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Original article

Marine Fungi Associated with the Seagrass *Posidonia oceanica* (L.) Delile from the West Coast of Libya: Diversity, Taxonomy, and Ecological Significance

Huda Benzayed*[1], Taher El Masri[1], Shada Alkharraz[1], Rayan Sharif[1], Massuda Ghenghish[2]

[1]*Department of Biology, Faculty of Education, University of Tripoli, Tripoli, Libya*
[2] *Botany Department, Faculty of Science, University of Tripoli, Tripoli, Libya*
Corresponding email. h.benzayed@uot.edu.ly

Abstract
Posidonia oceanica (L.) Delile, a marine endemic seagrass, serves as a critical ecosystem engineer in coastal marine environments of the Mediterranean Sea. This study investigates the fungal communities associated with *P. oceanica* along the west coast of Libya, thereby complementing previous research conducted on the eastern coast of the country. Fungal assemblages were examined through microscopy and culture-dependent methods applied to surface-sterilized samples of roots, rhizomes, and leaves collected from depths of 5–12 m from the beaches of Tripoli extending from Al-Qarabolli to Al-Siyahiyya. A total of 34 fungal isolates belonging to twelve taxa were obtained and identified. The identified taxa encompass: *Halotthia posidonia* and *Chaetomium* sp. (Ascomycetes); *Alternaria* sp., *Aspergillus fumigatus*, *A. niger*, *Cladosporium* sp., *Humicola* sp., *Penicillium* sp., *Fusarium* sp., and *Scopulariopsis* sp. (Hyphomycetes); and the Mucoromycetes representatives *Mucor* sp. and *Rhizopus* sp. Modern taxonomic classification assigns ten taxa to Ascomycota (83.3%) and two to Mucoromycota (16.7%). The highest recorded frequency of appearance was shared equally by *Alternaria* sp. and *Aspergillus fumigatus* (17.65% each), followed by *A. niger* (14.71%). The lowest frequency (2.94%) was recorded for both *Cladosporium* sp. and *Penicillium* sp. Statistical analysis confirmed significant variation in the frequency distribution among the isolated taxa ($x^2 = 32.18$, df = 9, $p < 0.001$). Most isolated taxa exhibit characteristics of terrestrial-derived (facultative marine) fungi, while *Halotthia posidonia* and *Humicola* sp. are considered obligate marine species. These findings expand our knowledge of the mycobiome of *P. oceanica* meadows along the Libyan coastline and underscore the need for further molecular-based surveys.
Keywords. Marine Mycology; *Posidonia oceanica*; Seagrass Fungi; Obligate Marine Fungi; Libya.

Introduction

Seagrasses are the only truly marine angiosperms, forming extensive meadows in shallow coastal and estuarine environments worldwide. They are currently classified into six families—Zosteraceae, Hydrocharitaceae, Posidoniaceae, Cymodoceaceae, Ruppiaceae, and Zannichelliaceae—encompassing twelve genera and approximately 72 recognized species [1] [2]. Along the Mediterranean Libyan coastline, three species are recorded: *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Ascherson, and *Halophila stipulacea* (Forsskål), with *P. oceanica* being the predominant and most ecologically significant species.

Posidonia oceanica meadows are recognized as critical coastal ecosystem engineers. They provide essential services including primary production, stabilization of seabed sediments, oxygenation of the water column, provision of nursery habitat for marine fauna, and—critically—long-term sequestration of "blue carbon" within their characteristic belowground structure, the matte [2] [3]. The matter accumulates over millennia and represents one of the most carbon-dense ecosystems on Earth.

Despite the ecological significance of *P. oceanica*, its associated fungal communities remain comparatively understudied relative to terrestrial plant-fungus interactions [4] [5] [6]. Early comprehensive surveys provided foundational data on fungal diversity in *P. oceanica* in Italian waters [7] [8] [9]. Notably, previous research compared fungal diversity across *P. oceanica* and associated macroalgae, demonstrating that the seagrass harbours a unique and diverse mycobiota [3]. Recent culture-independent, high-throughput sequencing studies have dramatically expanded our understanding of seagrass mycobiomes, revealing dominance of Ascomycota alongside previously cryptic groups [10] [11] [12].

In Libya, published information on marine fungi associated with *P. oceanica* along the eastern coastline dates to 2021, when confirmed [13] the isolation of 12 fungal species using the humid-chamber method was confirmed, representing new records for the eastern Libyan coast. Similarly, marine fungi associated with *Cymodocea nodosa* from the western coast have been reported [14]. The present study extends this work by documenting fungi associated with *P. oceanica* from the western coast of Libya, providing a more complete biogeographical picture of the fungal diversity associated with this seagrass across the Libyan coastline. Modern taxonomic placement of all isolated taxa according to the current Outline of Fungi is also provided [15].

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Materials and Methods

Sample Collection

Samples of roots, rhizomes, and leaves of *Posidonia oceanica* (L.) Delile were collected by divers from the beaches of Tripoli extending from Al-Qarabolli to Al-Siyahiyya along the west coast of Libya at depths of 5–12 m. Collected material was placed in sterile plastic bags, transported in cool boxes to the laboratory, and processed within 24 hours of collection.

Surface Sterilisation and Isolation

Surface stabilisation was performed by immersing plant material in 70% ethanol solution for 1–3 min, followed by three rinses with sterile distilled water. Surface-sterilised root, rhizome, and leaf segments were cut into approximately 5 cm pieces and plated onto Potato Dextrose Agar (PDA). Plates were incubated at room temperature (23 ± 2°C) for 3–4 weeks. Streptomycin was added to the PDA to suppress bacterial growth.

Identification

Following incubation, fungal colonies exhibiting distinct morphologies (variation in texture, colour, and growth pattern) were sub-cultured and examined under a light microscope. Reproductive structures—including ascospores, basidiospores, and conidia—were used for definitive identification at genus and/or species level. Standard identification keys were employed [16] [17] [18], and modern taxonomic placement follows the Outline of Fungi [15].

Statistical Analysis

The frequency of appearance (FA%) of each fungal taxon was calculated as: FA% = (number of isolates of a given taxon / total number of isolates) × 100. A Chi-squared goodness-of-fit test (x^2) was employed to determine whether the frequency distribution among the isolated taxa deviated significantly from a uniform (equal) distribution, using the formula: $x^2 = \Sigma\ ((\text{Observed} - \text{Expected})^2 / \text{Expected})$. All statistical analyses were performed using standard procedures ($\alpha = 0.05$).

Results

Fungal Taxa Identified

A total of 34 fungal isolates representing twelve taxa were identified from roots, rhizomes and leaves of *Posidonia oceanica* (Table 1). The identified taxa belong to two fungal phyla: Ascomycota (ten taxa, 83.3%) and Mucoromycota (two taxa, 16.7%). Within Ascomycota, three fungal classes were represented: Dothideomycetes (three taxa), Eurotiomycetes (three taxa), and Sordariomycetes (four taxa). The Mucoromycota were represented solely by the class Mucoromycetes (two taxa).

Table 1. Distribution of fungal taxa across roots, rhizomes and leaves of Posidonia oceanica.

Fungal Taxon	Roots	Rhizomes	Leaves
Alternaria sp.	-	+	+
Aspergillus fumigatus	+	+	-
Aspergillus niger	+	+	-
Chaetomium sp.	-	-	+
Fusarium sp.	+	+	-
Cladosporium sp.	-	-	+
Humicola sp.	+	-	-
Halotthia posidonia	-	+	-
Mucor sp.	+	-	-
Penicillium sp.	-	+	-
Rhizopus sp.	+	-	-
Scopulariopsis sp.	+	+	+

Modern Taxonomic Classification

(Table 2) presents the modern taxonomic classification of all identified taxa according to the current Outline of Fungi and relevant specialist literature [15]. Two taxa—*Halotthia posidonia* and *Humicola* sp.—are classified as obligate marine fungi, while the remaining ten are considered facultative (terrestrial-derived) marine fungi.

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Table 2. Modern taxonomic classification of fungal taxa isolated from Posidonia oceanica.

Taxon	Phylum	Class	Order	Family	Marine Status
Halotthia posidonia	Ascomycota	Dothideomycetes	Pleosporales	Halotthiaceae	Obligate
Alternaria sp.	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Facultative
Cladosporium sp.	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Facultative
Aspergillus fumigatus	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Facultative
Aspergillus niger	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Facultative
Penicillium sp.	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Facultative
Chaetomium sp.	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Facultative
Humicola sp.	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Obligate
Fusarium sp.	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Facultative
Scopulariopsis sp.	Ascomycota	Sordariomycetes	Microascales	Microascaceae	Facultative
Mucor sp.	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	Facultative
Rhizopus sp.	Mucoromycota	Mucoromycetes	Mucorales	Rhizopodaceae	Facultative

Frequency of Appearance and Statistical Analysis

The frequency of appearance (FA%) of each fungal taxon among the 34 isolates is presented in (Table 3). *Alternaria* sp. and *Aspergillus fumigatus* showed the highest frequencies (17.65% each, n = 6 isolates), followed by *A. niger* (14.71%, n = 5). *Mucor* sp. accounted for 11.76% (n = 4), while *Chaetomium* sp., *Fusarium* sp. and *Humicola* sp. each contributed 8.82% (n = 3). *Rhizopus* sp. represented 5.88% (n = 2), and *Cladosporium* sp. and *Penicillium* sp. each had the lowest frequency of 2.94% (n = 1).

Table 3. Frequency of appearance of fungal taxa isolated from Posidonia oceanica (n = 34 total isolates).

Fungal Taxon	No. of Isolates	Frequency of Appearance (%)
Alternaria sp.	6	17.65
Aspergillus fumigatus	6	17.65
Aspergillus niger	5	14.71
Mucor sp.	4	11.76
Chaetomium sp.	3	8.82
Humicola sp.	3	8.82
Fusarium sp.	3	8.82
Rhizopus sp.	2	5.88
Cladosporium sp.	1	2.94
Penicillium sp.	1	2.94
Total	34	100.00%

Statistical Summary — Chi-Squared Goodness-of-Fit Test

- H_0: Fungal taxa are equally distributed among isolates.
- Observed values (n): 6, 6, 5, 4, 3, 3, 3, 2, 1, 1
- Expected value (uniform): 34/10 = 3.40 per taxon
- $x^2 = \Sigma ((O-E)^2 / E) \approx 32.18$ df = 9 p-value < 0.001
- Conclusion: The distribution is significantly non-uniform ($p < 0.05$). Dominant taxa (*Alternaria*, *A. fumigatus*, *A. niger*) account for 50.00% of all isolates.

Methodological Justification for Statistical Exclusion:

Although *Halotthia posidonia* and *Scopulariopsis* sp. were morphologically identified and confirmed within the seagrass tissues, they were excluded from the quantitative Frequency of Appearance (FA%) calculations and the subsequent Chi-squared test. This exclusion is methodologically necessary because these two taxa were detected via direct microscopy or humid-chamber techniques rather than yielding quantifiable, independent culturable isolates on the primary isolation media (n = 34). Incorporating non-overlapping qualitative observation data into a purely quantitative frequency distribution matrix would introduce significant statistical bias and violate the foundational assumptions of the Chi-squared uniform distribution test. Therefore, maintaining this distinction ensures rigorous statistical compliance while comprehensively reporting the qualitative taxonomic richness of the *P. oceanica* mycobiome.

Graphical Representation of Results

(Figure 1) presents the frequency of appearance of fungal taxa isolated from *Posidonia oceanica*, rendered with a modern aesthetic. The dashed line indicates the mean frequency (11.05%), providing a clear reference point for comparison.

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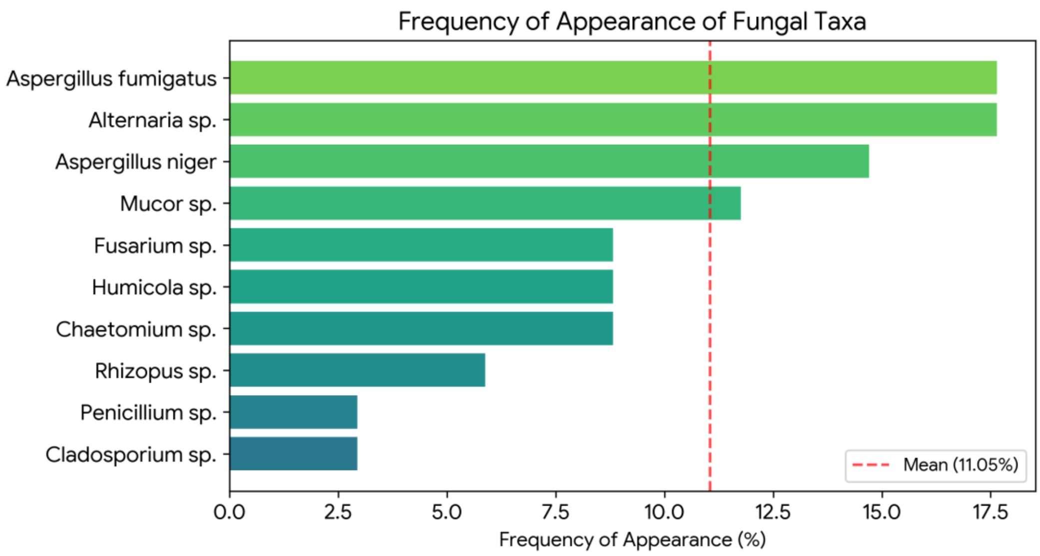

Figure 1. Frequency of appearance (%) of fungal taxa isolated from Posidonia oceanica.

(Figure 2) illustrates the phylum-level composition of fungal taxa associated with *Posidonia oceanica* from the west coast of Libya, utilizing a contemporary donut chart for enhanced readability.

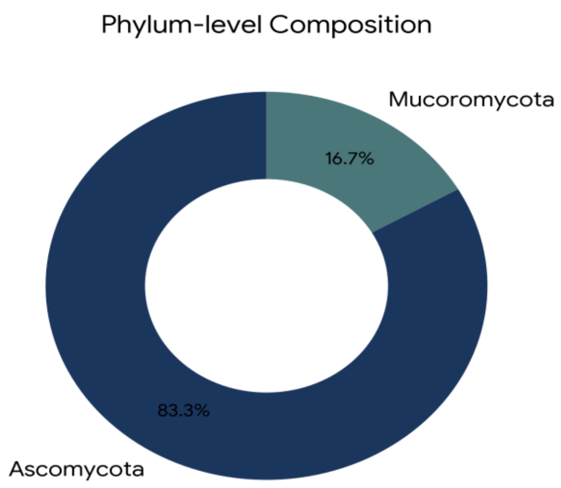

Figure 2. Phylum-level composition of fungal taxa associated with Posidonia oceanica.

Figure 3 displays the presence/absence distribution of fungal taxa across the three plant compartments (roots, rhizomes, and leaves) of *Posidonia oceanica*, presented as a clean and informative heatmap.

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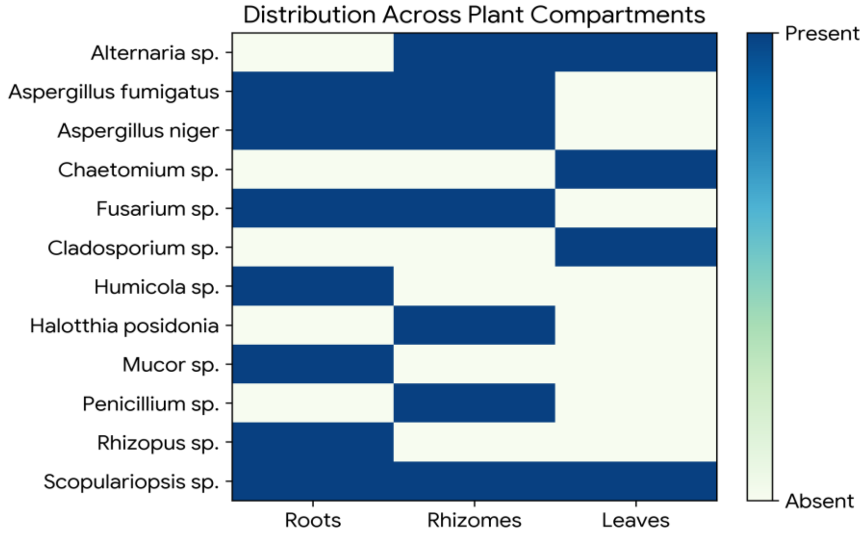

Figure 3. Presence/absence distribution of fungal taxa across plant compartments.

Figure 4 shows the class-level taxonomic diversity of fungal taxa isolated from *Posidonia oceanica*, depicted in a modern bar chart with clear value annotations.

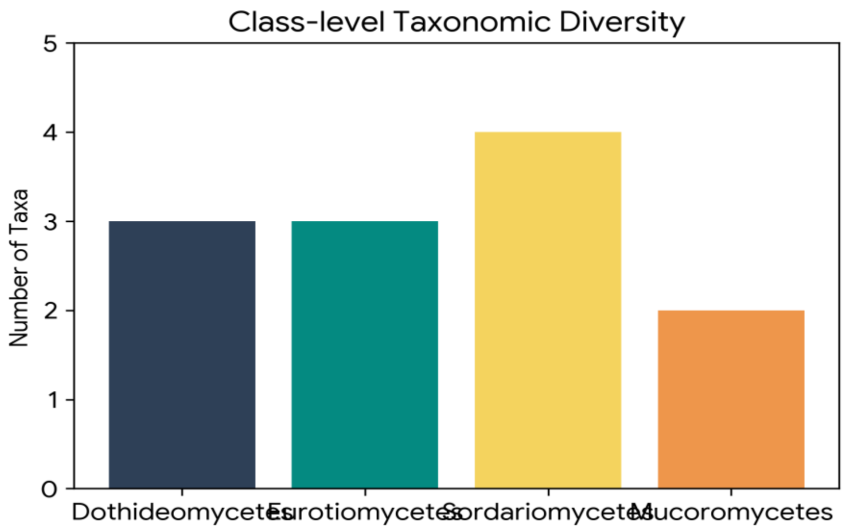

Figure 4. Class-level taxonomic diversity of fungal taxa isolated from Posidonia oceanica.

Discussion

The present study identified twelve fungal taxa associated with *Posidonia oceanica* from the west coast of Libya using culture-dependent methods. This diversity, while relatively modest, is consistent with the findings of other culture-based studies that typically report between 7 and 20 taxa from seagrasses [8] [9]. The limitation of culture-dependent methods in capturing the full mycobiome diversity—particularly non-sporulating taxa and obligate biotrophic fungi—is well documented. Recent metabarcoding studies have revealed substantially higher diversity, with hundreds of OTUs identified from *P. oceanica* tissues [11] [12].

The dominance of Ascomycota (83.3% of taxa) in the present study aligns with the global pattern observed across seagrass mycobiomes. A comprehensive review of culture-dependent studies found that Eurotiales fungi—specifically Aspergillus and Penicillium—account for over 25% of all listed taxa in seagrass-associated fungal surveys [19]. The consistent recurrence of these two genera across geographically distant studies [3] [7] [18] reflects their cosmopolitan nature and ability to colonise marine substrates. Their detection in the present Libyan study confirms a pattern repeatedly documented in the literature [11] [13].

Of particular ecological significance is the obligate marine fungus *Halotthia posidonia*, which, as its name implies, is specifically and consistently associated with *P. oceanica*. This ascomycete belongs to the order Pleosporales (class Dothideomycetes) and was detected here on rhizome samples, consistent with its typical substrate preference [17] [20]. A second obligate marine taxon, *Humicola* sp., was isolated exclusively from roots. This hyphomycete, characterised by its solitary, thick-walled, pyriform to clavate conidia, was previously reported from rhizomes of *Cymodocea nodosa* along the western Libyan coast [14], suggesting it may be a consistent component of the seagrass mycobiome in this region.

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The detection of Mucoromycota—represented by *Mucor* sp. and *Rhizopus* sp.—in a marine environment is noteworthy. Members of the class Mucoromycetes are predominantly soil organisms; however, their recovery from seagrass tissues has been reported in several studies and likely reflects their dispersal from adjacent terrestrial or riverine environments, or persistence in the sediment microbiome [3] [15]. In the modern classification, these taxa are placed in the phylum Mucoromycota (replacing the former Zygomycota), order Mucorales, family Mucoraceae (Mucor) and Rhizopodaceae (Rhizopus) respectively [15] [21].

Chaetomium sp., detected here from leaf samples, has been reported sporadically in association with *P. oceanica* in the Mediterranean [7]. This ascomycete (Sordariomycetes, Sordariales) is characterised by superficial, hair-covered ascomata and is capable of degrading cellulosic substrates, suggesting a saprotrophic role in the decomposition of senescent seagrass leaves [12] [22].

The statistical analysis ($x^2 = 32.18$, df = 9, $p < 0.001$) confirms a significantly non-uniform distribution among taxa, with *Alternaria* sp. and *A. fumigatus* collectively accounting for 35.3% of all isolates. This uneven distribution is consistent with patterns described in broader meta-analyses of seagrass mycobiomes, where a few dominant genera account for the majority of culture-based recoveries, likely reflecting competitive advantages in nutrient-rich seagrass tissues or greater sporulation rates on standard media such as PDA [11] [19].

Comparison with the eastern Libyan coast study [13] reveals both shared and unique taxa. Genera such as Alternaria, Aspergillus, Cladosporium, Penicillium, and Fusarium were common to both surveys, suggesting these are widespread components of the *P. oceanica* mycobiome along the Libyan coast. However, the western coast survey documented Scopulariopsis sp.—a member of the Microascaceae—that was not reported in the eastern study, indicating some biogeographic variation. Future studies employing molecular barcoding (ITS, LSU) and culture-independent approaches are strongly recommended to fully characterise the mycobiome of *P. oceanica* across the Libyan coastline.

Conclusion

This study documents twelve fungal taxa associated with *Posidonia oceanica* from the west coast of Libya, providing modern taxonomic classifications at the phylum, class, order, and family levels. The mycobiota is dominated by Ascomycota (83.3%), with Mucoromycota representing the remaining taxa. Two obligate marine species were confirmed: *Halotthia posidonia* and *Humicola* sp. Statistical analysis revealed a significantly non-uniform frequency distribution, with *Alternaria* sp. and *Aspergillus fumigatus* as the most frequently isolated taxa. These findings complement the existing knowledge of the eastern Libyan coastline and contribute to the broader understanding of fungal diversity in Mediterranean *P. oceanica* meadows. Molecular-based surveys and ecological studies investigating the functional roles of these fungi—including their potential as producers of bioactive secondary metabolites—are recommended for future research.

Conflict of interest. Nil

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