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# First report and diversity analysis of endophytic fungi associated with *Ulva* sp. from Iran

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**Endophytic fungi are diverse microorganisms that colonize plants symbiotically without causing overt infections. While numerous studies have focused on endophytes in terrestrial plants, there are no prior reports of endophytes associated with algae in Iran. Samples of *Ulva* sp. were collected during the fall of 2022 from the Bandar Abbas Fishery Coast, Iran, and transported to the laboratory. Following surface sterilization, the samples were cultured on potato dextrose agar (PDA) medium and incubated at 25 °C for 3 weeks. The resulting isolates were purified using the hyphal tip method. This study identified 33 fungal isolates from *Ulva* sp. collected at the Bandar Abbas Fishery Coast, Iran. Morphological and molecular analyses classified these isolates into 7 species across 6 genera: *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Penicillium*, and *Syncephalastrum*. *Aspergillus* was the most abundant genus (34% of isolates), while *Alternaria* and *Syncephalastrum* were the least frequent (9% each). Phylogenetic analyses of ITS, *β-tubulin*, *GAPDH*, *TEF*, and LSU gene sequences supported the morphological identification of the isolates. Species identified included *Alternaria alternate*, *Aspergillus caespitosus*, *Aspergillus terreus*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Penicillium digitatum*, and *Syncephalastrum racemosum*. All species are reported here for the first time as endophytes of *Ulva* sp. in Iran. Furthermore, this study represents the first documentation of endophytic fungi associated with the marine alga *Ulva* sp. in Iranian waters. This research enhances understanding of the ecological interactions between fungal endophytes and marine algae in Iranian ecosystems, emphasizing the diversity of symbiotic relationships in aquatic environments.**

**Keywords** Algal endophytes, Bandar abbas, Bioactive metabolites, Marine fungi

The southern coasts of Iran, bordering the Persian Gulf and the Sea of Oman, are recognized for their rich biological resources, particularly marine macroalgae (seaweeds). Seaweed communities rank among the most productive and biodiverse marine ecosystems, serving as primary producers and a significant contribution to global oxygen production. Investigating endophytic fungi associated with these macroalgae is essential to understanding the ecological dynamics and biodiversity of Iran's coastal regions. Globally, over 6,000 seaweed species have been identified, with approximately 300 species documented in the Persian Gulf and the Sea of Oman[1]. The southern coasts of Iran, bordering the Persian Gulf and the Sea of Oman, exhibit remarkable biodiversity, particularly in marine macroalgae (seaweeds). Seaweed communities rank among the most productive marine ecosystems globally, with primary production rates even those from tropical regions' rainforests[2]. The high productivity of multicellular seaweeds and eukaryotic organisms[3] is critical to marine ecosystems. *Ulva* species are key elements in the stability of coastal ecosystems by playing important roles in primary production, biogeochemical cycles, and bioremediation. In addition, many *Ulva* species serve as food for invertebrates and fish, thereby supporting biodiversity[4]. Endophytic fungi, a diverse group of microorganisms, colonize plants symbiotically without causing overt harm[5–6]. Over recent decades, these fungi have attracted considerable research interest due to their functional traits, including enhancing plant growth and tolerance to biotic and abiotic stresses[7–9]. They offer promising strategies for mitigating yield losses caused by abiotic stress[10] and can protect host plants from pathogens[6]. Additionally, endophytes produce bioactive metabolites with applications in medicine, agriculture, and industry[11–12].

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Endophytic colonization has been documented across diverse plant ecosystems, from tropical rainforests to temperate herbaceous communities. However, studies on endophytic fungi associated with marine macroalgae, such as *Ulva* sp. (family: Ulvaceae), remain limited. The coastal regions of Bandar Abbas, Iran, host abundant *Ulva* populations, yet their fungal endophytes remain understudied. This study addresses this gap by investigating endophytic fungi in *Ulva* sp. from the Shilat-Bandar Abbas coastline. Insights from this research could advance understanding of fungal-algal symbioses and their potential applications in agriculture and ecology.

## Materials and methods

### Collection of plant material and isolation of endophytes

In autumn 2022, during one sampling session, thirty-eight fresh, healthy, and disease-free *Ulva* sp. specimens were collected from the Fishery Coast in Bandar Abbas, Iran. Samples were rinsed with seawater to remove sand and epiphytes, stored in sterile plastic bags, and transported to the mycology laboratory of the Faculty of Agriculture at Tarbiat Modares University. Samples were washed with sterile distilled water and refrigerated at 4 °C. A multi-step surface sterilization protocol was applied:

1. Immersion in 70% ethanol for 1 min.
2. Rinsing with sterile distilled water.
3. Immersion in 70% ethanol for 15 s.
4. Triple rinsing with sterile distilled water.

To validate sterilization efficacy, aliquots from the final rinse water were cultured on potato dextrose agar (PDA). The absence of fungal growth in these controls confirmed successful surface disinfection. Samples were air-dried, cut into 1 $cm^2$ fragments, and placed on three culture media:

- PDA (Potato Dextrose Agar),
- PDA-SW (PDA supplemented with 200 mL $L^{-1}$ sterilized seawater),
- PDA-SLE (PDA supplemented with 200 mL $L^{-1}$ sterilized *Ulva* extract).

Petri dishes were incubated at 25 °C for 3 weeks. Emerging fungal colonies were isolated using the hyphal tip method and identified morphologically with standard taxonomic keys. Initial genus-level identification relied on macroscopic traits (colony morphology, pigmentation) and microscopic traits (spore structure, hyphal characteristics). Isolates were further cultured on Czapek's Yeast Extract Agar (CYA), Malt Extract Agar (MEA), and Potato Carrot Agar (PCA) for detailed species-level identification. An Olympus BX51 light microscope was used to observe the morphological features of the fungal isolates.

The identification of fungal species was conducted using established taxonomic keys from authoritative sources: *Alternaria alternata* was identified following Simmons (2007)[13], *Aspergillus* species identification was based on Klich (2002)[14]. For *Chaetomium globosum*, identification relied on the key by Watanabe (2002)[15]. *Cladosporium cladosporioides* was identified using the key by Bensch et al.. (2012)[16]. The identification of *Penicillium digitatum* was based on the works of Carmichael (1955)[17] and Pitt and Hocking (2009)[18]. Finally, *Syncephalastrum racemosum* was identified using keys from Benjamin (1966)[19], Domsch and Gams (1980)[20], and Zycha et al.. (1969)[21].

### Molecular identification

Morphological analysis classified the isolates into six genera: *Aspergillus*, *Penicillium*, *Chaetomium*, *Cladosporium*, *Alternaria*, and *Syncephalastrum*. For molecular identification, genomic DNA was extracted from eight isolates. Fungi were cultured on PDA at 28 °C for 7 days, after which mycelia were harvested, frozen in liquid nitrogen, and disrupted using a mortar and pestle. Genomic DNA from fungi was extracted using the cetyltrimethylammonium bromide (CTAB) method, with slight modifications, following the protocol of Gardes and Bruns (1993)[22].

### PCR amplification

Gene targets for amplification included the ITS rDNA region, *β-tubulin*, *TEF*, LSU, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and were selected based on taxonomic requirements. To amplify the genetic regions ITS, *TUB2*, *TEF-1α*, *GAPDH*, and D1/D2, the following primers were utilized respectively: ITS1 5′-CTTGGTCATTTAGAGGAAGTAA-3′, ITS4 5′-TCCTCCGCTTATTGATATGC-3′[23], T1 5′-AACATGCGT GAGATTGTAAGT-3′, T22 5′-TCTGGATGTTGTTGGGAATCC-3′[24], EF1-728 F 5′-CATCGAGAAGTTCGA GAAGG-3′, EF1-986R 5′-TACTTGAAGGAACCCTTACC-3′[25], gpd1 5′-CAACGGCTTCGGTCGCATTG-3′, gpd2 5′-GCCAAGCAGTTGGTTGTGC-3′[26], D1 5′-AACTTAAGCATATCAATAAGCGGAGGA-3′, and D2 5′-GGTCCGTGTTTCAAGACGG-3′[27].

PCR reactions (25 µL total volume) were prepared with the following components:

- 2.5 µL 10× PCR buffer,
- 1.5 mM $MgCl_2$,
- 200 µM dNTPs,
- 0.1 µM forward and reverse primers,
- 0.04 U/µL Taq DNA polymerase (Cinagene, Iran),
- 10 ng template DNA.

| Genomic region | Initial denaturation (Temperature// time) | 36 thermal cycles (Temperature//time) | | | Final extension (Temperature// time) |
|---|---|---|---|---|---|
| | | Denaturation | Annealing | Extension | |
| ITS-rDNA | 95 °C// 1 min | 95 °C//1 min | 56 °C// 30 S | 72 °C// 1 min | 72 °C// 5 min |
| *β-tubulin* | 94 °C// 1 min | 94 °C//30 S | 47 °C//30 S | 72 °C//150 S | 72 °C// 10 min |
| LSU | 94 °C// 5 min | 94 °C// 60 S | 52 °C// 60 S | 72 °C// 120 S | 72 °C// 10 min |
| *EF- 1α* | 94 °C// 5 min | 94 °C// 15 S | 61 °C// 30 S | 72 °C// 30 S | 72 °C// 8 min |
| *GAPDH* | 95 °C// 5 min | 95 °C//30 S | 57 °C// 30 S | 72 °C// 1 min | 72 °C// 7 min |

**Table 1**. Thermal cycling protocol for amplification using different primers.

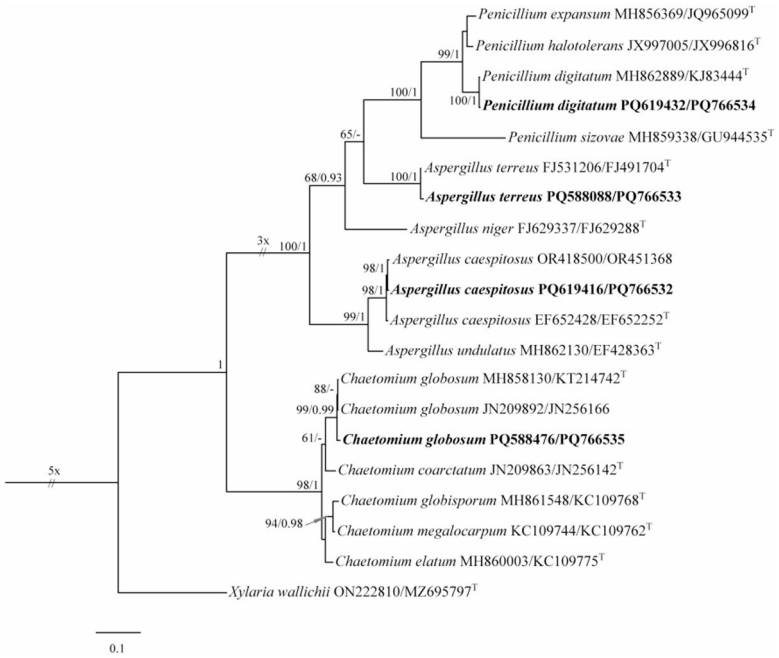


**Fig. 1**. The MrBayes phylogenetic tree was constructed for various species within the *Aspergillus*, *Penicillium*, and *Chaetomium* genera by integrating the ITS-rDNA region and the *β-tubulin* gene, utilizing the CIPRES Science Gateway. ML/PP bootstrap support values and posterior probabilities (PP) are shown at the nodes. The phylogenetic tree was rooted with *Xylaria wallichii*(Accession: ON222810/MZ695797) (Specimen No.: FCATAS911 (HT)). Bold font denotes the species investigated in this study.

The thermal cycling protocol for DNA amplification employing different primers is detailed in Table 1. The gene region was amplified using a SimpliAmp thermal cycler (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

## Sequencing and phylogenetic analysis

PCR products were visualized via agarose gel electrophoresis under UV light. Amplicons were sequenced bidirectionally (Codon Genetics, Iran) and edited using BioEdit v7.2.5. Sequences were aligned with reference data from GenBank using MAFFT v7.4 and curated in Mesquite v3.6.

Phylogenetic trees in Fig. 1 (*Aspergillus*, *Penicillium*, and *Chaetomium* genera), Fig. 3 (*Cladosporium*), and Fig. 4 (*Syncephalastrum*) were constructed using the Bayesian method, while the tree in Fig. 2 (*Alternaria*) was constructed using maximum parsimony. All analyses were performed through the CIPRES Science Gateway, and final visualizations were prepared in Adobe Illustrator 2019.

## Results

This study aimed to isolate and identify endophytic fungi associated with the genus *Ulva* in Iran. Morphological characterization identified the seaweed as *Ulva* sp., characterized by vivid grass-green, tubular fronds with unbranched thalli[28– 29]. A total of 33 fungal isolates were obtained from *Ulva* sp. collected at the Bandar Abbas Fishery Coast, Iran. Three different culture media were employed to isolate fungi. According to the findings and observations, the highest number of isolates was obtained from the PDA-SLE medium, which was therefore chosen as the optimal culture medium for this study. Based on cultural and morphological characteristics, all isolates were classified into 7 species across 6 genera: *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Penicillium* (Ascomycota), and *Syncephalastrum* (Mucoromycota) (Table 2).

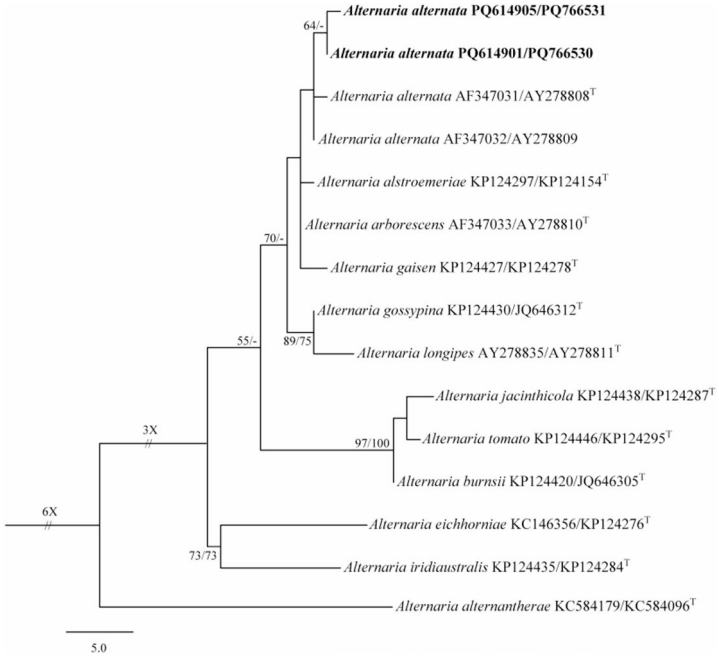


**Fig. 2**. The Maximum Parsimony (MP) phylogenetic tree for *Alternaria* spp. was constructed by integrating the ITS-rDNA region and the *GAPDH* gene, using the CIPRES Science Gateway. ML/MP bootstrap support values and posterior probabilities are shown at the nodes. The phylogenetic tree was rooted with *Alternaria alternantherae* strain CBS 124,392(Accession: KC584179/KC584096). Bold font denotes the species investigated in this study.

| Taxa | Phylum | GenBank Accession Numbers | Gene regions |
|---|---|---|---|
| *Alternaria alternata* | Ascomycota | PQ614901.1 | ITS |
| *Alternaria alternata* | | PQ766530.1 | *GAPDH* |
| *Alternaria alternata* | Ascomycota | PQ614905.1 | ITS |
| *Alternaria alternata* | | PQ766531.1 | *GAPDH* |
| *Aspergillus caespitosus* | Ascomycota | PQ619416.1 | ITS |
| *Aspergillus caespitosus* | | PQ766532.1 | *β-tubulin* |
| *Aspergillus terreus* | Ascomycota | PQ588088.1 | ITS |
| *Aspergillus terreus* | | PQ766533.1 | *β-tubulin* |
| *Chaetomium globosum* | Ascomycota | PQ588476.1 | ITS |
| *Chaetomium globosum* | | PQ766535.1 | *β-tubulin* |
| *Cladosporium cladosporioides* | Ascomycota | PQ614852.1 | ITS |
| *Cladosporium cladosporioides* | | PQ766529.1 | *TEF* |
| *Penicillium digitatum* | Ascomycota | PQ619432.1 | ITS |
| *Penicillium digitatum* | | PQ766534.1 | *β-tubulin* |
| *Syncephalastrum racemosum* | Mucoromycota | PP176476.1 | ITS |
| *Syncephalastrum racemosum* | | PQ665121.1 | LSU |

**Table 2**. Taxa and phyla of endophytic fungi isolated from *Ulva* sp. with GenBank accession numbers and gene regions used in the phylogenetic analyses.

## Taxonomic distribution

- *Aspergillus* (11 isolates, 34% of total),
- *Penicillium* (7 isolates, 21%),
- *Chaetomium* (5 isolates, 15%),
- *Cladosporium* (4 isolates, 12%),
- *Alternaria* (3 isolates, 9%),
- *Syncephalastrum* (3 isolates, 9%).

*Aspergillus* was the most abundant genus, while *Alternaria* and *Syncephalastrum* were the least frequent.

## Molecular validation

Eight isolates were selected for molecular analysis. Sequences of the ITS rDNA region, *β-tubulin*, *TEF*, LSU, and *GAPDH* genes were compared to GenBank databases using BLAST. Morphological identifications were confirmed for all isolates.

## Species descriptions

*Alternaria alternata*

- Morphology: Grayish-green colonies (50 mm) on PCA. Conidiophores were vertical (40–70 × 3–4 µm), with ellipsoidal conidia (24.5–35 × 5.5–9 µm).
- Molecular: ITS and *GAPDH* sequences matched *A. alternata* (3 isolates).

*Aspergillus caespitosus*

- Morphology: Colonies grew 50 mm in 7 days; green/olive conidia with light yellow reverse. Conidiophores were smooth (150–250 × 5–6 µm), with hemispherical vesicles (9–15 µm). Conidia were globose, rough-walled (3.5–4.5 µm).
- Molecular: ITS and *β-tubulin* sequences matched *A. caespitosus* (1 isolate).

*Aspergillus terreus*

- Morphology: Colonies on MEA reached 55 mm in 7 days at 25 °C, with pale orange-buff surfaces and yellow reverse. Conidial heads were dense, and double-columned; conidiophores were smooth to slightly rough (148–247 µm), with globose vesicles (14.5–20 µm). Conidia were globose (2–2.5 µm).
- Molecular: ITS and *β-tubulin* sequences matched *A. terreus* (10 isolates).

*Chaetomium globosum*

- Morphology: Olive-green colonies (8 cm) on PDA. Perithecia were spherical with club-shaped asci (45–57 × 10–12 µm) and lemon-shaped ascospores.
- Molecular: ITS and *β-tubulin* sequences matched *C. globosum* (5 isolates).

*Cladosporium cladosporioides*

- Morphology: Olive colonies (41 mm) on SNA. Conidiophores were cylindrical (27–160.5 × 2.9–4 µm), with ovoid conidia (3.5–5 × 2–3 µm).
- Molecular: ITS and *TEF* sequences matched *C. cladosporioides* (4 isolates).

*Penicillium digitatum*

- Morphology: Green colonies (41 mm) on MEA. Conidiophores were thin (62–154 × 5–6.5 µm), with cylindrical phialides and oval conidia (6.3–9.5 × 3–6 µm).
- Molecular: ITS and *β-tubulin* sequences matched *P. digitatum* (7 isolates).

*Syncephalastrum racemosum*

- Morphology: Rapid growth on PDA (white to black colonies). Sporangiophores were branched (3–4 µm), with spherical vesicles (11–16 µm) and ovoid sporangiospores (2–6 µm).
- Molecular: ITS and LSU sequences matched *S. racemosum* (3 isolates).

In this study, phylogenetic analyses were performed using gene regions selected for their taxonomic utility in fungal classification. Phylogenetic trees for *Aspergillus*, *Penicillium*, and *Chaetomium* were constructed using sequences from the ITS rDNA region and *β-tubulin* gene (Fig. 1). For *Cladosporium*, the ITS region and translation elongation factor (*TEF*) gene were analyzed (Fig. 3). *Alternaria* phylogenies were resolved using the ITS region and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene (Fig. 2). Finally, *Syncephalastrum* was analyzed using the ITS region and large subunit (LSU) rDNA (Fig. 4).

For the phylogenetic analysis of *Aspergillus*, *Penicillium*, and *Chaetomium*, a dataset containing 19 ingroup taxa and the outgroup *Xylaria wallichii* (Accession: ON222810/MZ695797) (Specimen No.: FCATAS911 (HT)) was analyzed. The results supported the morphological identification of the isolates from the genera *Aspergillus*, *Penicillium*, and *Chaetomium*. Specifically, the *A. terreus* isolate grouped with the reference strain CBS 117.37 (Accession: FJ531206/FJ491704), and the *A. caespitosus* isolate clustered with strains V313-04(Accession: OR418500/OR451368) and NRRL 1929 (Accession: EF652428/EF652252) in the phylogenetic tree based on ITS and *β-tubulin* gene sequences. Additionally, the isolates of *P. digitatum* and *C. globosum* formed clusters with their respective reference strains CBS 112,082 (Accession: MH862889/KJ83444) and strain CBS 160.62 (Accession: MH858130/KT214742), showing strong genetic similarity and confirming the accuracy of their identification (Fig. 1).

Phylogenetic analysis of *Alternaria* involved selecting three isolates identified as *A. alternata* based on morphological features, which were analyzed using ITS and *GAPDH* gene sequences. A dataset comprising 14

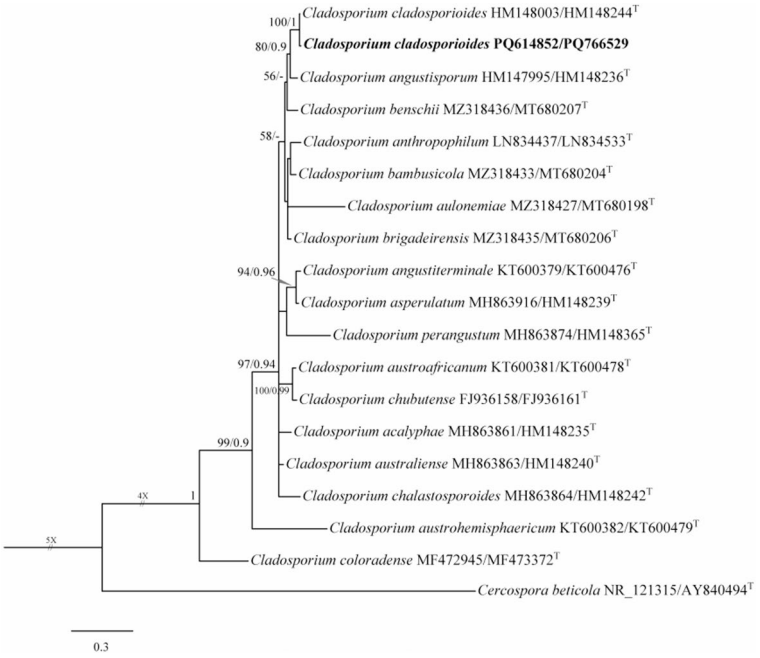


**Fig. 3**. The MrBayes phylogenetic tree was constructed for various species within the *Cladosporium* genus by integrating the ITS-rDNA region and the *TEF* gene, utilizing the CIPRES Science Gateway. ML/PP bootstrap support values and posterior probabilities (PP) are shown at the nodes. The phylogenetic tree was rooted with *Cercospora beticola* strain CBS 116,456(Accession: NR_121315/AY840494). Bold font denotes the species investigated in this study.

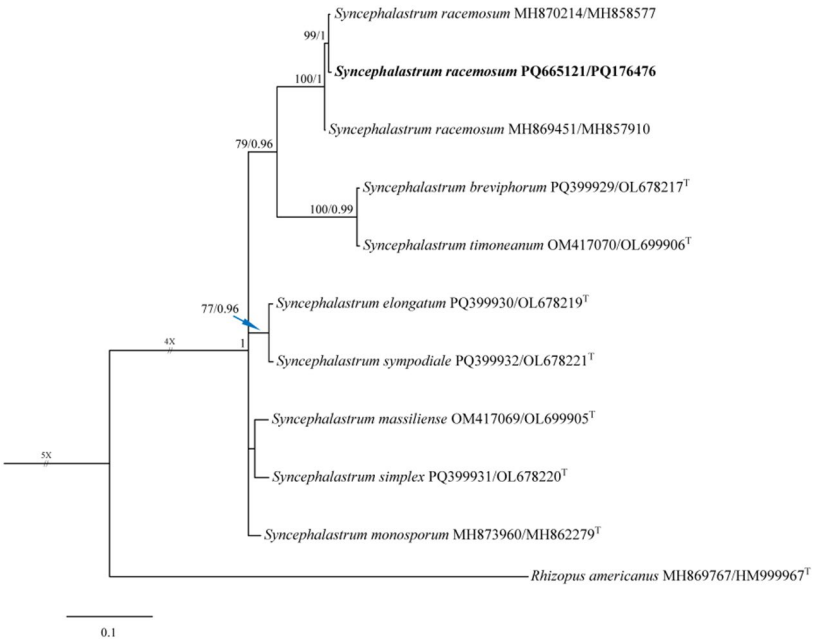


**Fig. 4**. The MrBayes phylogenetic tree was constructed for various species within the genus of *Syncephalastrum* by integrating the ITS-rDNA region and the D1/D2 gene, utilizing the CIPRES Science Gateway. ML/PP bootstrap support values and posterior probabilities are shown at the nodes. The phylogenetic tree was rooted with *Rhizopus americanus* strain CBS 340.62(Accession: MH869767/HM999967). Bold font denotes the species investigated in this study.

ingroup taxa and the outgroup *Alternaria alternantherae* strain CBS 124,392 (Accession: KC584179/KC584096) was used. The resulting phylogenetic tree (Fig. 2) showed that two isolates of *A. alternata* clustered within the subclade of section *Alternata* and grouped with reference strains EGS 34 – 016 1 (accession: AF347031/AY278808) and strain EGS 34 – 015 (accession: AF347032/AY278809). Furthermore, the genetic variation within the *Alternata* section led to the formation of distinct subclades, indicating considerable genetic heterogeneity within this section.

The morphological analysis identified the four isolates as *C. cladosporioides*. To confirm this, a phylogenetic analysis was performed using ITS-rDNA and translation elongation factor (*TEF*) gene sequences. The dataset included 18 ingroup taxa and the outgroup *Cercospora beticola* strain CBS 116,456(Accession: NR_121315/AY840494). BLAST comparisons revealed high sequence similarity between the isolate under study and reference strains of *C. cladosporioides*. Phylogenetic analysis (Fig. 3) placed *C. cladosporioides*, the species obtained in this study, within the *C. cladosporioides* clade, clustering it with the reference strain CBS 112,388(Accession: HM148003/HM148244), thereby confirming the morphological observations.

BLAST analysis revealed that the ITS-rDNA and LSU sequences of the isolate identified as *S. racemosum* based on morphological characteristics closely matched *S. racemosum* reference sequences in the NCBI GenBank. To resolve species boundaries, a multigene phylogenetic analysis was performed using MrBayes, incorporating 10 ingroup taxa and the outgroup *Rhizopus americanus* strain CBS 340.62(Accession: MH869767/HM999967). Phylogenetic results placed the isolate from this study within the *S. racemosum* clade, clustering with strains CBS 302.65 (Accession: MH870214/MH858577) and CBS 441.59 (Accession: MH869451/MH857910) (Fig. 4). Morphological traits characterized by fast spore production, round vesicle structures, and oval-shaped sporangiospores further supported this identification.

## Discussion

Fungi represent one of Earth's most diverse organisms, with approximately 156,000 documented species globally (Species Fungorum, 2024). Marine fungi, despite their ecological significance in nutrient cycling and symbiotic interactions, remain understudied. This study offers the first report of endophytic fungi associated with *Ulva* sp. in Iran, identifying seven species across six genera (*Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Chaetomium*, and *Syncephalastrum*), thus expanding knowledge of fungal diversity in Iran's coastal ecosystems and revealing novel host-fungus associations. *Aspergillus* dominated the isolates (34%), consistent with its widespread occurrence in marine algae[30– 31], while *Alternaria* and *Syncephalastrum* had the lowest percentages, each at 9%.

*A. caespitosus* and *S. racemosum* were documented as endophytes of *Ulva* sp. for the first time globally. Although previously isolated from soil and terrestrial plants, respectively[32– 33], their presence in marine algae underscores their adaptability to diverse environments. Also, the fungi *A. alternata*, *P. digitatum*, and *C. cladosporioides* were identified as endophytes of *Ulva* sp. for the first time globally. This study reports, for the first time, the isolation of *C. globosum* and *A. terreus* as endophytic fungi from the green macroalga *Ulva* sp. in Iran. *A. alternata* and *A. tenuissima* were recorded in Iranian waters for the first time, suggesting biogeographic variability in algal-fungal partnerships[34].

Robust species delimitation was achieved through the combined morphological and multi-gene phylogenetic analyses (ITS, *β-tubulin*, *GAPDH*, *TEF*, LSU). For instance, *A. terreus* was characterized by pale orange-buff colonies and globose conidia (2–2.5 µm), confirmed by ITS/*β-tubulin* sequencing, while *S. racemosum* exhibited rapid sporulation and ovoid sporangiospores (2–6 µm), validated by LSU/ITS data. Comparisons with global studies reveal consistency with prior records of *A. terreus* and *C. globosum* as marine endophytes[35– 36].

Previous studies in Iran have primarily focused on fungi associated with mangroves or fish, while endophytes in *Ulva* remain largely unexplored. In a recent study investigating fungal endophytes associated with algae from the southern shores of the Persian Gulf and the Sea of Oman, several species were isolated and reported. The fungi identified include *A. niger*, *A. flavus*, *A. terreus*, *A. puniceus*, *A. carlsbadensis*, *A. egyptiacus*, *A. chevalieri*, *P. chrysogenum*, *Penicillium* sp., *C. spicifera*, and *C. macrocarpum*. Notably, this study did not report any endophytic fungi from the genus *Ulva*. This highlights a substantial gap in our understanding of the diversity of endophytic fungi associated with marine algae within the Iranian ecosystem[37]. Globally, only a few studies have investigated *Ulva* endophytes, such as research conducted in Bangladesh[38]. The endophytic fungus *Beauveria* sp. has been reported to be isolated from the algae *Ulva* sp., specifically collected along the Mediterranean coastline[39]. *Rigidoporus vinctus*, a basidiomycete fungus, and *Candida railenensis*, a yeast, were identified in a study focused on the multi-functional bioactive secondary metabolites derived from endophytic fungi associated with marine algae. Specifically, these fungi were sourced from algae belonging to the genera *Enteromorpha* and *Ulva*, both of which are part of the Ulvaceae family[40].In a study focused on antioxidant activities, cytotoxicity and anticancer properties of extracts of endophytic fungi isolated from algae, different species of *Ulva* spp. were identified. The isolated endophytic fungi included *Chaetomium* sp., *Phomopsis* sp., *Acremonium* sp., *A. niger* and *Cladosporium* sp.[41]. *Ulva lactuca* algae as host of fungal endophytes *A. niger*, *A. terreus*, *Chatomium* sp., *Cladosporium* sp., *Eurotium* sp., *Leotiomyceta* sp., *P.chrysogenum*, and *Wigrospora* sp. and *Ulva linza* algae as the host of the fungal endophyte *Alternaria* sp. have been reported. Additionally, fungal endophytes such as *Aspergillus* sp. from the algae *Ulva intestinalis* and *Penicillium* sp. from *Ulva* sp. have been reported[42]. The species *S. racemosum* has been isolated and identified as an endophytic fungus from the red algae *Gracilaria corticata*[34]. In a study conducted on 14 different algae species from the Bay of Fundy in Canada, researchers aimed to explore the natural compounds produced by endophytic fungi associated with macroalgae. In this study, fungal species *Bionectria ochroleuca*, *Cordyceps* sp., *Penicillium* sp., and *Leptosphaeria* sp. were identified and isolated in connection with *Ulva intestinalis*[31]. From the *Ulva fasciata* algae gathered along the coast of Tamil Nadu, South India, endophytic fungi have been identified, including *A. niger*, *Curvularia* species (*Curvularia* sp. and *Curvularia lunata*), *Chaetomium* species, *Aspergillus* species (*Aspergillus* sp. and *A. terreus*), and *Paecilomyces* species[35]. The fungal endophyte

*C. globosum* has been isolated from the marine green alga *Ulva pertusa* from China[36]. The functional roles of these endophytes, such as nutrient exchange or stress tolerance, remain unexplored but deserve further study. For example, *A. terreus* is recognized for its production of bioactive compounds[43]. Endophytes support host plant growth through processes like nitrogen fixation, phosphate solubilization, and biological control of plant pathogens[44]. Broadening the scope of sampling to include additional Iranian coastal areas, such as the Persian Gulf and Sea of Oman, and diverse macroalgal hosts like *Gracilaria* and *Sargassum*, may reveal more extensive biogeographic patterns and co-evolutionary relationships. Additionally, endophytes like *A. caespitosus* may aid algal adaptation to climate change-induced stressors, such as warming oceans, a critical avenue for future research[45].

This study examines fungal endophytes associated with *Ulva* species in a specific marine region of Iran. Given the widespread distribution of *Ulva* species along both the southern and northern coasts of Iran, this research could serve as an important step toward expanding future studies and enhancing our understanding of the ecological interactions between fungal endophytes and seaweeds in Iranian marine ecosystems.

## Conclusion

This study offers the first report of endophytic fungi in Iranian *Ulva* sp., revealing 7 new records and highlighting the need for integrative approaches (morphology + genomics) in mycological research.

## Data availability

The datasets generated and/or analysed during the current study are available in the GenBank repository, https://www.ncbi.nlm.nih.gov/ (Table 2.).

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## Author contributions

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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