Fungal Diversity in the Neptune Forest: Comparison of the Mycobiota of Posidonia oceanica, Flabellia petiolata, and Padina pavonica

Anna Poli, Elena Bovio, Lucrezia Ranieri, Giovanna Cristina Varese* and Valeria Prigione

Department of Life Sciences and Systems Biology, Mycotheca Universitatis Taurinensis, University of Torino, Turin, Italy

Fungi are widely distributed in the Oceans, interact with other organisms and play roles that range from pathogenic to mutualistic. The present work focuses on the characterization of the cultivable mycobiota associated with the seagrass Posidonia oceanica (L.) Delile collected off the Elba Island (Italy). We identified 102 taxa (mainly Ascomycota) by the mean of a polyphasic approach. Leaves, rhizomes, roots and mate were characterized by unique mycobiota revealing a "plant-part-specificity." The comparison with the mycobiota associated with the green alga Flabellia petiolata and the brown alga Padina pavonica underlined a "substrate specificity." Indeed, despite being part of the same phytocoenosis, these photosynthetic organisms recruit different fungal communities. The mycobiota seems to be necessary for the host's defense and protection, playing, in this way, remarkable ecological roles. Among the 61 species detected in association with P. oceanica (including two species belonging to the newly introduced genus Paralulworthia), 37 were reported for the first time from the Mediterranean Sea.

Keywords: marine fungi, Mediterranean Sea, seagrass, algae, phylogeny, Lulworthiales

INTRODUCTION

Posidonia oceanica (L.) Delile, otherwise known as Neptune grass, is the most important seagrass in the Mediterranean Sea (Personnic et al., 2014). This endemic long-lived plant (Magnoliophyta, kingdom Archaeplastida) relies mainly on vegetative reproduction, and its largest clones can spread over 15 km (Arnaud-Haond et al., 2012). Neptune grass meadows, or rather forests, are a spawning ground, a nursery and a permanent habitat for many species: the presence of over 400 different seaweeds species and several thousands of animal species makes this underwater habitat a unique biodiversity hotspot (Boudouresque, 2004).

Rhizomes, roots and senescent leaf sheaths of P. oceanica form a characteristic peat-like seabed layer (mate) that can be several meters thick and thousands of years old. Mate are extremely resistant to microbiological decay, however, herbivores can feed on them with the aid of symbiotic microorganisms such as fungi or bacteria capable of degrading organic matters (Vohnik et al., 2017). To this respect, it is important to underline that likewise other seagrasses, P. oceanica supports a complex ecosystem that supplies large amounts of nutrients to the organisms living within and in adjacent waters. Fungi play an important role in the functioning of this ecosystem: while parasites and pathogens can negatively affect the wellness of the meadows, mutualists may be
essential for their health (Vohnik et al., 2019). Finally, saprobes transform dead plants into nutritive detritus through their degrading activity (Raghukumar, 2017). Overall, Posidonia forests are home to 20–25% of Mediterranean species and represent the climax community of the Mediterranean Sea (Minelli et al., 2008). However, in the aftermath of the anthropic impact, of the presence of exotic species and of the climate change, this climax community is seriously threatened and since 1992 is a priority in Annex I of the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora.

Marine fungi are a huge part of the microbial diversity hosted in the sea. Indeed, they have been retrieved worldwide from biotic and abiotic substrates such as algae, sediments, invertebrates, drift wood, etc. (Jones and Pang, 2012; Garzoli et al., 2014, 2015, 2018; Gnavi et al., 2017; Raghukumar, 2017; Bovio et al., 2018). Nevertheless, despite the total fungal diversity has been estimated to range between 10,000 and 12,500 taxa (Jones et al., 2019), only 1281 species have been described to date1. These organisms are important primary decomposers and living as mutualists (ecto- and endosymbionts), parasites, pathogens and saprobes, contribute greatly to nutrient cycling and food web (Amend et al., 2019; Grossart et al., 2019).

As part of a thorough investigation aimed to uncover the fungal diversity of the Mediterranean Sea, we focused on the isolation and identification of the cultivable mycobiota associated with P. oceanica, collected off the Elba Island (Tuscany). Once defined, the mycobiota was compared to those associated with the green and brown algae Flabellia petiolata (Gnavi et al., 2017) and Padina pavonica (Garzoli et al., 2018), two important components of the same phytocoenosis, collected during the same sampling and in close proximity. Finally, this paper introduces the novel genus Paralulworthia represented by two new species P. gigaspora and P. padinae.

MATeRIALS AND METHODS

Sampling

Plants of P. oceanica were collected from two sampling sites in the coastal waters of the Elba Island (Livorno, Italy): (1) Ghiaie (UTM WGS84 42°49′04″N, 10°19′20″E) and (2) Margidore (UTM WGS84 42°45′29″N, 10°18′24″E). Twelve plants (6 per sampling site) with the surrounding fluctuating senescent leaves (matte) were harvested at depth ranging from 5 to 15 m below sea level (bsl). Plants were maintained in sterile dark containers at 4 °C and processed within 36 h from sampling. Specific permissions to operate in the protected area of “Le Ghiaie” (Ghiaie site) and to the freely accessible Margidore site, were obtained by the port authority of Portoferraio (Livorno, Italy).

Fungal Isolation

Each plant was divided into four parts: leaves, rhizomes, roots and matte (Figure 1). In order to remove unrefined sediments, each sample was individually sonicated (30″ each time) and serially washed (three times) in sterilized artificial SeaWater (SW, 3.4% w/v Sea Salt mix – Sigma-Aldrich, St. Louis, MO, United States – in distilled water). Individual samples were homogenized in 20 mL of sterile filtered seawater by means of Ultra-Turrax (IKA, Staufen, Germany). One mL of homogenate was plated onto Petri dishes (15 cm Ø) containing the following culture media: Corn Meal Agar SeaWater (CMASW; 17 g CMA – Sigma-Aldrich, St. Louis, MO, United States – in 1 L of filtered SW) or Agar Posidonia (AP; 20 g of P. oceanica heated in 100 mL seawater – 60°C, 30 min – were filtered prior to the addition of 18 g agar and SW up to 1L). Each medium was supplemented with antibiotics (Gentamicin 80 mg L⁻¹, Piperacillin and Tazobactam 100 mg L⁻¹ – Sigma-Aldrich, St. Louis, United States).

Three replicates per medium and per sample were performed. A total of 288 plates were incubated at 15°C (spring average temperature of submerged meadows between 5 and 15 m bsl) for 15 days. Afterward, the temperatures was raised to 25°C for 45 days. This procedure allowed the isolation of both psychrophilic and mesophilic fungi. Colony forming units per gram of dry weight of each plant part (CFU g⁻¹ dw) were recorded. Isolated fungi were maintained in pure culture.

Fungal Identification

Fungi were identified by combining morpho-physiological, molecular and phylogenetic studies. Following preliminary determination of genera according to macroscopic and microscopic features (Domsch et al., 1980; von Arx, 1981; Kiffer and Morelet, 1997), fungal isolates were transferred to the media recommended by the authors of selected genus monographs for species identification. Molecular identification was performed by amplifying and sequencing the appropriate genetic marker as previously described (Poli et al., 2016; Garzoli et al., 2018). PCR products were purified and sequenced at Macrogen Europe Laboratory (Madrid, Spain). The resulting ABI chromatograms were visually inspected, trimmed and assembled to obtain consensus sequences using Sequencer 5.0 (GeneCodes Corporation, Ann Arbor, MI, United States)2. The newly generated sequences were compared by BLASTn analyses (default settings) to those available in public nucleotide databases provided by the NCBI (Bethesda MD, United States) and by the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands). Similarity values equal or higher than 98% (e-value > e-100) were considered credible.

Representative strains of each species isolated in pure culture during this work are preserved at Mycotheca Universitatis Taurinensis (MUT)3. The accession numbers of the sequences deposited in GenBank are: MK578237 – MK578251; MK581058 – MK581072; MK626711 – MK626722; MN165499 – MN165504; MN173595 – MN173610.

---

1www.marinefungi.org

2http://www.genecodes.com

3www.mut.unito.it
Paralulworthia gigaspora and Paralulworthia posidoniae: Growth Condition, Morphological, and Molecular Study

Following pre-growth, MUT 435, MUT 672, MUT 5413, and MUT 5261 were inoculated in triplicate onto Petri dishes (9 cm Ø) containing MEASW and incubated at 21°C. The fungal growth, as well as macro- and microscopic features, was monitored at 4, 7, 14, 21 and 28 days from the inoculum. In an attempt to induce sporulation, sterile specimens of Quercus ruber cork and Pinus pinaster wood (species autochthonous to the Mediterranean area) were placed on 3 weeks old fungal colonies (Panebianco et al., 2002) which were further incubated for 4 weeks at 21°C. Colonized wood specimens were transferred into 50 mL tubes containing 20 mL sterile sea water and incubated at 21°C for 1 month. Mature reproductive fungal structures were observed and images captured with an optical microscope (Leica DM4500B, Leica Microsystems GmbH, Germany) equipped with a camera (Leica DFC320, Leica Microsystems GmbH, Germany). For a better inspection of ascomata growing inside wood tissues, scraps of colonized wood embedded in melted paraffin were dissected with a cryostat (Leica CM1850, Leica Microsystems GmbH, Germany).

Genomic DNA was extracted as mentioned above and the partial sequences of the internal transcribed spacers including the 5.8S rDNA gene (nrITS), 28S large ribosomal subunit (nrLSU) and 18S small ribosomal subunit (nrSSU) were amplified by PCR using the primer pairs ITS1/ITS4 (White et al., 1990), LR0R/LR7 (Vilgalys and Hester, 1990), NS1/NS4 (White et al., 1990). A dataset consisting of nrSSU, nrITS, and nrLSU, was assembled on the basis of BLASTn results and of the most recent phylogenetic study focused on Lulworthiales (Azevedo et al., 2017). Reference sequences were retrieved from GenBank (Table 2). Alignments were generated using MUSCLE (default conditions for gap openings and gap extension penalties), implemented in MEGA v. 7.0 (Molecular Evolutionary Genetics Analysis), visually inspected and trimmed by TrimAl v. 1.2⁴ to delimit and discard ambiguously aligned regions. No incongruence was observed among single-loci phylogenetic trees and alignments were concatenated into a single data matrix with SequenceMatrix (Vaidya et al., 2011). The best evolutionary model under the Akaike Information Criterion (AIC) was determined with jModelTest 2 (Darriba et al., 2012). Phylogenetic inference was estimated using Maximum Likelihood (ML) and Bayesian Inference (BI) criteria. The ML analysis was produced using RAxML v. 8.1.2 (Stamatakis, 2014) under GTR + I + G evolutionary model and 1,000 bootstrap replicates. BI was performed with MrBayes 3.2.2 (Ronquist et al., 2012) with the same substitution model (GTR + I + G). The alignment was run for 5 million generations with two independent runs each containing four Markov Chains Monte Carlo (MCMC) and sampling every 100 iterations. The first 25% of generated trees were discarded as “burn-in.” A consensus tree was generated using the “sumt” function of MrBayes and Bayesian posterior probabilities (BYPP) were calculated. Consensus trees were

⁴http://trimal.cgenomics.org
visualized in FigTree v. 1.4.2\(^6\). Due to topological similarity of the two resulting trees, only BI analysis with BYPP and MLB values was reported.

**Statistical Analysis**

Differences among the total fungal loads (CFU g\(^{-1}\) dwt) of each plant portion were inferred by applying the analysis of variance (ANOVA), Bonferroni post hoc test (\(p < 0.05\)), using GraphPad Prism 5 for Windows, GraphPad Software (San Diego, CA, United States)\(^8\).

Significant differences among mycobiota of individual plant parts and organisms were evaluated by applying the PERmutational Multivariate ANalysis Of Variance (PERMANOVA; pseudo-F index; \(p < 0.05\)) and visualized by the Canonical Analysis of Principal Coordinates (CAP) or Principal Coordinate Analysis (PCO). The contribution of single species (in percentage) to the diversity observed within and between groups was assessed by SIMilarity PERCenrage (SIMPER) analysis. These analyses were performed with the statistical package PRIMER 7 (Plymouth Routines in Multivariate Ecological Research, Albany Auckland, New Zealand).

**RESULTS**

All plants of *P. oceanica* were colonized by fungi. The average fungal abundance (CFU g\(^{-1}\) dw) of the four parts ranged from \(5.0 \times 10^2\) CFU g\(^{-1}\) dw to \(1.4 \times 10^4\) CFU g\(^{-1}\) dw (Figure 2). In Ghiaie, rhizomes displayed the highest fungal load, followed by matte, leaves and roots, whereas in Margidore leaves were the richest part, followed by rhizomes and matte evenly, and finally by roots. Considering the individual plant parts, only roots did not differ significantly between the two sampling sites.

Overall, 287 isolates, representative of 102 taxa were retrieved: 43 from leaves, 70 from matte, 118 from rhizomes, and 56 from roots. The majority of taxa needed a specific medium and incubation temperature: 50 taxa were exclusively isolated from CMA with 31 from AP and only 21 from both media; 76 taxa grew exclusively at 25\(^\circ\) C and 11 at 15\(^\circ\) C. In comparison to the plants harvested at Ghiaie, the number of taxa retrieved from the plants harvested at Margidore was almost double (Table 1).

Sixty-eight percent of the strains were identified at species level and 12% at genus level. Those fungi that remained identified at higher rank were sterile in axenic culture and did not accomplished a univocal molecular result. Most of the taxa belonged to Ascomycota (91) while Basidiomycota (8) and Mucoromycota (3) were scarcely represented (Table 3).

In general, 10 classes, 12 orders and 32 families were represented. With the exception of Leotiomycetes (3%), Ascomycota were homogeneously distributed in three classes: Dothideomycetes (30%, mainly Pleosporales and Capnodiales – 18% and 12%, respectively), Sordariomycetes (29%, mainly Hypocreales and Microascaceae – 13% and 6%, respectively) and Eurotiomycetes (26%, mainly Eurotiales – 24%). As for Basidiomycota, the most represented classes were Agaricomycetes (4%), Tremellomycetes (1%), Ustilaginomycetes (1%) and Wallemiomycetes (1%). The single representative of Mucoromycota was *Cunninghamella bertholletiae* (Mucorales).

*Penicillium* and *Cladosporium* were the most abundant genera with 17 and 9 species, and were isolated from all sites and plant parts, regardless of the culture conditions used.

Out of the 102 taxa isolated, only *Cladosporium cladosporioides* and *Penicillium commune* were detected in all four plant parts; 7 and 18 taxa were isolated from three and two parts, respectively. The rest was exclusive to rhizomes (32), matte (15), leaves (14), and roots (13) (Figure 3).

In terms of fungal species diversity, plant parts were significantly different (PERMANOVA; \(p < 0.05\); Figure 4), with the exception of leaves vs matte (PERMANOVA; \(p = 0.06\)). The most frequent species were *Penicillium chrysogenum* (72.5%) in leaves, *Corollospora* sp. (39.8%), *Corollospora maritima* (21.0%)

---

\(^{6}\)http://tree.bio.ed.ac.uk/software/figtree

\(^{8}\)www.graphpad.com

**TABLE 1** Number of exclusive fungal taxa per site (Ghiaie and Margidore), medium (CMA and AP), temperature (15\(^\circ\) C and 25\(^\circ\) C) and plant part (leaves, roots, rhizomes, and matte).

|                | Ghiaie | Margidore |
|----------------|--------|-----------|
| Total          | 27 (53)| 49 (75)   |
| Medium         |        |           |
| CMA            | 23 (43)| 35 (53)   |
| AP             | 10 (29)| 22 (40)   |
| Temperature    |        |           |
| 15\(^\circ\) C | 3 (15) | 9 (23)    |
| 25\(^\circ\) C | 38 (50)| 52 (66)   |
| Plant part     |        |           |
| Leaves         | 6 (20) | 9 (21)    |
| Roots          | 4 (14) | 9 (18)    |
| Rhizomes       | 14 (31)| 22 (38)   |
| Matte          | 4 (24) | 11 (31)   |

In brackets total number of taxa retrieved.
**TABLE 2 | Dataset used for phylogenetic analysis.**

| Species                                                                                 | Strain    | Source                                      | nrITS       | nrSSU       | nrLSU       |
|-----------------------------------------------------------------------------------------|-----------|---------------------------------------------|-------------|-------------|-------------|
| **Achroceratosphaeria** potamia Réblová, Fourn. & Hyde                                  | JF 08139  | Submerged wood of *Platanus* sp.            | –           | GQ966541    | GQ966538    |
| **Bimuria** novae-zelandiae Hawkesw., Chea & Sheridan                                    | CBS 107.79| Soil                                        | –           | AY016338    | AY016356    |
| **Cumulospora marina** Schmidt                                                         | MF46      | Submerged wood                             | –           | GU252136    | GU252135    |
| **C. varia** Chatmala & Somrithipol                                                    | GC53      | Submerged wood                             | –           | GU256625    | GU256626    |
| **Halazon mehlae** Abdel-Aziz, Abdel-Wahab & Nagah.                                    | GR78      | Submerged wood                             | –           | EU848593    | EU848578    |
| **H. fuscus** (Schmidt) Abdel-Wahab, Pang, Nagah., Abdel-Aziz & Jones                  | NBRC 105256| Driftwood                                   | –           | GU252148    | GU252147    |
| **Hydea pigmaea** (Kohlm.) Pang & Jones                                                | NBRC 33069| Driftwood                                   | –           | GU252134    | GU252133    |
| **Kohlmeyeriella crassa** (Nakagiri) Kohlm., Volkm.-Kohlm., Campb., Spatafora & Gräfenhan | NBRC 32133| Sea foam                                    | LC146741    | AY879005    | LC146742    |
| **K. tubulata** (Kohlm.) Jones, Johnson & Moss                                        | PP115     | Marine environment                         | –           | AY878998    | AF491265    |
|                                                                                       | PP0989    | Marine environment                         | –           | AY878997    | AF491264    |
| **L. helminthicola** (Berk. & Broome) Weese ex Petch                                    | CBS 884.85| Yerba mate                                  | EU715680    | AY016345    | AY016362    |
| **Lindra thalassiae** Orpurt, Meyers, Boral & Simms                                   | JK 509A   | Marine environment                         | –           | U46874      | U46891      |
|                                                                                       | AFTOL 413 | Marine environment                         | DQ491508    | DQ470994    | DQ470947    |
|                                                                                       | JK 5090   | Marine environment                         | –           | AF195634    | AF195635    |
|                                                                                       | JK 4322   | Thalassia testudinum leaves                 | –           | AF195632    | AF195633    |
| **L. obtusa** Nakagiri & Tubaki                                                       | NRBC 31317| Sea foam                                    | LC146744    | AY879002    | AY878960    |
| **L. marinera** Meyers                                                                | AFTOL 5012| Marine environment                         | –           | FJ176847    | FJ176902    |
| **Lulworthia lignoarenaria** (Koch & Jones) Kohlm., Volkm.-Kohlm., Campb., Spatafora & Gräfenhan | AFTOL 5013| Marine environment                         | –           | AY879000    | AY878958    |
| **L. fucicola** Sutherland.                                                            | ATCC 64288| Intertidal wood                             | –           | AY879007    | AY878965    |
| **L. grandispora** Meyers                                                             | PP1249    | Marine environment                         | –           | AY879008    | AY878966    |
| **L. medusa** (Ellis & Everh.) Cribb & Cribb                                          | AFTOL 424 | Dead *Rhizophora* sp. branch                | –           | DQ522855    | DQ522856    |
|                                                                                       | NTOU3841  | Driftwood                                   | –           | KY026044    | KY026048    |
|                                                                                       | NTOU3847  | Decayed mangrove wood                       | –           | KY026046    | KY026049    |
|                                                                                       | NTOU3849  | Decayed mangrove wood                       | –           | KY026047    | KY026050    |
| **L. atlantica** Azevedo, Caeiro & Barata                                             | FCUL170907CP5| Sea water                                   | KT347219    | KT347201    | JN866824    |
|                                                                                       | FCUL280207CF9| Sea water                                   | KT347218    | KT347202    | JN866808    |
| **L. opaca** (Linder) Cribb & J.W. Cribb                                              | CBS 21860 | Driftwood in seawater                       | –           | AY879003    | AY87896    |
| **Matsusporium tropicale** (Kohlm.) Jones & Pang                                      | FCUL210208SP4| Sea water                                   | KT347205    | KT347193    | JN866843    |
| **Moleospora maritima** Abdel-Wahab, Abdel-Aziz & Nagah.                              | NTOU3849  | Submerged wood                              | –           | GU252142    | GU252141    |
|                                                                                       | MF836     | Drift stems of *Phragmites australis*       | –           | GU252138    | GU252137    |
| **Paralulworthia gigaspora** sp. nov.                                                 | MUT 435   | *P. oceanica* – rhizomes                    | MN649242    | MN649246    | MN649250    |

(Continued)
TABLE 2 | Continued

| Species                        | Strain   | Source                | nrITS          | nrSSU          | nrLSU          |
|--------------------------------|----------|-----------------------|----------------|----------------|----------------|
|                               | MANA 672 | P. oceanica – rhizomes| MN649244       | MN649248       | MN649252       |
|                               | MANA 5413| P. oceanica – rhizomes| MN649243       | MN649247       | MN649251       |
| Paralulworthia posidoniae sp. nov. | MANA 5261| P. oceanica – rhizomes| MN649245       | MN649249       | MN649253       |
| Zalerion maritima (Linder) Anastasiou | FCUL280207CP1 | Sea water            | KT347216       | KT347203       | JN886806       |
|                               | FCUL010407SP2 | Sea water            | KT347217       | KT347204       | JN886805       |
| Lulwoana uniseptata (Nakagiri) Kohlmeyer et al. | NBRC 32137 | Submerged wood       | LC146746       | LC146746       | LC146746       |
|                               | CBS 16760 | Driftwood            | –              | AY879034       | AY878991       |
| Satosphaeria monoceras Alcorn  | CBS 154,26 | n.d.                 | DO337380       | DO238603       | AO163668       |

Genbank sequences include newly generated nrITS, nrLSU and nrSSU amplicons relative to the novel species Paralulworthia gigaspora and P. posidoniae.

and Penicillium chrysogenum (9.25%) in matte, Penicillium antarcticum (28.1%), Lulworthiales sp. (24.1%), Aspergillus sydowii (14.5%) and Microascales sp. (14.1%) in rhizomes, and finally Lulworthiales sp. (59.0%) and Penicillium antarcticum (23.8%) in roots (SIMPER analysis). No significant difference (PERMANOVA; p = 0.471) was observed between sampling sites.

The mycobiota of P. oceanica included four strains of Lulworthiaceae (MUT 435, MUT 672, MUT 5413, and MUT 5261) that are representatives of a novel genus consisting of two species. The phylogenetic tree based on nrITS, nrSSU, and nrLSU (Figure 5), together with the morphological inspection confirmed the novelty of Paralulworthia gigaspora sp. nov. and of Paralulworthia posidoniae sp. nov.

Phylogenetic Inference and Taxonomy of Paralulworthia gigaspora and Paralulworthia posidoniae

Preliminary analyses carried out individually with nrITS, nrSSU, and nrLSU denoted no incongruence in the topology of the trees. The combined dataset, built on the basis of BLASTn results and of recent phylogenetic studies (Azevedo et al., 2017), consisted of 45 taxa (including MUT isolates) that represented 15 genera and 27 species. Twelve sequences (4 nrITS, 4 nrSSU, and 4 nrLSU) were newly generated, while 94 were retrieved from GenBank.

The combined dataset had an aligned length of 2135 characters, of which 1096 were constant, 352 were parsimony-uninformative and 687 parsimony informative.

The strains under investigation formed a strongly supported and separated clade (BYPP = 1.00; MLB = 100%) within Lulworthiaceae, unique family of the order Lulworthiales (Figure 5). Since this clade was close to the genus Lulworthia sensu latu, we introduced the novel genus Paralulworthia that included the two species Paralulworthia gigaspora and Paralulworthia posidoniae. The development of the sexual morphotype of MUT 435 and MUT 5261 on maritime pine wood confirmed the divergence between the two species. Paralulworthia gen. nov. MB 833285.
### TABLE 3 | Fungal taxa isolated from Posidonia oceanica.

| Taxa                                      | From Mediterranean Sea | From *P. oceanica* |
|-------------------------------------------|------------------------|-------------------|
| **Acrodontium pigmentosum** Videira & Crous* | FR                     | FR                |
| **Acrostalagmus luteoalbus** (Link) Zare, W. Gams & Schroers | [1], [2]               | FR                |
| **Alfaria dandenongensis** Crous*          | FR                     | FR                |
| **Alteraria sp.**                         | –                      | –                 |
| **Arthrinium arundinis** (Corda) Dyko & B. Sutton | [1], [2], [3]        | [3]               |
| **Arthrinium sp.**                        | –                      | –                 |
| **Aspergillus conicus** Blochwitz         | [4]                    | FR                |
| **Aspergillus fumigatus** Fresen.         | [2], [3]               | [3]               |
| **Aspergillus pseudoglaucus** Blochwitz*  | FR                     | FR                |
| **Aspergillus sydowii** (Bainier & Sartory) Thom & Church* | [2], [3]               | FR                |
| **Aspergillus versicolor** (Vuill.) Tirab.* | [2], [3]               | FR                |
| **Aureobasidium pullulans** (De Bary) G. Arnaud ex Cif., Ribaldi & Corte | [4] | FR |
| **Bjerkandera adusta** (Willd.) P. Karst. | [4]                    | FR                |
| **Cadophora sp.**                         | –                      | –                 |
| **Capnodium sp.**                         | –                      | –                 |
| **Cerrena unicolor** (Bull.) Murrill*     | FR                     | FR                |
| **Chrysosporium lobatum** Scharapov*      | FR                     | FR                |
| **Chrysosporium undulatum** P. Vidal, Guarro & Ulig* | FR                         | FR                |
| **Cladosporium aggregatocirocaticatum** Bensch, Crous & U. Braun* | FR                         | FR                |
| **Cladosporium allicinum** [Fr.: Fr.] Bensch, U. Braun & Crous | [1], [2], [4]            | FR                |
| **Cladosporium cladosporioides** (Fresen.) G.A. de Vries | [1], [2], [3], [6], [7] | [3] |
| **Cladosporium halotolerans** Zalar, de Hoog & Gunde-Cim. | [2], [6] | FR |
| **Cladosporium herbarum** (Pers.) Link | [1], [3] | FR |
| **Cladosporium pseudocladosporioides** Bensch, Crous & U. Braun | [2], [4], [6], [7] | FR |
| **Cladosporium sphaerospermum** Penz. | [1], [4] | [3] |
| **Cladosporium velox** Zalar, de Hoog & Gunde-Cim.* | FR | FR |
| **Cladosporium xylophilum** Bensch, Shabunin, Crous & U. Braun | [4] | FR |
| **Cordyceps farinosa** (Holmsk.) Kepler, B. Shrestha & Spatafora* | FR | FR |
| **Corollospora angulosa** Abdul-Wahab & Nagah.* | [8] | FR |
| **Corollospora maritima** Werderm.* | FR | FR |
| **Corollospora sp.** | – | – |
| **Cunninghamella bertholletiae** Stadel* | FR | FR |
| **Dasyascus virgineus** (Batsch) Gray* | FR | FR |
| **Didymella tabae** G.J. Jellis & Punith.* | FR | FR |
| **Dioszia sp.** | – | – |
| **Elbamycella rosea** A. Poli, E. Bovio, V. Prigione & G.C. Varese | [16] | [16] |
| **Engyodontium album** (Limber) de Hoog* | [6], [17] | FR |
| **Fusarium venenatum** Nirenberg* | FR | FR |
| **Gibellulopsis nigrescens** (Pethybr.) Zare, W. Gams & Summerb. | [1], [2], [3], [4], [6] | [3] |
| **Halosarpea japonica** Abdul-Wahab & Nagah.* | FR | FR |
| **Hypocreales sp.** | – | – |
| **Irpex lacteus** (Fr.) Fr.* | [16] | FR |
| **Lophiotrema rufa** (Fuckel) Y. Zhang ter, C.L. Schoch & K.D. Hyde | [1], [3] | [3] |
| **Lulworthiales sp.** | – | – |
| **Massarina sp.** | – | – |
| **Metarrhizium carneum** (Duché & R. Heim) Kepler, S.A. Rehner & Humber* | FR | FR |
| **Microascales sp.** | – | – |
| **Microascaceae sp.** | – | – |
| **Microsphaeropsis arundinis** (S. Ahmad) B. Sutton* | [1], [2], [3] | [3] |
| **Neoroussosella solani** (Crous & M.J. Wingf.) Jayasiri & K.D. Hyde* | FR | FR |
| **Neoroussosella sp.** | – | – |
| **Nigrospora oryzae** (Berkt. & Broome) Petch* | FR | FR |

(Continued)
### TABLE 3 | Continued

| Taxa                                      | From Mediterranean Sea | From P. oceanica |
|-------------------------------------------|------------------------|------------------|
| Onygenaceae sp.                           | –                      | –                |
| Ophiognomonia setacea (Pers.) Sogonov*    | FR                     | FR               |
| Paracamarosporium psoralae Crous & M.J. Wingf. * | FR                  | FR               |
| Paraconiothyrium variabile Riccioni, Damm, Verkley & Crous | [4]                 | FR               |
| Paralulworthia gigaspora sp. nov.*        | FR                     | FR               |
| Paralulworthia posidoniae sp. nov.*       | FR                     | FR               |
| Paraphaeosphaeria michotii (Westend.) O.E. Erkss.* | FR                  | FR               |
| Penicillium antarcticum A.D. Hocking & C.F. McRae | [1], [2], [4]     | FR               |
| Penicillium brevicompactum Dierckx        | [1], [2], [3], [4]    | [3]              |
| Penicillium canescens Sopp.*              | [2]                    | FR               |
| Penicillium chrysogenum Thom               | [1], [2]               | [3]              |
| Penicillium commune Thom                   | [1]                    | FR               |
| Penicillium cremeogriseum Chaib.*         | FR                     | FR               |
| Penicillium crustosum Thom                 | [1]                    | FR               |
| Penicillium cyjetkowici S. W. Peterson, Z. Jurjevic & J. C. Frisvad* | FR            | FR               |
| Penicillium glabrum (Wehrme) Westling*    | FR                     | FR               |
| Penicillium janczewskii K.M. Zalesky*     | [3]                    | [3]              |
| Penicillium panicum Frisvad*              | FR                     | FR               |
| Penicillium pinophilum Hedg.*             | FR                     | FR               |
| Penicillium roseo purpureum Dierckx       | [4]                    | FR               |
| Penicillium solitum Westling              | [1]                    | FR               |
| Penicillium sp.                           | –                      | –                |
| Penicillium steckii K.M. Zalesky          | [4], [18]              | FR               |
| Penicillium vinaceum J.C. Gilman & E.V. Abbott* | [2]                | FR               |
| Phoma sp.                                 | –                      | –                |
| Plectosphaerellaceae sp.                  | –                      | –                |
| Pleosporales sp.                          | –                      | –                |
| Pleurophoma sp.                           | –                      | –                |
| Porostereum spadiceum (Pers.) Hjortstam & Rywarden | FR             | FR               |
| Preussia funiculata (Preuss) Fückel*      | FR                     | FR               |
| Preussia terricola Cain*                  | FR                     | FR               |
| Pseudozyma prolific Bandoni               | FR                     | FR               |
| Pseudureocctium ilacium (Thom) Luangsa-ard, Houbreken, Hywel-Jones & Samson* | [6]             | FR               |
| Pyrenochaetopsis sp.                      | –                      | –                |
| Pyrenochaetopsis leptospora (Sacc. & Briard) Gruyter, Aveskamp & Verkley* | FR | FR               |
| Ramularia sp.                             | –                      | –                |
| Rheocercosporidium carotae (Årsello) U. Braun | [1], [19]            | [19]             |
| Roussellaceae sp.                         | –                      | –                |
| Sarocladium bacilisporum (Onions & G.L. Barron) Summerb.* | FR       | FR               |
| Scedosporum apiospermum (Sacc.) Sacc. ex Castell. & Chalm.* | [6]       | [6]              |
| Schizophyllum commune Fr.                 | [1], [3], [4]         | [3]              |
| Sordariomycetes sp.                       | –                      | –                |
| Tairomycetes assutensis Samson & Abdel-Fattah* | FR               | FR               |
| Tairomycetes trachyspermus (Shear) Stolik & Samson* | FR       | FR               |
| Tolypocladium cylindrosporum W. Gams*     | FR                     | FR               |
| Trichoderma atroviride P. Karst.*         | [6]                    | FR               |
| Trichoderma harzianum Rifai               | [2], [3], [4], [18]   | [3]              |
| Wallemia sebi (Fr.) Arx                   | [3], [4]               | [3]              |
| Zoophila ebrissa Guarro, P.F. Cannon & Aa* | FR                   | FR               |

FR, First Record from the Mediterranean Sea and/or P. oceanica. *Species isolated only from P. oceanica within the phytocoenosis. References: [1] Gnavi et al. (2017); [2] Bovio et al. (2017); [3] Panno et al. (2013); [4] Garzoli et al. (2018); [5] Greco et al. (2017); [6] Garzoli et al. (2015); [7] Garzoli et al. (2014); [8] Abdel-Wahab et al. (2009); [9] Vohník et al. (2016); [10] Jones et al. (1972); [11] Montemartini (1979); [12] Furtado and Jones (1980); [13] Grasso et al. (1985); [14] Cuomo et al. (1988); [15] Grasso et al. (1990); [16] Poli et al. (2018); [17] Proksch et al. (2010); [18] Paz et al. (2010); [19] Gnavi et al. (2014).
Type species: Paralulworthia gigaspora sp. nov.

Etymology: in reference to the phylogenetic closeness to the genus Lulworthia sensu latu.

Phylogenetic placement: Lulworthiaceae, Lulworthiales, Sordariomycetes, Ascomycota. The genus Paralulworthia gen. nov. clustered together with the genera Lulworthia sensu latu and Halazoon (Figure 5).

Description: growing efficiently on wood. Ascomata subglobose to ellipsoidal, single, scattered, immersed in the cortex or erumpent, brown to black at maturity; peridium dark brown composed of several layers of thick–walled cells of textura angularis. Asci containing 8 spores, dehiscent. Sterile elements not observed. Ascospores uni- to 3-septate at maturity, constricted at septa (particularly the central one), ellipsoid to fusiform, pointed at both ends, thin-walled, from light to dark brown at maturity, straight.

Paralulworthia gigaspora sp. nov. MB 833288 (Figure 6).

Type: Europe, Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghiaie ISL, 3–5 m depth, 42°49’04”N, 10°19’20”E March 2010. R. Mussat-Sartor and N. Nurra, on the sea grass P. oceanica rhizomes, MUT 435 holotype, living culture permanently preserved in metabolically inactively state by deep-freezing at MUT. MUT 672 and MUT 5413 = paratypes.

Etymology: in reference to the large size of the ascospores.

Description: growing actively on P. pinaster. Hyphae 3.2 µm wide, septate, from brownish to hyaline. Chlamydospores numerous, solitary and multicellular, from one to four celled, 2 × 3 µm diam., from light to dark brown, globose and subglobose.

Sexual morph: Ascomata 480-540 × 160–270 µm (\( \bar{x} = 506.7 \times 205 \) µm, \( n = 10 \)), subglobose to ellipsoidal, single, scattered, immersed in the cortex or erumpent, brown to black at maturity; peridium dark brown 73-88 µm thick composed of several layers of thick–walled cells of textura angularis. Asci containing 8 spores, dehiscent, fusiform to cylindrical, containing 8 spores, dehiscent, hyaline, vertically oriented to the host surface. Sterile elements not observed. Ascospores 76-83 × 19-25 µm (\( \bar{x} = 79.3 \times 21.7 \) µm, \( n = 10 \)), uni- to 3-septate at maturity, constricted at septa (particularly the central one), ellipsoid to fusiform, pointed at both ends, thin-walled, from light to dark brown at maturity, straight.

Asexual morph not observed.

Colony description: growing on MEASW from 60 to 90 mm Ø after 28 days at 21°C, mycelium from feltrose to floccose, from whitish to ochre, reverse from light brown to bordeaux. A caramel colored diffusible pigment and exudates were often present.

Paralulworthia posidoniae sp. nov. MB 833291 (Figure 7).
Type: Europe, Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghiaie ISL, 3–5 m depth, 42°49′04″ N, 10°19′20″ E. March 2010, R. Mussat-Sartor and N. Nurra, on the sea grass Posidonia oceanica rhizomes, MUT 5261 holotype, living culture permanently preserved in metabolically inactively state by deep-freezing at MUT.

Etymology: in reference to the original substrate, the seagrass Posidonia oceanica.

Description: growing actively on P. pinaster. Hyphae 2.5–3.5 μm (\(\bar{x} = 2.9\ \mu m, n = 10\)) wide, septate, from hyaline to dematiaceous.

Sexual morph: Ascomata 365–450 × 155–230 μm (\(\bar{x} = 405 \times 191.3\ \mu m, n = 10\)), ellipsoidal, single, scattered, immersed in the cortex or erumpent, brown to black at maturity; peridium dark brown 23-50 μm thick composed of several layers of thick-walled cells of textura angularis. Asci 150–190 × 40–60 μm (\(\bar{x} = 171.3 \times 52\ \mu m, n = 10\)), containing 8 spores, clavate and early dehiscent. Sterile elements not observed. Ascospores oblong, 32–43 × 12–16 μm (\(\bar{x} = 38.4 \times 13.2\ \mu m, n = 10\)), uni- to 3-septate at maturity, constricted at septa (particularly the central one), ellipsoid to fusiform, pointed at both ends, thin-walled, from light to dark brown at maturity, straight.

Asexual morph not observed.

Colony description: growing on MEASW, reaching 42 mm Ø after 28 days at 21°C, mycelium feltrose with irregular edges, whitish with slight shades of purple in the middle, gray-green at the edges; reverse caramel. A caramel colored diffusible pigment and exudates were often present.

**DISCUSSION**

Broadening the knowledge of marine fungi associated with a vast range of biotic and abiotic substrates is becoming more and more important. The reason behind this lies in the ecological relevance that these organisms may play in the oceans and in their possible exploitation as a source of potentially novel enzymes and
bioactive compounds (Bovio et al., 2019; Grossart et al., 2019; Overy et al., 2019; Youssef et al., 2019).

Nowadays, *P. oceanica* is rapidly disappearing from the Mediterranean Sea, being seriously threatened by a number of factors. Its mycobiota was partially studied in the past (Cuomo et al., 1985; Panno et al., 2013). Recently Vohnik and collaborators described the endophytes colonizing the roots of this endemic seagrass (Vohnik et al., 2017, 2019). Here, we investigated the cultivable mycobiota of *P. oceanica* with the intent of comparing it with the fungal communities of two photobionts of the same phytocoenosis.

**Mycobiota of *P. oceanica***

The observed total fungal load ranged from $5 \times 10^2$ to $1.4 \times 10^4$ CFU g$^{-1}$ dw, approximately one order of magnitude higher than what reported by Panno et al. (2013) (up to $1.6 \times 10^3$ CFU g$^{-1}$ dw). Since the two sampling occurred in the same period of the year (March), we hypothesize that the climate factor did not affect the total load. The fungal abundance through the plant parts was also slightly different: in the present study, the fungal load followed the distribution rhizomes > leaves > mate > roots, whereas in the previous investigation mate and leaves were swapped (Panno et al., 2013). The use of specific culture media and of two incubation temperatures maximized the number of taxa isolated. Indeed, a lower temperature (15°C) allowed the growth of psychrotolerant/psychrophilic fungi such as four species of *Penicillium* (*P. canescens*, *P. glabrum*, *P. paneum*, *P. vinaceum*) (Table 2). Most probably, this is one of the reasons why we retrieved a number of taxa higher than what reported in 2013 by Panno (102 vs. 88).

As expected, Ascomycota prevailed, confirming once again the dominance of this phylum in the oceans. In detail, *Penicillium* and *Cladosporium*, were widely represented in all plant parts and sampling sites, and were characterized by the highest number of species (17 and 9, respectively). Members of *Penicillium* and *Cladosporium* are commonly reported in marine environments worldwide, probably due to the high adaptation to the peculiar chemical-physical conditions (Raghukumar, 2017). On the other side, it is not unreasonable to relate the high abundance of these genera to their extraordinary sporulation rate that may end in an overestimation of the substrate colonization (Imhoff et al., 2011).

Interestingly, 37 species were retrieved for the first time from the Mediterranean Sea and 61 from *P. oceanica* (Table 2), while only 19 species were previously detected in *P. oceanica* (Cuomo et al., 1985; Panno et al., 2013; Gnavi et al., 2014). Several species, besides being isolated for the first time from the Mediterranean Sea and from *P. oceanica*, were never found in any marine habitat before. For instance, this is the case of *Alfaria dandenongensis*, *Aspergillus pseudoglaucus*, *Cerrena unicolor*, *Didymella fabae*, and *Neorossoella solani*. *A. pseudoglaucus* is an opportunistic human pathogen (Siqueira et al., 2018) as well as *N. solani*, a causal agent of keratomycosis (Mochizuki et al., 2017), while their underwater ecological role is still obscure. On the contrary, we can hypothesize the role of the plant pathogen *D. fabae* and of the leaf litter saprobe *A. dandenongensis*. Similarly, it is possible to assume an active role for white rot fungi (i.e., *Bjerkandera adusta*, *C. unicolor* and *Schizopyllum commune*) in transforming recalcitrant matrixes (Poli et al., 2018). Finally, entomopathogenic fungi like *Cordyceps farinosa*, due to their ability to degrade chitin, may be capable of attacking the shellfish carapace.

**Distribution Among Plant Parts**

The mycobiota of *P. oceanica* changed significantly among the four parts of the plant, with the exception of leaves vs. mate. This last observation could be ascribable to the similar structure of the substrates: the main components of mate are the decaying and fluctuating leaves (Vohnik et al., 2017). The presence of antagonistic organisms, both micro- and macro-, together with the specific localization of toxic metabolites, such as tannic acid in the leaves (Pergent et al., 2008), may affect the distribution among plant parts. It is not unexpected to retrieve unique mycobiota on differently organized and composed parts of a complex plant, as occurs in terrestrial phanerogams. The only two species common to the four regions were *C. cladosporioides* and *P. commune*: the former is a usual inhabitant of the sea (Garzoli et al., 2015), the latter, although observed for the first time on *P. oceanica*, was already found associated with *F. petiolata* (Gnavi et al., 2017). *P. chrysogenum* dominated the mycobiota of the leaves, whereas the marine genus *Corollospora* prevailed in mate. With respect to rhizomes and roots, two taxa (*Lulworthiales sp.* and *P. antarcticum*) were the main components of the fungal communities, even though their proportions were different in the two plant parts. Vohnik et al. (2016) found members of the order *Lulworthiales* in the rhizomes and roots of *P. oceanica*. *Lulworthiales* degrade wood and marsh plants in marine and estuarine environments and seem to be the most common seagrass decomposers followed by *Corollospora maritima*. Indeed, *C. maritima* and *Lulworthiales* spp., are well-known cellulases producers and can break down complex lignocellulosic compounds, making them available for other organisms nourishment (Raghukumar, 2017). Interestingly, two new species of *Lulworthiales* were found in rhizomes and described here for the first time.

The retrieval of *P. chrysogenum* in leaves and, to a lower extent in mate, may be related to defense mechanisms against predators and/or pathogens, as demonstrated in terrestrial plants, where the presence of this fungus stimulates the synthesis of secondary metabolites such as salicylic and jasmonic acids (Chen et al., 2018).

**The Mycobiota of *P. oceanica* Compared With the Fungal Communities of *F. petiolata* and *P. pavonica***

*F. petiolata* and *P. pavonica* are two algae that characterize the Neptune forests. Since their mycobiota were recently described
(Gnvi et al., 2017; Garzoli et al., 2018), we considered worthwhile to compare the fungal communities associated with these three organisms that grow only few centimeters apart. To this end, one must bear in mind that samplings occurred at the same time and in the same sites (Ghiaie and Margidore); it would be interesting to clarify whether a strict specificity between fungi and photobionts exists.

In terms of number of taxa, *P. pavonica* displayed the richest mycoflora, followed by *P. oceanica* and finally by *F. petiolata*, where the number of taxa was almost halved. As shown in Figure 8, *P. oceanica* and the two algae had 11 species in common: *Acrostalagmus luteoalbus, Arthrinium arundinis, Cladosporium allicinum, C. cladosporioides, C. sphaerospermum, Gibellulopsis nigrescens, Massarina rubi, P. antarcticum, P. brevcompactum, P. roseopurpureum*, and *S. commune*. Besides their broad diffusion in the oceans, these fungi, due to the secretion of secondary metabolites (e.g., antialgal, antifungal, and/or antibacterial) may help in deterring colonization of their hosts by other microbes, and in warding off predators (Raghukumar, 2017). On the other side, the three substrates show specific mycobiota, significantly different from each other (Figure 9), with high and equivalent percentages of dissimilarity (94.68–95.74%). The uniqueness of each community derives from the prevalence of exclusive taxa (Figure 8).

Confirming the “substrate specificity” is also the fact that there is a significant difference between the mycobiota of the three organisms even if the "non-photosynthesizing" parts of *P. oceanica* were excluded from the analysis. The leaves of *P. oceanica* are in fact characterized by a mycobiota significantly different from that of the other two photobionts (data not shown).

This divergence in “substrate specificity” may be due to the different composition and morphology of the hosts (e.g., *P. pavonica* with its hairy surface could entrap a high number of fungal propagules, contrary to the slippery texture of *F. petiolata*) and/or to the different metabolites produced by seagrasses and algae. Extracts of *P. oceanica* were proved active against bacteria, dermatophytes, and yeasts (Bernard and Pesando, 1989) as well as against *Trypanosoma brucei rhodesiense* and *Leishmania donovani* (Orhan et al., 2006). Likewise, the activity of *P. pavonica* extracts against bacteria and fungi was comparable to the efficiency showed by antibiotics and antymycotics used as control (Al-Enazi et al., 2018). Antibacterial, antiviral, antimitic and antifungal properties were also detected in raw extracts of *F. petiolata* (Ballesteros et al., 1992). However, whether the macro-organisms, their microbiota, or the holobiont derived from their strict interaction is responsible for the production of the active compounds is still unclear. With the exclusion of cosmopolitan and/or widespread fungi, we can hypothesize the shaping of a relation between the microorganisms and their host, as recently demonstrated for three Atlantic sponges (Bovio et al., 2018). The establishment of a holobiont depends on both active (selection) and passive (drift) events. Due to the high connectivity of aquatic environments, differences in microbiota may be dependent on a combination of selection and drift. In most cases, fungi spread by passive dispersion of hyphal fragments and/or propagules that happen to adhere to the host. Their further development is consequent to the compatibility and interdependence between the individual organisms. In other words, the holobiont is an organism with its own features, modeled upon a mutual selection. The unimportance of the sampling site supports this idea. Ghiaie and Margidore are two extremely different sites both geographically (Ligurian
Sea and Tyrrhenian Sea, respectively) and for the anthropic influence: while the former is located in a marine protected area, the latter is subjected to an intense anthropic disturbance. Nevertheless, no significant difference was detected in terms of fungal biodiversity.

An often-underappreciated ecological function of holobionts is their ability to restrain and nourish rare species: the host provides a niche favorable for the growth of specific communities different from those of the surrounding environment (Dittami et al., 2019). To this regard, we may define “rare” the two novel species here introduced.

**Paralulworthia gigaspora and P. posidoniae**

This investigation introduces two new species that belong to a novel genus of Lulworthiaceae, the sole family of the order Lulworthiales (Sordariomycetes). Lulworthiaceae spp. are strictly marine species found on a range of substrates (e.g., submerged wood, seaweed, seafoam, etc.). This group includes the following genera: Cumulospora, Halazoon, Hydea, Kohlmeyerella, Lulwoana, Lulworthia, Lindra, Matsusprium, and Moleospora. The new genus Paralulworthia accommodates the four strains MUT 435, MUT 672, MUT 5413, and MUT 5261. *P. gigaspora* and *P. posidoniae* formed a strongly supported cluster segregated from the genus *Lulworthia sensu latu*. The previously suggested polyphyletic nature of *Lulworthia* spp. (Azevedo et al., 2017), that discriminate *Lulworthia sensu strictu* from *Lulworthia sensu latu* is here confirmed and justifies the introduction of the new genus *Paralulworthia*. Besides, members of the genus *Lulworthia* are morphologically characterized by filamentous hyaline ascospores (Kohlmeyer et al., 2000), well distinct from the ellipsoidal brown ascospores of the new genus. Furthermore, beyond the phylogenetic distance, the dimension of the ascocarps and ascospores allowed the recognition of two species. *P. gigaspora* and *P. posidoniae* were isolated exclusively from rhizomes that are characterized by a high content of lignin (Kaal et al., 2016). Reproductive structures were detected only following colonization of *P. pinaster* wood specimens submerged in sea-water. These two observations indicate a possible lignicolous nature of these species.

**DATA AVAILABILITY STATEMENT**

The sequences generated in this study can be found in GenBank. The accession numbers of the sequences deposited in GenBank are: MK578237–MK578251; MK581058–MK581072; MK626711–MK626722; MN165499–MN165504; MN173027–MN173033; MN177616–MN177627; MN173595–MN173610.

**AUTHOR CONTRIBUTIONS**

AP conducted molecular studies, phylogensis, data analysis, and manuscript writing. EB and LR carried morphological analysis. VP was responsible for morphological analysis and manuscript writing. GV supervised the work.

**FUNDING**

This study was funded by University of Torino (ex 60%) and Fondazione CRT (Turin, Italy).

**REFERENCES**

Abdel-Wahab, M. A., Nagahama, T., and Abdel-Aziz, F. A. (2009). Two new Corollospora species and one new anamorph based on morphological and molecular data. *Mycoscience* 50, 147–155. doi: 10.1007/s10267-008-0466-9

Al-Enazi, N. M., Awaad, A. S., Zain, M. E., and Alqasoumi, S. I. (2018). Antimicrobial, antioxidant and anticancer activities of *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* extracts. *Saudi Pharm. J.* 26, 44–52. doi: 10.1016/j.jsps.2017.11.001

Amend, A., Burgaud, G., Cunliffe, M., Edgcomb, V. P., Ettinger, C. L., Gutierrez, M. H., et al. (2019). Fungi in the marine environment: open questions and unsolved problems. *mBio* 10:e01189-18. doi: 10.1128/mBio.01189-18

Arnaud-Haond, S., Duarte, C. M., Diaz-Almela, E., Marba, N., Sintes, T., and Serrao, E. A. (2012). Implications of extreme life span in clonal organisms: millenary clones in meadows of the threatened seagrass *Posidonia oceanica*. *PLoS One* 7:e30454. doi: 10.1371/journal.pone.0030454

Azevedo, E., Barata, M., Marques, M. I., and Caeiro, M. F. (2017). *Lulworthia atlantica*: a new species supported by molecular phylogeny and morphological analysis. *Mycologia* 109, 287–295. doi: 10.1080/00275514.2017.1302255

**CONCLUSION**

The seagrass *P. oceanica* is home to a huge variety of fungi. The comparison of our data with those previously obtained on two algae collected in the same phytocoenosis underlines a substrate-specificity. Besides, we demonstrated a plant-part-specificity of the fungal communities associated with the host. Marine phanerogames, likewise their terrestrial counterparts, enroll distinct microbiomes in different regions (i.e., phylloplane, rhizoplane etc.). This selective distribution seems to increment the resistance of plant to pathogens and predators by producing chemo-attractants and anti-microbial compounds. This is even more important for *Posidonia* meadows that are seriously threatened by climate changes, allochthonous species and anthropic activities. Nonetheless, further studies will need to clarify the mechanisms (e.g., molecular, physiological, etc.) at the basis of the specific interaction between microorganisms and hosts responsible for the shaping of a holobiont.

**ACKNOWLEDGMENTS**

We are grateful to the non-profit cooperative society Pelagosphera s.c.r.l. for collecting the seagrass samples.
Ballesteros, E., Martin, D., and Uriz, M. J. (1992). Biological-activity of extracts from some Mediterranean macrophytes. *Bot. Mar.* 35, 481–485. doi: 10.1515/botm.1992.35.6.481

Bernard, P., and Pesando, D. (1989). Antibacterial and antifungal activity of extracts from the rhizomes of the Mediterranean seagrass *Posidonia oceanica* (L) Delile. *Bot. Mar.* 32, 85–88. doi: 10.1515/botm.1989.32.2.85

Boudouresque, C. F. (2004). Marine biodiversity in the Mediterranean: status, species, populations and communities. *Trav. Sci. Parc Nat. Port Cros* 20, 97–146.

Bovio, E., Garzoli, L., Poli, A., Luginani, A., Villa, P., Musumeci, R., et al. (2019). Marine fungi from the sponge *Gratella compressa*: biodiversity, chemodiversity, and biotechnological potential. *Mar. Drugs* 17:220. doi: 10.3390/md17042020

Bovio, E., Garzoli, L., Poli, A., Prigione, V., Firsova, D., McCormack, G., et al. (2018). The culturable mycobionta associated with three Atlantic sponges, including two new species: *Thelephalus balaustiformis* and *T. spongiae*. *Fungal Syst. Evol.* 1, 141–167. doi: 10.3114/fuse.2018.01.07

Bovio, E., Gnavi, G., Prigione, V., Spina, F., Denaro, R., Yakimov, M., et al. (2017). *Penicillium chrysogenum* mycelium of *Asparagopsis taxiformis* activates defense via gene regulation of salicylic acid and jasmonic acid signaling in *Arabidopsis*. *Physiol. Mol. Plant Pathol.* 103, 54–61. doi: 10.1016/j.pmpp.2018.04.006

Cuomo, V., Jones, E. B. G., and Grasso, S. (1988). Occurrence and distribution of marine fungi along the coast of the Mediterranean sea. *Progr. Oceanogr.* 21, 189–200. doi: 10.1016/0079-6611(88)90038-9

Cuomo, V., Vanzanella, F., Fresi, E., Cinelli, F., and Mazzella, L. (1985). Fungal flora of *Posidonia oceanica* and its ecological significance. *Trans. Br. Mycol. Soc.* 84, 35–40. doi: 10.1016/s0070-6611(85)80217-5

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2012). *jModelTest* 2: more models, new heuristics and parallel computing. *Nat. Methods* 9:772. doi: 10.1038/nmeth.2109

Dittami, S. M., Arboleda, E., Auguet, J.-C., Bigalke, A., Briand, E., Cardenas, P., et al. (2019). A community perspective on the concept of marine holobionts: current status, challenges, and future directions. *Prog. Oceanogr.* 189–200. doi: 10.1016/j.pocean.2015.05.005

Domsch, K. H., Gams, W., and Anderson, T. H. (1980). *Les Deuteromycetes: Classification et cles d'Identification Generique*. Paris: INRA Editions.

Kohlmeyer, J., Spatapora, J. W., and Volkmann-Kohlmeyer, B. (2000). *Lulworthiales*, a new order of marine Ascomycota. *Mycolologia* 92, 453–458. doi: 10.2307/3761504

Minelli, A., Rufio, S., and Stoch, F. (2008). *Seagrass Meadows, Flowering Plants in the Mediterranean Sea*. Udine: Mueseo Friulano di Storia Naturale.

Mochizuki, K., Nishida, T., Murata, K., Ishida, K., Sunada, A., Asari, S., et al. (2017). *Roussea solani* causing keratomycosis, with an observed both sexual and asexual morpha. *J. Infect. Chemother.* 23, 651–654. doi: 10.1007/s10157.2017.03.005

Montemartini, A. C. (1979). La micoflora marina della baia di Portofino. *Plant Biosyst.* 113, 297–325. doi: 10.1080/112635079094226650

Orhan, I., Sener, B., Atici, T., Brun, R., Perozzo, R., and Tasdemir, D. (2006). Turkish freshwater and marine macrophyte extracts show in vitro antiprotozoal activity and inhibit Fab, a key enzyme of *Plasmodium falciparum* fatty acid biosynthesis. *Phytomedicine* 13, 388–393. doi: 10.1016/j.phymed.2005.10.010

Overy, D. P., Rama, T., Oosterhuis, R., Walker, A. K., and Pang, K. L. (2019). The neglected marine fungi, *Sussus stricto*, and their isolation for natural products’ discovery. *Mar. Drugs* 17:42. doi: 10.3390/md171001042

Paz, Z., Komon-Zelazowska, M., Druzhinina, I. S., Aveskamp, M. M., Shnaiderman, A., Alumà, Y., et al. (2010). Diversity and potential antifungal properties of fungi associated with a Mediterranean seagrass. *Fungal Divers.* 42, 17–26. doi: 10.1007/s13225-010-0020-x

Pergent, G., Boudouresque, C.-F., Dumay, O., Pergent-Martini, C., and Wallis-Echeverria, S. (2008). Competition between the invasive macrophyte *Caulerpa taxifolia* and the seagrass *Posidonia oceanica*: contrasting strategies. *BMC Ecol.* 8:20. doi: 10.1186/1472-6785-8-20

Persson, C., Boudouresque, C. F., Astruch, P., Ballesteros, E., Blouet, S., Bellan-Santini, D., et al. (2014). An ecosystem-based approach to assess the status of a Mediterranean ecosystem, the *Posidonia oceanica* seagrass meadow. *PLoS One* 9:e89984. doi: 10.1371/journal.pone.0098994

Poli, A., Lazari, A., Prigione, V., Vazcones, S., Spadaro, D., and Varese, G. C. (2016). Influence of plant genotype on the cultivable fungi associated to tomato rhizosphere and roots in different soils. *Fungal Biol.* 120, 862–872. doi: 10.1016/j.funbio.2016.03.008
Poli, A., Vizzini, A., Prigione, V., and Varese, G. C. (2018). Basidiomycota isolated from the Mediterranean sea – phylogeny and putative ecological roles. *Fungal Ecol.* 36, 51–62. doi: 10.1016/j.funeco.2018.09.002

Proksch, P., Putz, A., Ortlepp, S., Kjer, J., and Bayer, M. (2010). Bioactive natural products from marine sponges and fungal endophytes. *Phytochem. Rev.* 9, 475–489. doi: 10.1007/s11101-010-9178-9

Raghukumar, S. (2017). *Fungi in Coastal and Oceanic Marine Ecosystems: Marine Fungi.* Basel: Springer.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., et al. (2012). MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. doi: 10.1093/sysbio/sys029

Siqueira, J. P. Z., Sutton, D. A., Gene, J., Garcia, D., Wiederhold, N., and Guarro, J. (2017). *Species of Aspergillus section Aspergillus from clinical samples in the United States.* *Med. Mycol.* 56, 541–550. doi: 10.1093/mmy/myx085

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. doi: 10.1093/bioinformatics/btu033

Vaidya, G., Lohman, D. J., and Meier, R. (2011). *SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information.* *Cladistics* 27, 171–180. doi: 10.1111/j.1096-0031.2010.00329.x

Vilgalys, R., and Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 172, 4238–4246. doi: 10.1128/JB.172.8.4238-4246.1990

Vohník, M., Borovec, O., Kolaríkova, Z., Sudova, R., and Reblova, M. (2019). Extensive sampling and high-throughput sequencing reveal *Posidonomycetes atricolor* gen. et sp. nov. (Aigialaceae, Pleosporales) as the dominant root mycobiont of the dominant Mediterranean seagrass *Posidonia oceanica.* *Mycokeys* 55, 59–86. doi: 10.3897/mycokeys.55.35682

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Poli, Bovio, Ranieri, Varese and Prigione. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.