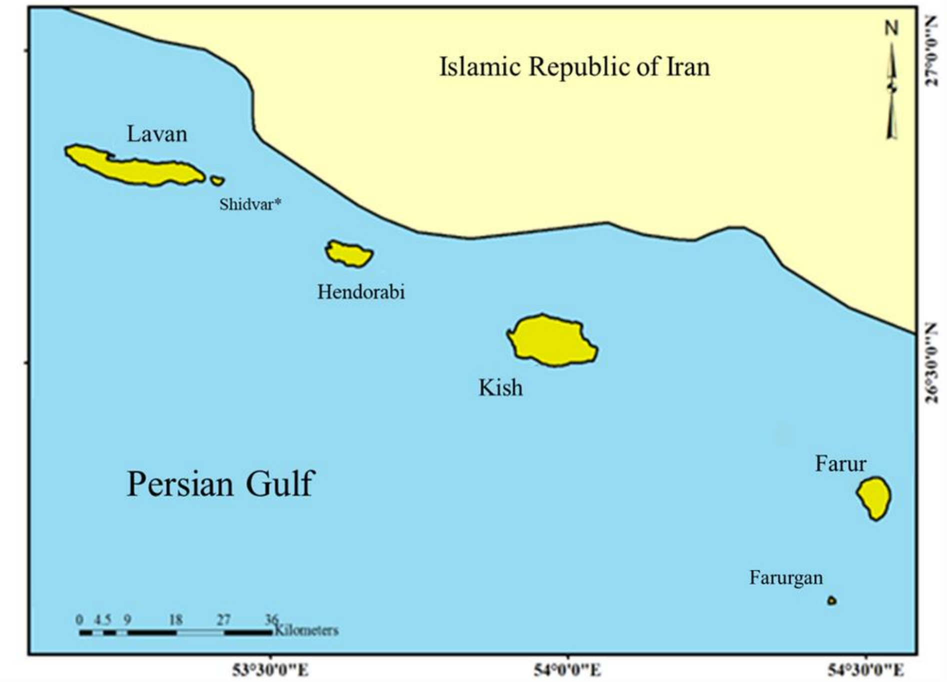

Figure 1: Geographical location of Shidvar Island.

Molecular analysis

DNA was extracted by the CTAB-Chloroform method (Baker, 1999; Bazzaz *et al.,* 2024; Kaveh *et al.,* 2025) and then the target gene ITS2 (Pochon *et al.,* 2007) were amplified with the primers ITS2F: 5′-GTGAATTGCAGAACTCCGTG-3′ and ITS2R: 5′ CCTCCGCTTACTTTATATGCTT-3′ with the following thermal profile: initial 94°C, 5 min following 30 cycles of 30 s at 94°C, 1 min at 60°C, 30 s at 72°C and a final extension 5 min at 72°C. PCR product sequencing was done by the Dideoxy Chain Termination method at Alpha Sequencing Company in the United States using an ABI model 23730x machine. All of the symbiotic microalgae sequences obtained from 17 coral samples were used for phylogenetic analyses. As some of the sequences are exactly similar, 16 sequences were aligned using ClustalX (Version: 2,0,12-win-msi) (Thompson *et al.,* 1994). After checking all of the electrophoregram 9 of the sequences have been submitted in GenBank, the accession number of them were shown in Table 2. for this purpose, the submitted sequences with highest identity in NCBI have been used, all the alignment sequences were used to draw phylogenetic tree using MrBayes software (Version 3.2.7a), Bootstraps 1000, GTR+G model, outgroup *Gymnodinium beii* and tree was rooted on outgroup.

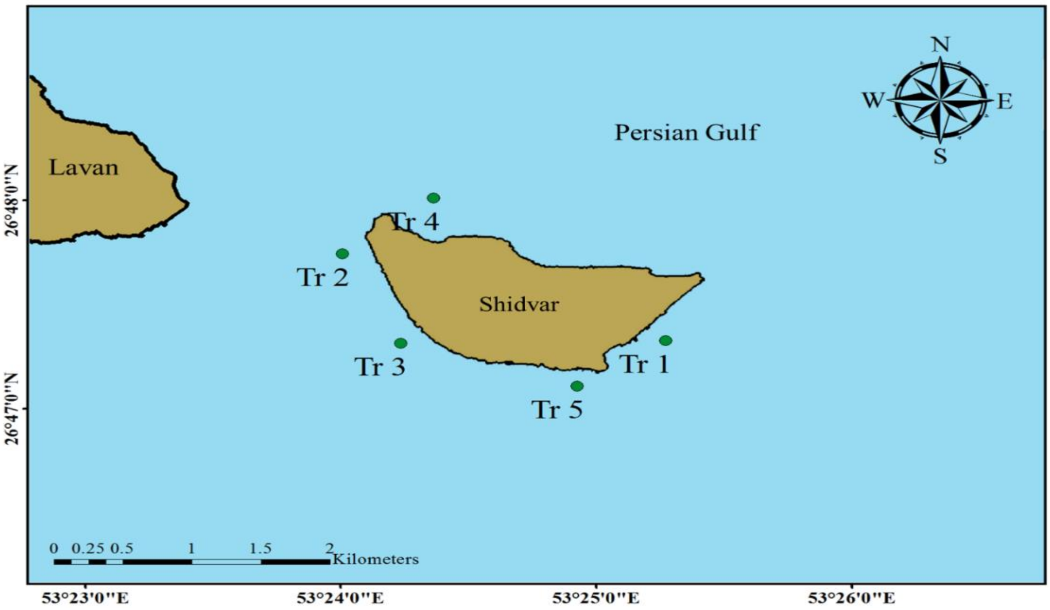

Figure 2: Sampled location (Transects 1-5).

Table 1: Geographical location and depth of sampled areas.

Transect number	Longitude (E)	Latitude (N)	Depth
1	53° 25′ 5.94″	26° 47′ 21.72″	3.5 – 4.5
2	53° 24′ 6.66″	26° 47′ 40.38″	3.5 – 4.5
3	53° 24′ 16.80″	26° 47′ 19.80″	2.5 – 3.2
4	53° 24′ 21.60″	26° 47′ 52.02″	2.2 – 2.7
5	53° 32′ 48.90″	26° 47′ 13.02″	4.5 – 4.9

Results

Coral Diversity

After surveying all the transects, the results showed that there are 17 distinct Species of reef-building corals around Shidvar Island. The names of coral species and where they were located are as follows:

Transect 1

The 8 corals observed in Transect 1 were: *Porites lobate*, *Dipsatrea pallida*, *Porites compressa*, *Dipsatrea matthaii*, *Acropora downingi*, *Platygyra daedalea*, *Plesiastrea versipora*, and *Cyphastrea serailia* (Figs. 3 and 4).

Table 2: Accession number of submitted sequences in GenBank.

	Accession Number	Microalgae type	Host
1	PQ637480	*Cladocopium* (Clade C)	*Porites compressa*
2	PQ637481	*Cladocopium* (Clade C)	*Porites lobata*
3	PQ637482	*Cladocopium* (Clade C)	*Psammocora stellata*
4	PQ637483	*Cladocopium* (Clade C)	*Porites* sp.
5	PQ637484	*Durusdinium* (Clade D)	*Platygyra sinensis*
6	PQ637485	*Durusdinium* (Clade D)	*Cyphastrea microphthalma*
7	PQ637486	*Durusdinium* (Clade D)	*Cyphastrea serailia*
8	PQ637487	*Durusdinium* (Clade D)	*Pavona decussata*
9	PQ637488	*Symbiodinium* (Clade A)	*Leptastrea transversa*

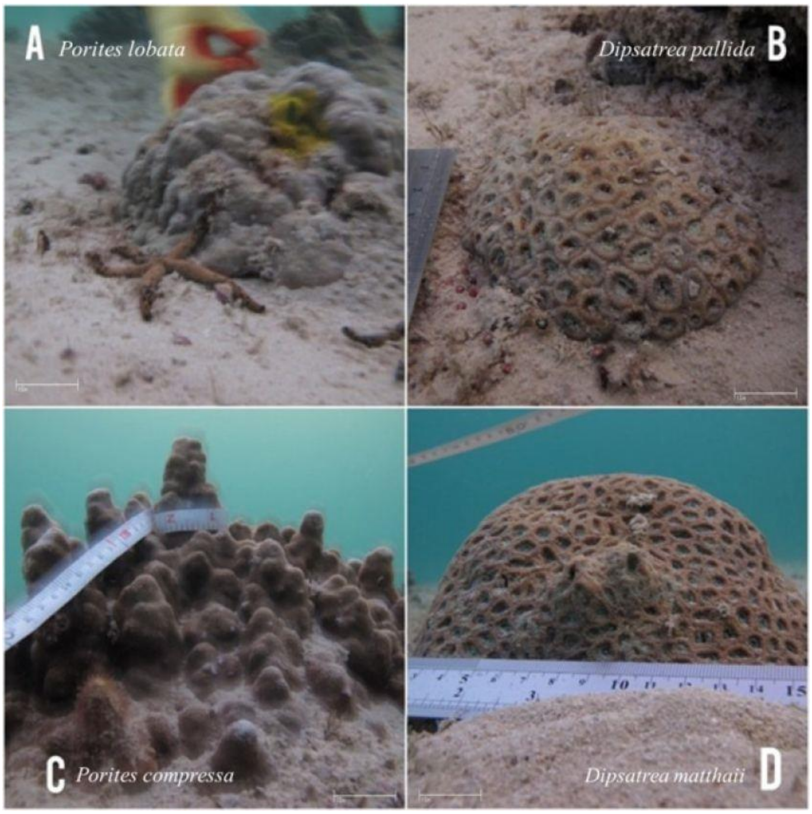

Figure 3: (A) *Porites lobata*, (B) *Dipsatrea pallida*, (C) *Porites compressa*, (D) *Dipsatrea matthaii*.

Transect 2

The 4 coral species observed in Transect 2 were: *Cyphastrea microphthalma*, *Leptastrea travsversa*, *Platygyra sinensis*, and *Porites* sp. (Fig. 5).

Transect 3

Two coral species were observed in Transect 3: *Pavona decussata*, and *Psammocora stellata* (Fig. 6).

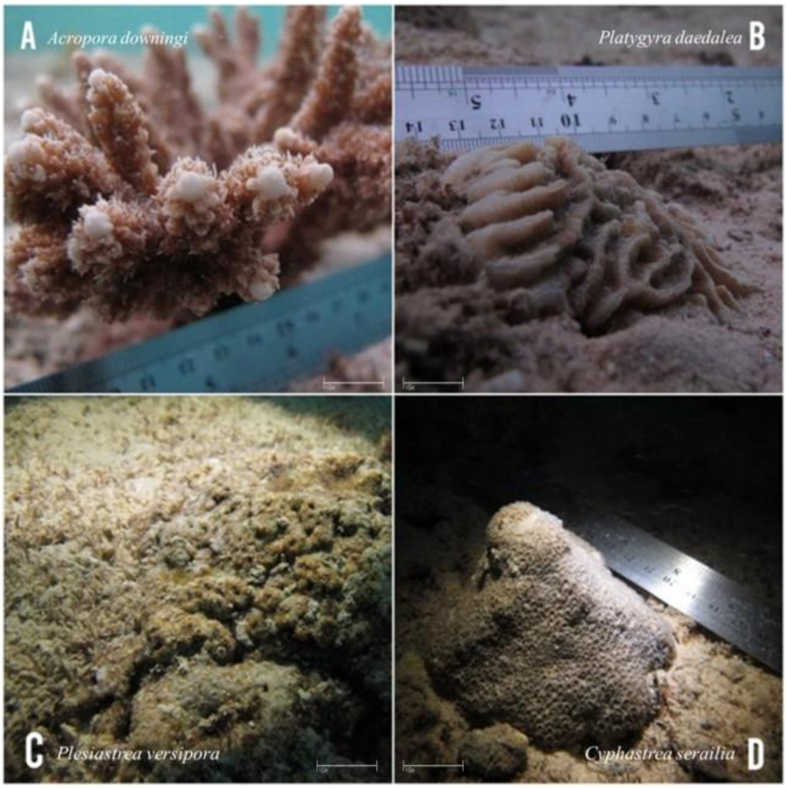

Figure 4: (A)*Acropora downingi*, (B) *Platygyra daedalea*, (C) *Plesiastrea versipora*, (D) *Cyphastrea serailia*.

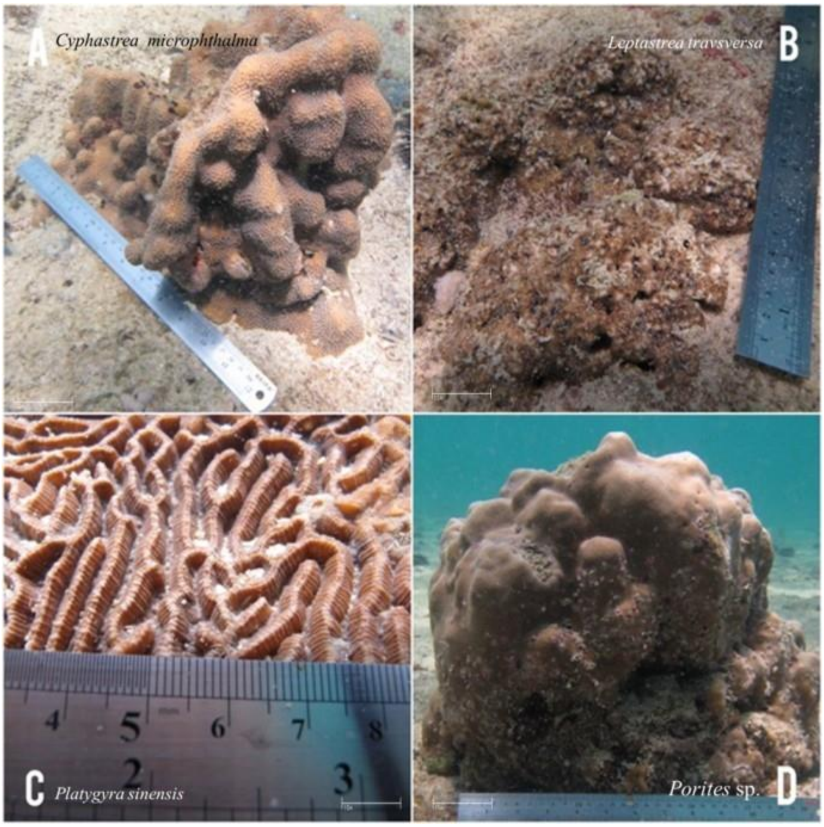

Figure 5: (A) *Cyphastrea microphthalma*, (B) *Leptastrea transversa*, (C) *Platygyra sinensis*, (D) *Porites* sp.

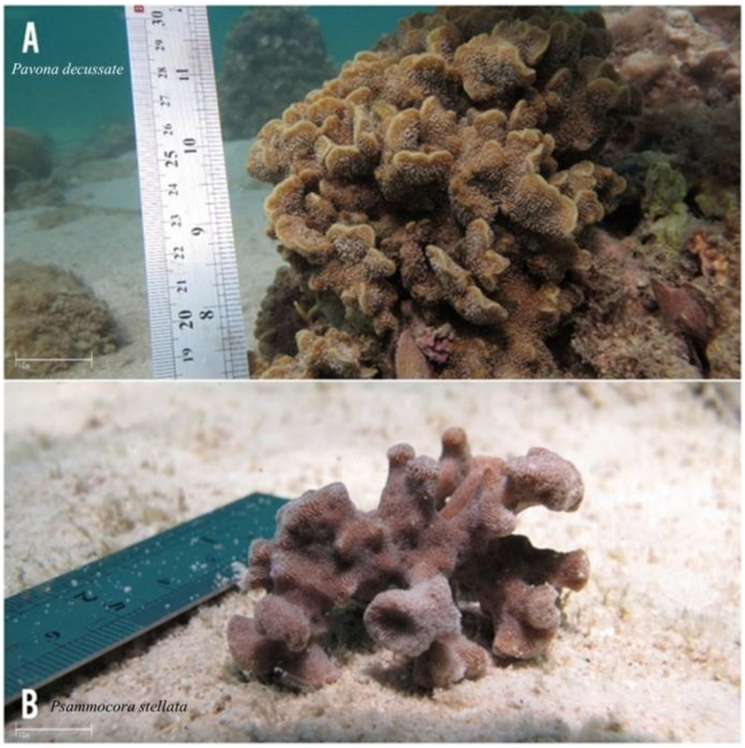

Figure 6: (A) *Pavona decussate*, (B) *Psammocora stellata*.

Transect 4

Four species were observed in Transect 4*:* *Favites pentagona*, *Psammocora stellata*, *Platygyra sinensis,* and *Porites* sp. (Fig. 7).

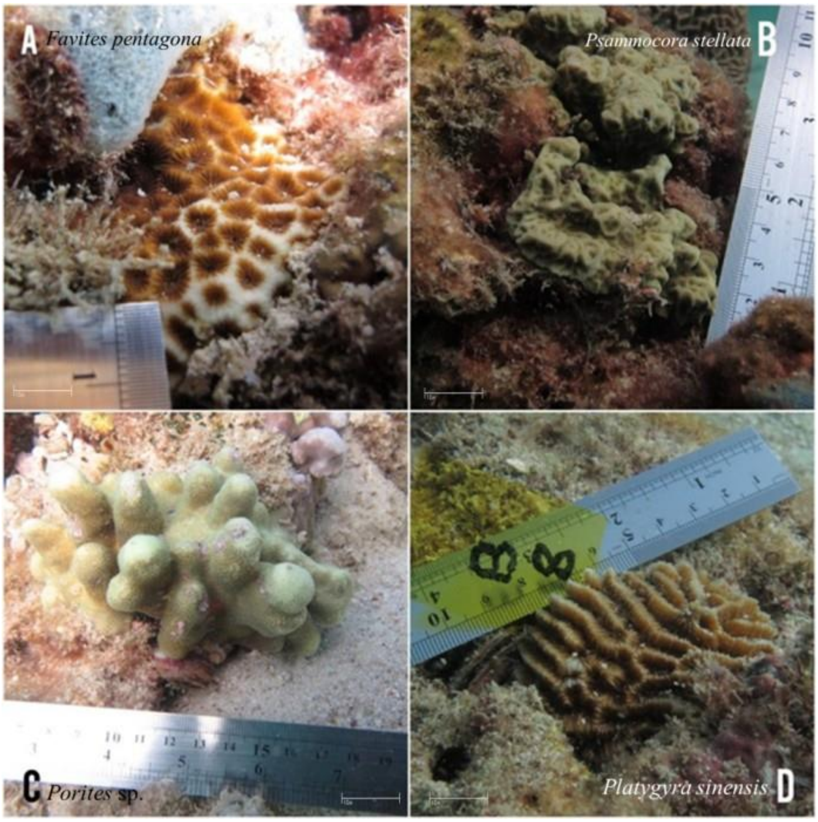

Figure 7: (A) *Favites pentagona*, (B) *Psammocora stellata*, (C) *Porites* sp., (D) *Platygyra sinensis*.

Transect 5

The 2 coral species observed in Transect 5 were: *Anomastrea irregularis*, and *Acropora arabensis* (Fig. 8).

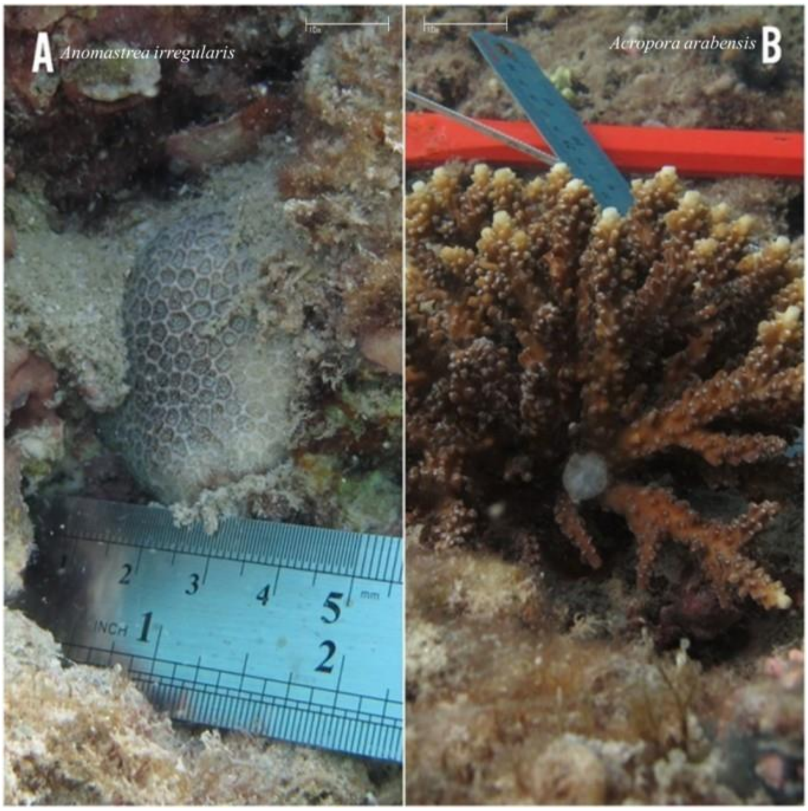

Figure 8: (A) *Anomastrea irregularis*, (B) *Acropora arabensis*.

Phylogenetic analysis

PCR amplifications of the ITS2 region provided products of approximately 350 bp in length. The NCBI and GeoSymbio BLAST tools found that all of the ITS2 sequences obtained in this study had been previously reported and belong to three different types of symbiotic micro algae: C, D, and A. None of the sequences from this study had any indels or mismatches in the alignments with reference sequences from GenBank. Sixteen sequences, obtained from *P. lobata, D. pallida, P. compressa, D. matthaii, A. downingi, P. daedalea, P. versipora, C. serailia, C. microphthalma, L. travsversa, P. sinensis, Porites* sp., *P. decussata*, *P. stellata, F. pentagona, A. irregularis, and A. arabensis* colonies, showed a double peak in their chromatograms similar to endosymbiont type D1. Nonetheless, the presence of other non-dominant symbiotic microalgae populations is also possible (<10%; Mieog *et al.,* 2007) and therefore the sequences resulting from this study were considered as the dominant type of endosymbiont within each specimen rather than the only one. The seven remaining sequences of *Durusdinium* (formerly named clade D), all of which were acquired from *D. matthaii, D. pallida, A. downingi, P. daedalea, C. serailia, C. microphthalma,* and *P. decussata* colonies, were identified to be 100% similar to the previously reported subclade D1a; JX845339, while the other eight sequences of endosymbiont, hosted by *Porites* sp., *P.*

compressa, *P. lobata* and *Psammocora stellata* were identical to subclade C1; AF333515. Only one of the coral species *L. travsversa* hosted clade A, which showed more similarity to clade A; JQ518393. Details are shown in Table 2.

The phylogenetic reconstructions of ITS2 rDNA sequences divided clade D *Symbiodinium* into three different major subclades. As shown in Figure 9, subclade D1a from this study formed a fairly well-supported monophyletic subclade (ML=100%, PP = 78%) with the previously reported D1a (JX845339), Also clade C ITS -1 sequences from eight coral species together with previously reported sequences, including some sequences from zoantharians in Jamaica (AF333515), Southeast Africa (EU431996) and Kaohe Bay (DQ182634) formed a highly supported monophyly (ML= 87%, PP= 99%) within the clade C radiation (LaJeunesse*,* 2001; Apprill and Gates, 2007; Macdonald *et al.,* 2008).

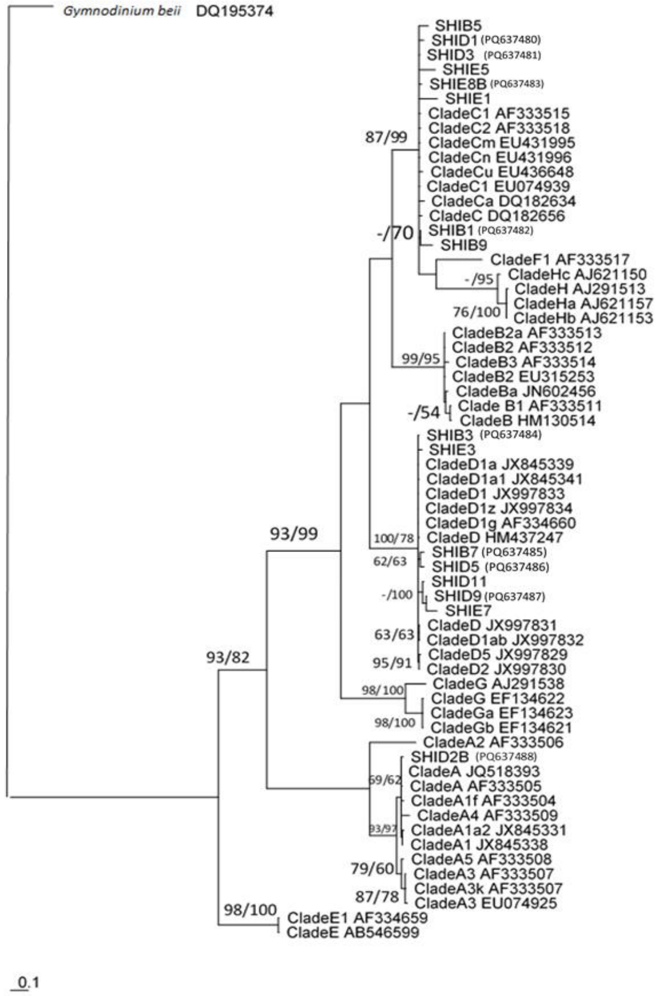

Figure 9: Bayesian tree of the Symbiotic microalgae ITS2 from coral colonies at sites off Shidvar Island (accession numbers listed in Table 2). Clade controls (A, B, C, D, E, F, and G) and an outgroup organism (*Gymnodinium beii*) were included in the analysis (accession numbers shown in the figure). Maximum likelihood / Bayesian posterior possibilities bootstrap percentages from 100 trees are shown at nodes. Distance represents the number of substitutions per 100 bases. Host coral species are: *Cyphastrea microphthalma*, *Cyphastrea serailia, Porites compressa*, *Porites lobata*, *Porites* sp., *Platygyra sinensis*, *Pavona decussata*, *Psammocora stellata* and *Leptastrea travsversa.*

The phylogenetic tree showed the presence of clade A only in one coral species (*Leptastrea travsversa*) strongly clustered (ML=69%, PP= 62%) with subclade A1 that is hosted by *Palythoa* sp. in the Persian Gulf (JX845338), *Condylactis gigantea* in Jamaica (AF333504) and *Zoanthus sansibaricus* in the Persian Gulf (JX845331) (LaJeunesse, 2001; Koupaei *et al.,* 2014).

Discussion

In this study, 17 reef-building corals: *P. lobata, D. pallida, P. compressa, D. matthaii, A. downingi, P. daedalea, P. versipora, C. serailia, C. microphthalma, L. travsversa, P. sinensis, Porites* sp., *P. decussata*, *P. stellata, F. pentagona, A. irregularis,* and *A. arabensis* were identified using the Veron 2000 identification key. Based on the previous studies, these species have been reported before off Iranian islands in the northern parts of the Persian Gulf (Fatemi and Shokri, 2001; Ghavam Mostafavi *et al.,* 2007; Shahhosseiny *et al.,* 2011; Samiei *et al.,* 2013; Ghavam Mostafavi *et al.,* 2013)

Clade D was the most prevalent symbiotic clade among the coral species that were examined in various locations in the northern Persian Gulf. According to the research done between 2005 and 2020 at various locations as well as the current study (Ghavam Mostafavi *et al.,* 2007; Shahhosseiny *et al.,* 2011; Ghavam Mostafavi *et al.,* 2013; Mashini *et al.,* 2017) *Durusdinium* is among the most common symbiotic microalgae in the Persian Gulf. In the present study, dominant reef-building corals i.e. *D. matthaii, D. pallida, A. downingi, P. daedalea, C. serailia, C. microphthalma,* and *P. decussata* harbored clade D, which is typically seen in higher numbers when corals are exposed to heat and fluctuating temperatures, stressful environments, and other conditions that negatively impact coral health (Baker, 2001; Stat and Gates, 2011). Clade D has a greater probability of surviving a coral bleaching event due to its higher resistance to thermal stress (1–1.5°C) than other clades (Toller *et al.,* 2001; Baker *et al.,* 2004; Goulet, 2006; Hume *et al.,* 2020; Kaveh *et al.,* 2025). Because clade D is thermally resistant, the corals that host them are more resilient to rising sea surface temperatures (Dehghani *et al.,* 2018).

Clade C was reported to be the second most abundant clade sampled in the northern islands (Kish and Larak Islands) of the PG (Ghavam Mostafavi *et al.,* 2007). Interestingly, studies from the southern PG have proved the prevalence of clade C and also reported the presence of thermo-tolerant *Symbiodinium thermophilum* sp. nov. (Hume *et al.,* 2013, 2015). In the present study, *Porites* sp., *P. compressa*, *P. lobata* and *Psammocora stellata* showed symbiosis with subclade C1. Subclade C1 is an Indo–Pacific generalist, known from multiple hosts and environments (LaJeunesse, 2005; Reimer *et al.,* 2006b). According to Reimer *et al.* (2006b) C1 is less adapted to high light radiation, while subclade A1 is adapted to highly irradiated environments. The clade C dominance reported here agrees with another study of Symbiotic microalgae from the southern Persian Gulf (Hume *et al.,* 2015). Although Clade C was more dominant symbiont, other clades, including A and D were commonly detected in corals off Iranian

Islands (Dehghani *et al.,* 2018; Bazzaz *et al.,* 2024).

Subclade A1 and A3 have been reported in Pacific and Atlantic Oceans (LaJeunesse, 2002; Reimer *et al.,* 2006b, 2007, 2011; Kamezaki *et al.,* 2013; Rabelo *et al.,* 2014) and in the Persian Gulf on *Zoanthus sansibaricus* in Hengam, Kish, Larak and Qeshm islands and *Palythoa tuberculosa* in Hengam island (Koupaei *et al.,* 2016b) as well as on reef-building corals, *Leptastrea transversa, Stylophora pistillata, Pocillopora damicornis* and *Dipsatrea pallida* in Larak island (Dehghani *et al.,* 2018). Corals that live in extremely shallow, high-irradiance waters have been identified as belonging to Clade A (Rowan *et al.,* 1997). Only clade A is known to be able to produce significant amounts of mycosporine-like amino acids (MAAs) (Banaszak *et al.,* 2000), which are substances that aid in preventing UV radiation damage (Neale *et al.,* 1998). However, whereas subclade A1 was only present in *P. tuberculosa* collected off Hengam Island, a variant of subclade A1 was common in colonies of *Z. sansibaricus*. Furthermore, it has been demonstrated that zoantharians belonging to the genus *Palythoa* worldwide are widely distributed in subclades C1 and C3 (Reimer *et al.,* 2006a; 2007; Reimer and Todd, 2009; Finney *et al.,* 2010; Reimer *et al.,* 2011).

Based on the present and previous studies (Ghavam Mostafavi *et al.,* 2007; Shahhosseiny *et al.,* 2011; Ghavam Mostafavi *et al.,* 2013; Mashini *et al.,* 2015;2017; Kaveh *et al.,* 2025) which were carried out in this region, it could be concluded that coral reefs around Iranian islands can survive in the face of climate change and global warming.

Conclusion

In the present study, the dominant reef-building corals around Shidvar Island were surveyed and identified in 5 transects. The study of symbiotic unicellular algae, showed that the genera *Durusdinium* and *Cladocopium* were the dominant symbiotic microalgae. These results are in accordance with previous findings in the Iranian waters of the Persian Gulf.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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