

Article

# News from the Sea: A New Genus and Seven New Species in the Pleosporalean Families Roussoellaceae and Thyridariaceae

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

Mycotheca Universitatis Taurinensis, Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; anna.poli@unito.it (A.P.); elena.bovio@inrae.fr (E.B.); lucrezia.ranieri@edu.unito.it (L.R.); valeria.prigione@unito.it (V.P.)

\* Correspondence: cristina.varese@unito.it; Tel.: +39-011-670-5964

Received: 19 March 2020; Accepted: 3 April 2020; Published: 6 April 2020



**Abstract:** Nineteen fungal strains associated with the seagrass *Posidonia oceanica*, with the green alga *Flabellia petiolata*, and the brown alga *Padina pavonica* were collected in the Mediterranean Sea. These strains were previously identified at the family level and hypothesised to be undescribed species. Strains were examined by deep multi-loci phylogenetic and morphological analyses. Maximum-likelihood and Bayesian phylogenies proved that *Parathyridariella* gen. nov. is a distinct genus in the family Thyridariaceae. Analyses based on five genetic markers revealed seven new species: *Neoroussoella lignicola* sp. nov., *Roussoella margidorensis* sp. nov., *R. mediterranea* sp. nov., and *R. padinae* sp. nov. within the family Roussoellaceae, and *Parathyridaria flabelliae* sp. nov., *P. tyrrhenica* sp. nov., and *Parathyridariella dematiacea* gen. nov. et sp. nov. within the family Thyridariaceae.

**Keywords:** marine fungi; new taxa; phylogeny; lignicolous fungi

## 1. Introduction

Marine fungi are a relevant and active component of the microbial communities that inhabit the oceans [1]. Fungi in the marine environment live as mutualists, parasites, pathogens and saprobes, and are pivotal to marine food webs because of the recycling of recalcitrant substrata [2]; besides, these widely dispersed organisms are a source of novel bioactive compounds [3].

Marine fungi have been recovered worldwide from a broad range of biotic and abiotic substrata, such as driftwood algae, sponges, corals, sediments, etc. [4,5]. Following the definition of Pang et al [6] that considered “a marine fungus” to be any fungus retrieved repeatedly from marine environment and that reproduces in the marine environment, Jones et al. [7] listed 1680 fungal species belonging to 693 genera, 223 families, 87 orders, 21 classes and six phyla. However, considering that the total number of marine fungi has been estimated to exceed 10,000 taxa [8], fungal diversity remains largely undescribed. With more than 900 species [9], the Ascomycota are the dominant fungal phylum in the sea; the most represented lineages include the order Pleosporales (class Dothideomycetes) with 36 families, 95 genera and 194 species described to date ([www.marinefungi.org](http://www.marinefungi.org)).

In recent surveys aimed to uncover the underwater fungal diversity, 19 unidentified Roussoellaceae were isolated from several substrates, as follows: 12 from the brown alga *Padina pavonica* (L.) Thivy [10], 4 from the green alga *Flabellia petiolata* (Turra) Nizamuddin [11], 2 from the seagrass *Posidonia oceanica* (L.) Delile, and 1 from the Atlantic sponge *Dysidea fragilis* (Montagu) [13]. The Roussoellaceae is a well-resolved family in the Pleosporales [14]. Others [15] have treated the family Roussoellaceae as a synonym of Thyridariaceae, based on phylogenetic affinities. However,

following the discovery of new genera in this group, delineated by high resolution multi-locus phylogenetic analyses, the Roussoellaceae and Thyridariaceae are now recognized as two distinct but closely related families [16–20].

Many new species of Roussoellaceae and Thyridariaceae have recently been described on terrestrial plants including bamboo, palms and mangroves [14,17,20,21]. This paper provides a more precise phylogenetic placement of the 19 strains isolated from marine substrata together with morphological insights of those strains that represent new species within these two families.

## 2. Materials and Methods

### 2.1. Fungal Isolates

The fungal isolates analyzed in this paper were retrieved in the Mediterranean Sea from *P. oceanica* (2), collected in Riva Trigoso bay and Elba island, *P. pavonica* (12), and *F. petiolata* (3) from the coastal waters of Elba island [10–12]. A single isolate was previously retrieved in association with *D. fragilis* in the Atlantic Ocean [13] (Table 1).

**Table 1.** Dataset used for phylogenetic analysis. Genbank sequences including newly generated nrITS, nrLSU, nrSSU, TEF1- $\alpha$  and RPB2 amplicons relative to the novel species of Roussoellaceae and Thyridariaceae, to *Parathyridaria robiniae* MUT 2452 and MUT 4893 and to *Parathyridaria ramulicola* MUT 4397.

Species	Strain Code	Source	nrITS	nrSSU	nrLSU	TEF-1 $\alpha$	RPB2
<b>Roussoellaceae</b>							
<i>Arthopyrenia salicis</i> Massal	CBS 368.94	<i>Salix</i> bark	KF443410	AY538333	AY538339	KF443404	KF443397
<i>Neorousoella bambusae</i> Liu and Hyde	MFLUCC 11-0124	Dead branch of <i>Bambusa</i>	KJ474827	–	KJ474839	KJ474848	KJ474856
<i>N. alishanense</i> Karunarathna, Kuo, Phookamsak and Hyde	AKTW 03 FU31016	<i>Pennisetum purpureum</i>	MK503816	MK503828	MK503822	MK336181	MN037756
	AKTW 11 FU31018	<i>Pennisetum purpureum</i>	MK503818	MK503830	MK503824	MK336182	MN037757
<i>N. entadae</i> Jones and Hyde	MFLUCC 18-0243	<i>Leucaena</i> sp.	MK347786	MK347893	MK348004	MK360065	MK434866
<i>N. heveae</i> Senwanna, Phookamsak and Hyde	MFLUCC 17-1983	Twig of <i>Hevea brasiliensis</i>	MH590693	–	MH590689	–	–
<i>N. leucaenae</i> Jones and Hyde	MFLUCC 18-1544	Decaying pod of <i>Leucaena</i>	MK347767	MK347874	MK347984	MK360067	MK434876
	MFLUCC 17-0927	<i>Pterocarpus</i> sp.	MK347733	MK347841	MK347950	MK360066	MK434896
<i>Neorousoella lignicolasp. nov.</i>	MUT 4904	<i>P. pavonica</i>	KT699129	MN556307*	MN556319*	MN605894*	MN605914*
	MUT 5008	<i>P. oceanica</i> leaves	MN556317*	MN556308*	MN556320*	MN605895*	MN605915*
	MUT 5373	<i>P. pavonica</i>	KU314953	KU314954	MN556321*	MN605896*	MN605916*
<i>Pararousoella juglandicola</i> Crous and Schumacher	CBS 145037		MK442607	–	MK442543	MK442699	MK442671
<i>P. mukdahanensis</i> (Phookamsak, Dai and Hyde) Crous	MFLUCC 11-0201	Bamboo	KU940129	KU872121	KU863118	–	–
<i>P. rosarum</i> Jones and Hyde	MFLUCC 17-0796	<i>Rosa</i> sp.	MG8289391	MG829154	MG829048	MG829224	–
<i>Pseudoneoconiothyrium rosae</i> (Phukhams., Camporesi and Hyde) Phukhams., Camporesi and Hyde	MFLUCC 15-0052	Dead aerial spines of <i>Rosa canina</i>	MG828922	MG829138	MG829032	–	–

Table 1. Cont.

Species	Strain Code	Source	nrITS	nrSSU	nrLSU	TEF-1 $\alpha$	RPB2
<i>Roussoella chiangraina</i> Phookamsak, Liu and Hyde	MFLUCC 10-0556	Dead branch of bamboo	KJ474828	–	KJ474840	KJ474849	KJ474857
<i>R. doimaesalongensis</i> Thambug. and Hyde	MFLUCC 14-0584	Dead branch of bamboo	KY026584	–	KY000659	KY651249	KY678394
<i>R. elaeicola</i> Konta. and Hyde	MFLUCC 15-0276a	Dead petiole of <i>Elaeis guineensis</i>	MH742329	–	MH742326	–	–
	MFLUCC 15-0276b	Dead petiole of <i>Elaeis guineensis</i>	MH742330	–	MH742327	–	–
<i>R. euonymi</i> Crous and Akulov	CBS 143426	Fallen branches of <i>Euonymus europaeus</i>	MH107915	–	MH107961	–	MH108007
<i>R. hysteroioides</i> (Ces.) Höhn.	CBS 546.94	<i>Phyllostachys</i>	KF443405	AY642528	KF443381	KF443399	KF443392
<i>R. intermedia</i> Ju, Rogers and Huhndorf	CBS 170.96	Bamboo	KF443407	KF443390	KF443382	KF443398	KF443394
<i>R. japonensis</i> Kaz. Tanaka, Liu and Hyde	MAFF 239636	Twigs of <i>Sasa veitchii</i>	KJ474829	AB524480	AB524621	AB539114	AB539101
<i>R. kunmingensis</i> Jiang, Phookamsak and Hyde	KUMCC 18-0128	Dead bamboo	MH453491	–	MH453487	MH453480	MH453484
<i>R. mangrovei</i> Phukhams. and Hyde	MFLUCC 16-0424	Dead branches of <i>Rhizophora</i>	MH025951	–	MH023318	MH028246	MH028250
<i>Roussoella margidorensis</i> sp. nov.	MUT 5329	<i>P. pavonica</i>	KU314944	MN556309*	MN556322*	MN605897*	MN605917*
<i>Roussoella mediterranea</i> sp. nov.	MUT 5306	<i>P. pavonica</i>	KU255054	MN556310*	MN556323*	MN605898*	MN605918*
	MUT 5369	<i>P. pavonica</i>	KU314947	KU314948	MN556324*	MN605899*	MN605919*
<i>R. mexicana</i> Crous and Yáñez-Mor.	CPC 25355	Leaf spots of <i>Coffea arabica</i>	KT950848	–	KT950862	–	–
<i>R. neopustulans</i> Dai, Liu and Hyde	MFLUCC 11-0609	Bamboo	KJ474833	–	KJ474841	KJ474850	–
	MFLUCC 12-0003	Bamboo	KU940130	KU872122	KU863119	–	–
<i>R. nitidula</i> Sacc. and Paol.	MFLUCC 11-0182	Bamboo	KJ474835	–	KJ474843	KJ474852	KJ474859
	MFLUCC 11-0634	Bamboo	KJ474834	–	KJ474842	KJ474851	KJ474858
<i>Roussoella padinae</i> sp. nov.	MUT 5341	<i>P. pavonica</i>	KU158153	KU158176	MN556325*	MN605900*	MN605920*
	MUT 5365	<i>P. pavonica</i>	KU158170	KU158179	MN556326*	MN605901*	MN605921*
	MUT 5503	<i>P. pavonica</i>	KU158170	MN556312*	MN556327*	MN605902*	MN605922*
<i>R. pseudohysteroioides</i> Dai and Hyde	MFLUCC 13-0852	Bamboo	KU940131	KU872123	KU863120	KU940198	–
<i>R. pustulans</i> (Ellis and Everh.) Ju, Rogers and Huhndorf	KT 1709	Culms of <i>Sasa kurilensis</i>	KJ474830	AB524482	AB524623	AB539116	AB539103
<i>R. scabrispora</i> (Höhn.) Aptroot	MFLUCC 11-0624	Bamboo	KJ474836	–	KJ474844	KJ474853	KJ474860
	RSC	Bamboo	KX650566	–	KX650566	KX650537	–
<i>R. siamensis</i> Phookamsak, Liu and Hyde	MFLUCC 11-0149	Bamboo	KJ474837	KU872125	KJ474845	KJ474854	KJ474861
<i>R. thailandica</i> Dai, Liu and Hyde	MFLUCC 11-0621	Bamboo	KJ474838	–	KJ474846	–	–
<i>R. tuberculata</i> Dai and Hyde	MFLUCC 13-0854	Bamboo	KU940132	KU872124	KU863121	KU940199	–
<i>R. verrucispora</i> Kaz. Tanaka, Liu and Hyde	CBS 125434	<i>Sasa kurilensis</i>	KJ474832	AB52448	AB524622	AB539115	–

Table 1. Cont.

Species	Strain Code	Source	nrITS	nrSSU	nrLSU	TEF-1 $\alpha$	RPB2
<i>R. yunnanensis</i> Jiang, Phookamsak and Hyde	KUMCC 18-0115	Dead bamboo	MH453492	–	MH453488	MH453481	–
<i>Roussoellopsis macrospora</i> (Hino and Katum.) Hino and Katum	MFLUCC 12-0005	Bamboo	KJ739604	KJ739608	KJ474847	KJ474855	KJ474862
<i>Ro. tosaensis</i> (Hino and Katum.) Hino and Katum	KT 1659	Culms of bamboo	–	AB524484	AB524625	AB539117	AB539104
<b>Thyridariaceae</b>							
<i>Cycasicola goaensis</i> Jones and Hyde	MFLUCC 17-0754	<i>Cycas</i> sp.	MG828885	MG829112	MG829001	MG829198	–
<i>C. leucaenae</i> Jones and Hyde	MFLUCC 17-0914	<i>Leucaena leucocephala</i>	MK34772	MK347833	MK347942	MK360046	MK434900
<i>Liua muriformis</i> Phookamsak, Jiang and Hyde	KUMCC 18-0177	Dead hanging branches of <i>Lonicera maackii</i>	MK433599	MK433595	MK433598	MK426798	MK426799
<i>Parathyridaria percutanea</i> (Ahmed, Stevens, van de Sande and de Hoog) Jaklitsch and Voglmayr	CBS 868.95	Human	KF322118	KF366451	KF366449	KF407987	KF366452
	CBS 128203	Human	KF322117	KF366450	KF366448	KF407988	KF366453
<i>P. ramulicola</i> Jaklitsch, Fourn and Voglmayr	CBS 141479	Twigs of <i>Ribes rubrum</i>	NR_147657	KX650514	KX650565	KX650536	KX650584
	MUT 4397	<i>P. oceanica</i>	KC339235	MN556311*	KF636775	MN605913*	MN605933*
<i>P. robiniae</i> Mapook, Camporesi and Hyde	MFLUCC 14-1119	Dead branch of <i>Robinia pseudoacacia</i>	KY511142	–	KY511141	KY549682	–
	MUT 2452	<i>Dysidea fragilis</i>	MG813183	MN556312*	MG816491	MN605903*	MN605923*
	MUT 4893	<i>P. pavonica</i>	KM355998	KM355993	MN556328*	MN605904*	MN605924*
<i>Parathyridaria flabelliae</i> sp. nov.	MUT 4859	<i>F. petiolata</i>	KR014355	KT587315	KP671716	MN605909*	MN605929*
	MUT 4886	<i>F. petiolata</i>	KR014358	KT587317	KP671720	MN605910*	MN605930*
<i>Parathyridaria tyrrhenica</i> sp. nov.	MUT 4966	<i>F. petiolata</i> ,	KR014366	KT587309	KP671740	MN605911*	MN605931*
	MUT 5371	<i>P. pavonica</i>	KU314951	KU314952	MN556329*	MN605912*	MN605932*
<i>Parathyridariella dematiacea</i> sp. nov.	MUT 4419	<i>P. oceanica</i> rhizomes	KC339245	MN556313*	KF636786	MN605905*	MN605925*
	MUT 4884	<i>F. petiolata</i>	MN556317*	KT587329	KP671726	MN605906*	MN605926*
	MUT 5310	<i>P. pavonica</i>	KU255057	MN556314*	MN556330*	MN605907*	MN605927*
	MUT 5381	<i>P. pavonica</i>	KU314959	KU314960	MN556331*	MN605908*	MN605928*
<i>Thyridaria acaciae</i> (Crous and Wingf.) Jaklitsch and Voglmayr	CBS 138873	Leaves of <i>Acacia tortilis</i>	KP004469	–	KP004497	–	–
<i>T. broussonetiae</i> (Sacc.) Traverso	TB	<i>Hippocrepis emerus</i>	KX650567	–	KX650567	KX650538	KX650585
	TB1	<i>Amorpha fruticosa</i>	KX650568	KX650515	KX650568	KX650539	KX650586
<i>Thyridariella mahakoshae</i> Devadatha, Sarma, Wanas., Hyde and Jones	NFCCI 4215	Decaying wood <i>Avicennia marina</i>	MG020435	MG020441	MG020438	MG023140	MG020446
<i>Th. mangrovei</i> Devadatha, Sarma, Hyde, Wanas. and Jones	NFCCI 4213	Decaying wood <i>Avicennia marina</i>	MG020434	MG020440	MG020437	MG020443	MG020445
	NFCCI 4214	Decaying wood <i>Avicennia marina</i>	MG020436	MG020442	MG020439	MG020444	MG020447

Table 1. Cont.

Species	Strain Code	Source	nrITS	nrSSU	nrLSU	TEF-1 $\alpha$	RPB2
<b>Occultibambusaceae</b>							
<i>Occultibambusa bambusae</i> Dai and Hyde	MFLUCC 11-0394	Bamboo	KU940124	–	KU863113	KU940194	KU940171
	MFLUCC 13-0855	Bamboo	KU940123	KU872116	KU863112	KU940193	KU940170
<b>Ohleriaceae</b>							
<i>Ohleria modesta</i> Fuckel	MGC		KX650562	–	KX650562	KX650533	KX650582
	OM	Branches of <i>Chamaecytisus proliferus</i>	KX650563	KX650513	KX650563	KX650534	KX650583
<b>Torulaceae</b>							
<i>Dendryphion europaeum</i> Crous and Schumacher	CPC 22943	<i>Heracleum sphondylium</i>	KJ869146	–	KJ869203	–	–
<i>Torula herbarum</i> (Pers.) Link	CBS 111855	n.a.	KF443409	KF443391	KF443386	KF443403	KF443396
	CBS 595.96		KF443408	KF443387	KF443385	KF443402	KF443395
<i>Torula hollandica</i> Crous	CBS 220.69	Delphinium dead stem	KF443406	–	MH877717	–	–

\* = newly generated sequences; n.a. = not available.

The strains investigated were originally isolated on Corn Meal Agar medium supplemented with sea salts (CMAS; 3.5% w/v sea salt mix, Sigma-Aldrich, Saint Louis, USA, in ddH<sub>2</sub>O) and are preserved at the *Mycotheca Universitatis Taurinensis* (MUT), Italy.

## 2.2. Morphological Analysis

All isolates were pre-grown on Malt Extract Agar-sea water (MEASW; 20 g malt extract, 20 g glucose, 2 g peptone, 20 g agar in 1 L of sea water) for one month at 24 °C prior to inoculation in triplicate onto new Petri dishes (9 cm Ø) containing (i) MEASW, (ii) Oatmeal Agar-sea water (OASW; 30 g oatmeal, 20 g agar in 1 L of sea water), or (iii) Potato Dextrose Agar-sea water (PDASW; 4 g potato extract, 20 g dextrose, 20 g agar in 1 L of sea water). Petri dishes were incubated at 15 and/or 24 °C. The colony growth was monitored periodically for 28 days. Macroscopic and microscopic traits, were assessed for strains grown on MEASW at the end of the incubation period.

In an attempt to induce sporulation, sterile pieces of *Quercus ruber* cork and *Pinus pinaster* wood (species autochthonous to the Mediterranean area) were placed on 3 week old fungal colonies grown on MEASW ([22], modified). Petri dishes were further incubated for 4 weeks at 24 °C. Subsequently, cork and wood pieces were transferred to 50 mL tubes containing 20 mL of sterile sea water. Samples were incubated at 24 °C for one month. In parallel, the strains were also plated on Synthetic Nutrient Agar-sea water (SNASW; 1 g KH<sub>2</sub>PO<sub>4</sub>, 1 g KNO<sub>3</sub>, 0.5 g MgSO<sub>4</sub> • 7H<sub>2</sub>O, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 20 g agar in 1 L of sea water) supplemented with sterile pine needles. Petri dishes were incubated at 24 °C for one month.

Morphological structures were observed, and images captured using an optical microscope (Leica DM4500B, Leica microsystems GmbH, Germany) equipped with a camera (Leica DFC320, Leica microsystems GmbH, Germany). Macro- and microscopic features were compared with the available description of Roussoellaceae and Thyridariaceae [14,15,17,18,20].

## 2.3. DNA Extraction, PCR Amplification, and Data Assembling

Genomic DNA was extracted from about 100 mg of fresh mycelium grown on MEASW plates. Mycelium was disrupted by the mean of a MM400 tissue lyzer (Retsch GmbH, Haan, Germany) and DNA extracted using a NucleoSpin kit (Macherey Nagel GmbH, Duren, DE, USA) following the manufacturer's instructions. The quality and quantity of DNA were measured spectrophotometrically (Infinite 200 PRO NanoQuant; TECAN, Switzerland); DNA was stored at –20 °C.

The partial sequences of five genetic markers were amplified by PCR. Primer pairs ITS1/ITS4 [23], LR0R/LR7 [24], NS1/NS4 [23] were used to amplify the internal transcribed spacers, including the 5.8S rDNA gene (nrITS), 28S large ribosomal subunit (nrLSU) and 18S small ribosomal subunit (nrSSU). The translation elongation factor (TEF1 $\alpha$ ) and RNA polymerase II subunit (RPB2) were amplified by using primer pairs EF1-1018F/EF1-1620R [25] and fRPB2-5F/fPB2-7R [26].

Amplifications were run in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) programmed as described in Table 2. Reaction mixtures consisted of 20–40 ng DNA template, 10 $\times$  PCR Buffer (15 mM MgCl<sub>2</sub>, 500 mM KCl, 100 mM Tris-HCl, pH 8.3), 200  $\mu$ M each dNTP, 1  $\mu$ M each primer, 2.5 U Taq DNA Polymerase (Qiagen, Chatsworth, CA, USA), in 50  $\mu$ L final volume. For problematic cases, additional MgCl<sub>2</sub> and/or 2.5% DMSO facilitated the reaction.

**Table 2.** Primers and PCR conditions used to amplify specific gene marker.

	Forward and Reverse Primers	Thermocycler Conditions	References
ITS	ITS1- ITS4	95 °C: 5 min, (95 °C: 40 s, 55 °C: 50 s, 72 °C: 50 sec) $\times$ 35 cycles; 72 °C: 8 min; 4 °C: $\infty$	[23]
LSU	LR0R-LR7	95 °C: 5 min, (95 °C: 1 min, 50 °C: 1 min, 72 °C: 2 min) $\times$ 35 cycles; 72 °C: 10 min; 4 °C: $\infty$	[24]
SSU	NS1-NS4	95 °C: 5 min, (95 °C: 1 min, 50 °C: 1 min, 72 °C: 2 min) $\times$ 35 cycles; 72 °C: 10 min; 4 °C: $\infty$	[23]
TEF-1 $\alpha$	1018F/1620R	95 °C: 5 min, (95 °C: 1 min, 50 °C: 1 min; 72 °C: 2 min) $\times$ 40 cycles, 72 °C: 10 min; 4 °C: $\infty$	[25]
RPB2	fRPB2-5F/fPB2-7cR	94 °C: 3 min, (94 °C: 30 s; 55 °C: 30 s; 72 °C: 1 min) $\times$ 40 cycles, 72 °C: 10 min; 4 °C: $\infty$	[26]

Amplicons, together with a GelPilot 1 kb plus DNA Ladder, were visualized on a 1.5% agarose gel stained with 5 mL 100 mL<sup>-1</sup> ethidium bromide; PCR products were purified and sequenced at the Macrogen Europe Laboratory (Madrid, Spain). The resulting Applied Biosystem (ABI) chromatograms were inspected, trimmed and assembled to obtain consensus sequences using Sequencer 5.0 (GeneCodes Corporation, Ann Arbor, Michigan, USA <http://www.genecodes.com>). Newly generated sequences were deposited in GenBank (Table 1).

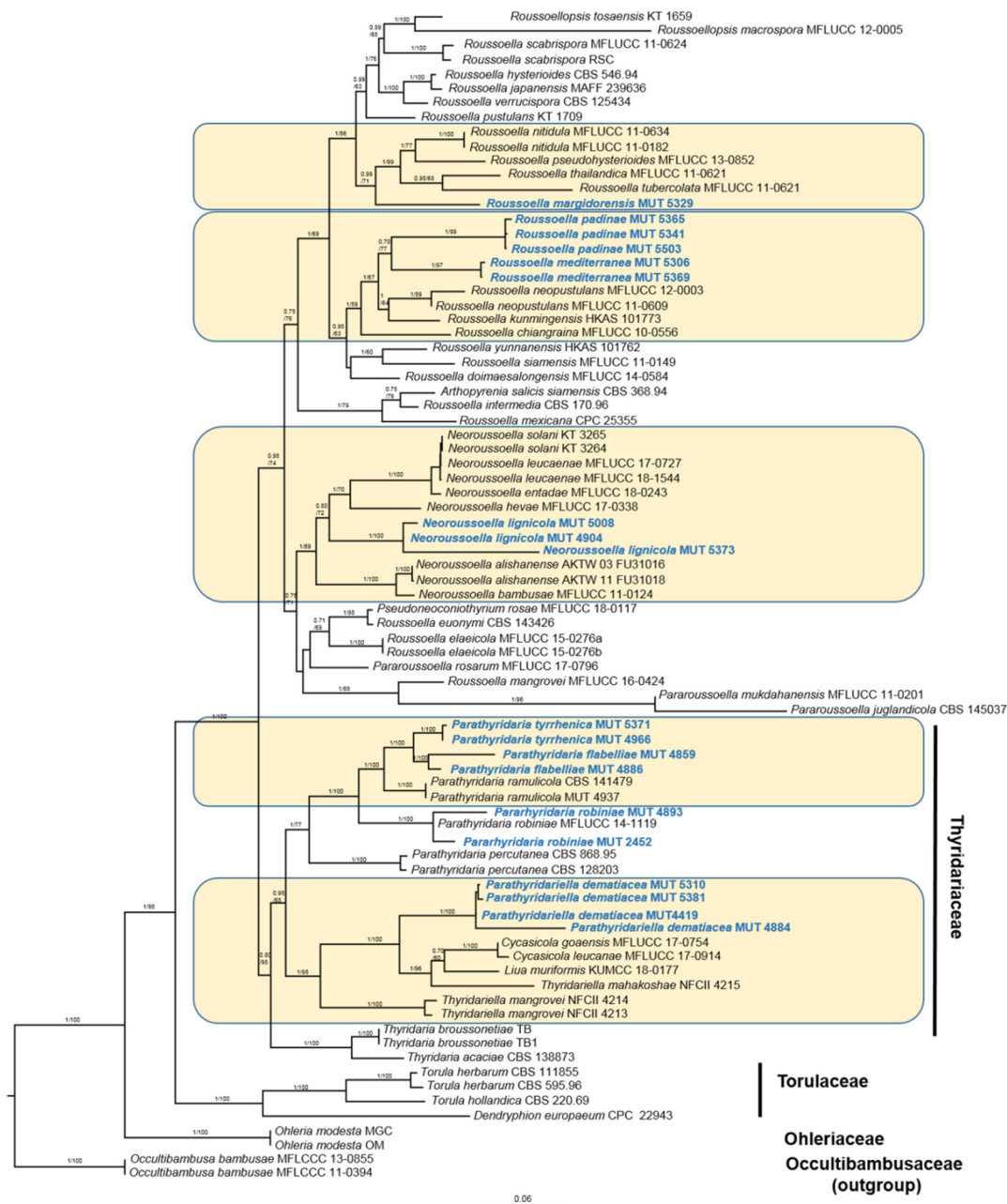
#### 2.4. Sequence Alignment and Phylogenetic Analysis

A dataset consisting of nrSSU, nrITS, nrLSU, TEF1 $\alpha$  and RPB2 was assembled on the basis of BLASTn results and of recent phylogenetic studies focused on Roussoellaceae and Thyridariaceae [18,20]. Reference sequences were retrieved from GenBank (Table 1).

Sequences were aligned 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. 1.2 (<http://trimal.cgenomics.org>) to delimit and discard ambiguously aligned regions. Since no incongruence was observed among single-loci phylogenetic trees, alignments were concatenated into a single data matrix with SequenceMatrix [27]. The best evolutionary model under the Akaike Information Criterion (AIC) was determined with jModelTest 2 [28].

Phylogenetic inference was estimated using Maximum Likelihood (ML) and Bayesian Inference (BI) criteria. The ML analysis was generated using RAxML v. 8.1.2 [29] under GTR + I + G evolutionary model and 1000 bootstrap replicates. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. BI was performed with MrBayes 3.2.2 [30] with the same substitution model (GTR + I + G). The alignment was run for 10 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 (BPP) were calculated. Consensus trees were visualized in FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>).

Two strains of *Occultibambusa bambusae* (Occultibambusaceae) were used to root the tree. Due to topological similarity of the two resulting trees, only ML analysis with MLB and BPP values was reported (Figure 1).



**Figure 1.** Phylogram generated from RAxML analysis based on a combined dataset of nrITS, nrSSU, nrLSU, TEF1 $\alpha$  and RPB2 partial sequences. The tree is rooted to *Occultibambusa bambusae*. Branch numbers indicate BYPP/MLB values; Bar = expected changes per site (0.06).

DNA diagnostic characters were visually identified by the presence of heterozygous bases. For each locus, aligned sequences of the individual clusters containing new species, were inspected. Nucleotide diversities of the novel species were annotated when occurred (Tables S1–S18).

Sequence alignments and phylogenetic trees were deposited in TreeBASE (<http://www.treebase.org>, submission number S24773).

Following phylogenetic tree inspection, isolates that clustered in the same group and that derived from the same substrate were subjected to PCR-fingerprinting by using the micro- and mini-satellite primers (GTG)<sub>5</sub> and M13 [31,32] to exclude duplicates from further analysis. DNA fingerprints were visualized with 1.5% agarose gel stained with 5 mL 100 mL<sup>-1</sup> ethidium bromide while a GelPilot 1 kb plusDNA Ladder was used as a reference. Images were acquired with a Gel Doc1000 System (Bio-Rad, Hercules, CA, USA) and fingerprints analyzed using Bionumerics v 7.6 (<http://www.applied-maths.com>).

### 3. Results

#### 3.1. Phylogenetic Inference

Preliminary analyses carried out individually with nrITS, nrSSU, nrLSU, TEF1 $\alpha$  and RPB2 denoted no incongruence in the topology of the single-locus trees. The combined five-markers dataset—built on the basis of BLASTn results and of recent phylogenetic studies [18,20]—consisted of 81 taxa (including MUT isolates) that represented 16 genera and 56 species (Table 1). A total of 63 sequences (2 nrITS, 8 nrSSU, 13 nrLSU, 20 TEF1 $\alpha$  and 20 RPB2) were newly generated while 261 were retrieved from GenBank.

The combined dataset had an aligned length of 3390 characters, of which 1683 were constant, 657 were parsimony-uninformative and 1050 parsimony informative (TL = 218, CI = 0.422018, RI = 0.825243, RC = 0.348267, HI = 0.877952).

Strains MUT 4893 and MUT 2452 were identified as *Parathyridaria robiniae*, the rest of the strains represented seven new species and one new genus (Figure 1). *Parathyridaria tyrrhenica* sp. nov. (MUT 5371 and MUT 4966) formed a sister clade to *Parathyridaria flabelliae* sp. nov. (MUT 4859 and MUT 4886) with high statistical support (BYPP = 1.00; MLB = 100%); these two novel species are closely related to *P. ramulicola* (BYPP = 1.00; MLB = 100%) and clustered with other *Parathyridaria* species in the Thyridariaeae family. Within this family, four isolates (MUT 5310, MUT 5381, MUT 4419 and MUT 4884) clustered together with the genera *Thyridariella*, *Liua* and *Cycasicola*, and formed a strongly supported monophyletic lineage (BYPP = 1.00; MLB = 100%). Therefore, we have introduced the novel genus *Parathyridariella*, typified by the new species *Parathyridariella dematiacea* sp. nov.

The three strains, MUT 4904, MUT 5373 and MUT 5008, represented a novel species *Neorousoella lignicola* sp. nov. and formed an independent and robust clade (BYPP = 1.00; MLB = 100%), within the *Neorousoella* group in the Rousoellaceae.

Two sister clades within the *Rousoella* group were represented by the new species *Rousoella padinae* sp. nov. (MUT 5503, MUT 5341 and MUT 5365) and *Rousoella mediterranea* sp. nov. (MUT 5306 and MUT 5369). Finally, MUT 5329 *Rousoella margidorensis* sp. nov. clustered together with *R. nitidula*, *R. pseudohysterioides*, *R. thailandica* and *R. tuberculata* (BYPP = 0.99; MLB = 71%) but was phylogenetically distant from these species.

Nucleotide divergence between each novel species and members of the same clusters were annotated for each locus, when occurred (Tables S1–S18).

#### 3.2. Taxonomy

*Parathyridariella* gen. nov. V. Prigione, A. Poli, E. Bovio and G.C. Varese

MYCOBANK: MB 832836

**Type species.** *Parathyridariella dematiacea* sp. nov.

**Etymology.** In reference to the phylogenetic proximity to the genus *Thyridariella*.

**Phylogenetic placement.** Thyridariaceae, Sordariomycetes, Ascomycota. The genus *Parathyridariella* gen. nov. clusters together with genera *Cycasicola*, *Liua* and *Thyridariella* (Figure 1).

*Parathyridariella dematiacea* sp. nov. V. Prigione, A. Poli, E. Bovio and G.C. Varese

MYCOBANK: MB 832837

Figure 2



**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghiaie ISL, 14–15 m depth, 42°49′04″N, 10°19′20″E, from the green alga *Flabellia petiolata*, 20 March 2010, R. Mussat-Sartor and N. Nurra, MUT 4884 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at Mycotheca Universitatis Taurinensis (MUT).

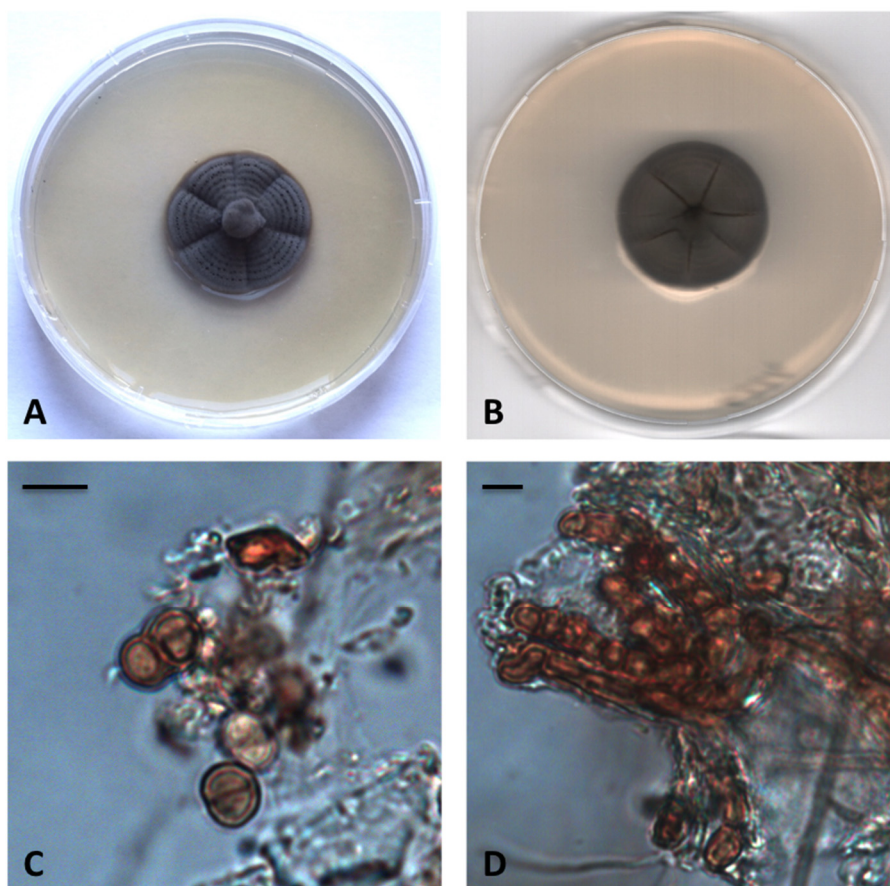
**Additional material examined.** Italy, Liguria, Mediterranean Sea, Riva Trigoso, Punta Manara (GE), 5–21 m depth, 44°15′08.62″N 9°24′17.64″E, from the seagrass *Posidonia oceanica*, March 2008, MUT 4419.

**Etymology.** In reference to the color of the colony on culture media.

**Description.** Growing actively on *Pinus pinaster* and *Quercus ruber* cork. Showing a floccose growth mainly on *Pinus pinaster*. Hyphae 2.8–4.8 µm wide, septate, hyaline to lightly pigmented. Chlamydospores numerous, mostly in chain, intercalary or solitary, globose to subglobose, from brownish to dark brown, 7–10 × 6–8 µm diameter.

Sexual morph not observed. Asexual morph with differentiated conidiogenesis not observed.

**Colony description.** Colonies on MEASW attaining 28–34 mm diam after 28 days at 24 °C, mycelium from dark grey/black to dark green, dense with radial grooves and concentric rings, submerged edges; reverse dark green. Brown exudate present above the concentric rings. Growth on OASW reaching 40–54 mm diam at 24 °C and 21–29 mm diam at 15 °C; colonies on PDA attaining 36–49 mm diam and 15.5–22.5 mm diam at 24 °C and 15 °C, respectively.



**Figure 2.** *Parathyridariella dematiacea* sp. nov. 28-days-old colony at 21 °C on MEASW (A) and reverse (B); solitary (C) and in chain (D) chlamydospores. Scale bars: 10 µm (C, D).

*Parathyridaria tyrrhenica* sp. nov. A. Poli, V. Prigione, E. Bovio and G.C. Varese  
MYCOBANK: MB 832838

Figure 3

**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghaie ISL, 14–15 m depth, 42°49′04″N, 10°19′20″E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5371 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at MUT.

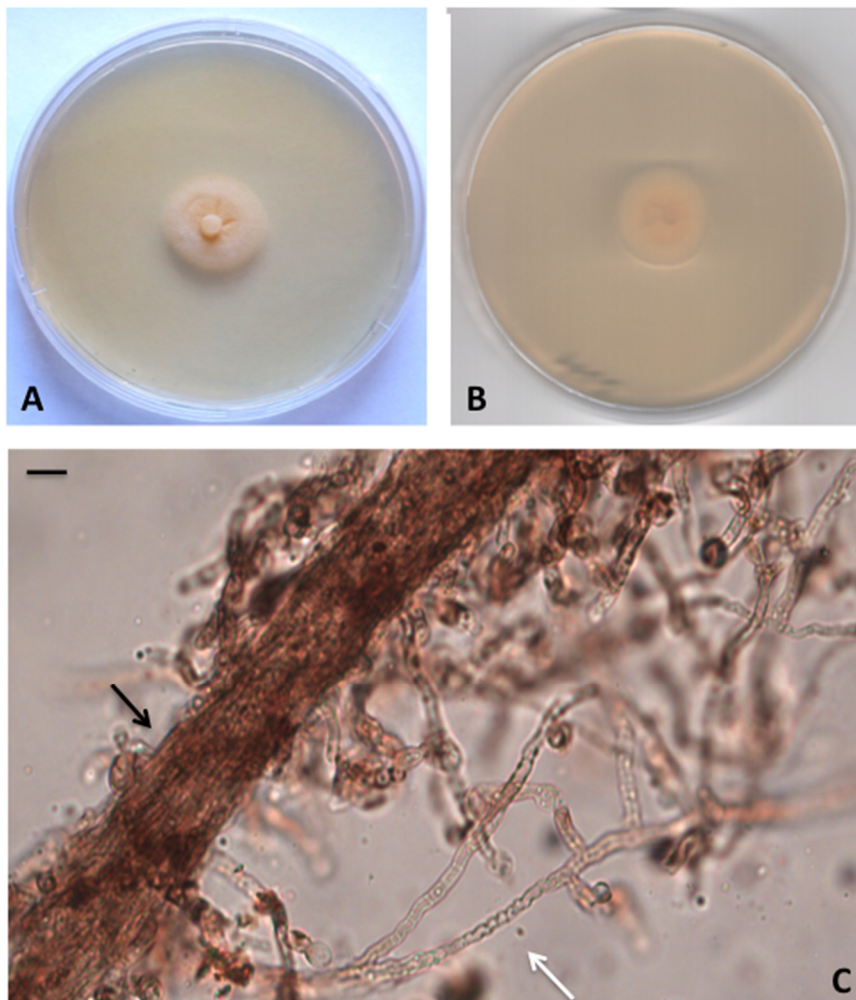
**Additional material examined.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghaie ISL, 14–15 m depth, 42°49′04″N, 10°19′20″E, from the green alga *Flabellia petiolata*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 4966.

**Etymology.** In reference to Tyrrhenian Sea.

**Description.** Growing actively on *Pinus pinaster* wood and *Quercus ruber* cork. Hyphae 5 µm diameter, septate, hyaline to brownish, sometimes wavy or swollen, forming hyphal strands.

Sexual morph not observed. Asexual morph with differentiated conidiogenesis: not observed.

**Colony description.** Colonies growing on MEASW, reaching 10 mm diam after 28 days, at 21 °C, mycelium funiculose, yellowish, lightly ochre at the edges; reverse light yellow, lighter at the edges. Growth on OASW reaching 48–50 mm diam at 24 °C and 26–29 mm diam at 15 °C; colonies on PDA attaining 31–46 mm diam and 16–19 mm diam at 24 °C and 15 °C, respectively.



**Figure 3.** *Parathyridaria tyrrhenica* sp. nov. 28-days-old colony at 21 °C on MEASW (A) and reverse (B); mycelium (C), black and white arrows indicate hyphal strands and wavy hyphae, respectively. Scale bar: 10 µm.

*Parathyridaria flabelliae* sp. nov. E. Bovio, A. Poli, V. Prigione and G.C. Varese  
MYCOBANK: MB 832839

Figure 4

**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghiaie ISL, 14–15 m depth, 42°49′04″N, 10°19′20″E, from the green alga *Flabellia petiolata*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 4859 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at MUT.

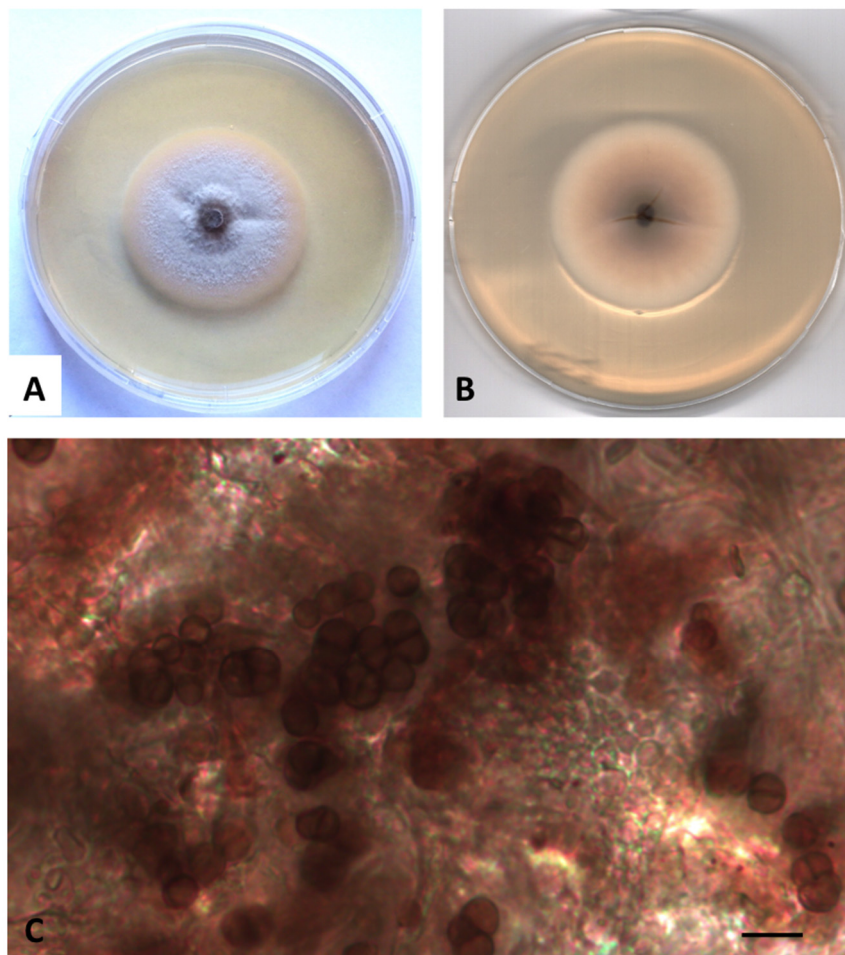
**Additional material examined.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Ghiaie ISL, 14–15 m depth, 42°49′04″N, 10°19′20″E, from the green alga *Flabellia petiolata*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 4886.

**Etymology.** In reference to the original substratum, the green alga *Flabellia petiolata*.

**Description.** Growing actively on *Pinus pinaster* and on *Quercus ruber* cork. *Hyphae* 2.6–5 µm wide, septate and hyaline. *Chlamydospores* numerous, globose or subglobose, from light to dark brown, unicellular (4 × 5 µm diameter) and multicellular (up to four-celled; 8 × 12 µm diameter).

Sexual morph not observed. Asexual morph with differentiated conidiogenesis not observed.

**Colony description.** Colonies growing on MEASW, reaching 37–44 mm diam after 28 days at 21 °C, funiculose, whitish with submerged edges; reverse brown in the middle, lighter at edges. Growth on OASW reaching 60 mm diam at 24 °C and 33–35 mm diam at 15 °C; colonies on PDA attaining 53–64 mm diam and 23–24 mm diam at 24 °C and 15 °C, respectively.



**Figure 4.** *Parathyridaria flabelliae* sp. nov. 28-days-old colony at 21 °C on MEASW (A) and reverse (B); unicellular and multicellular chlamydospores (C). Scale bar: 10 µm.

*Neorousoella lignicola* sp. nov. A. Poli, E. Bovio, V. Prigione and G.C. Varese  
MYCOBANK: MB 832840

Figure 5

**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45'29"N, 10°18'24"E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5373 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at MUT.

**Additional material examined.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45'29"N, 10°18'24"E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 4904.

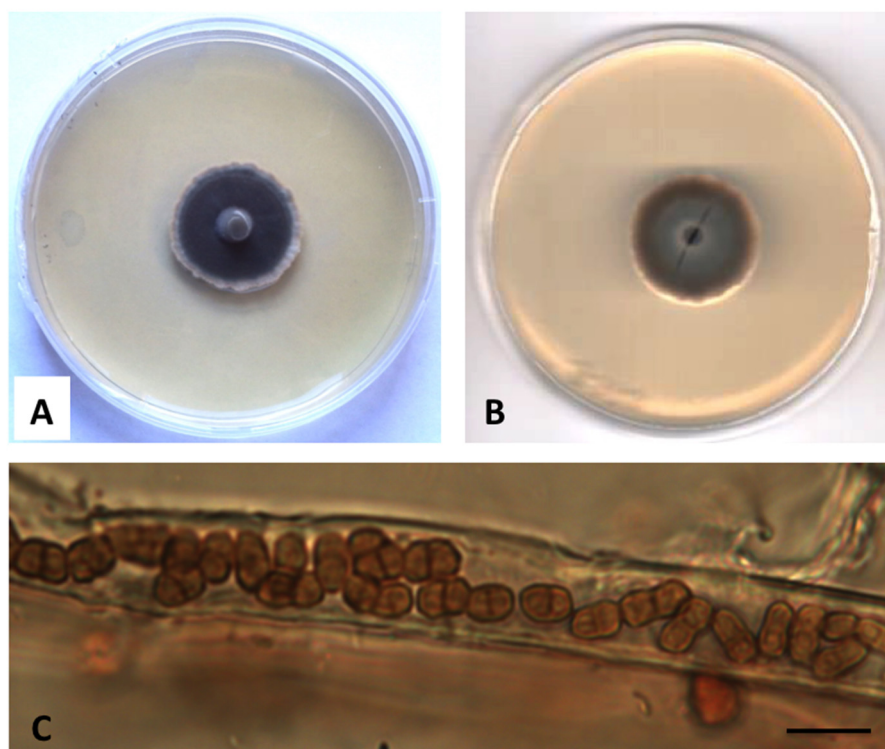
Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45'29"N, 10°18'24"E, from the seagrass *Posidonia oceanica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5008.

**Etymology.** In reference to the lignicolous behavior.

**Description.** Growing efficiently on *Pinus pinaster* wood. *Hyphae* 2–4.4  $\mu\text{m}$  wide, septate, hyaline, assuming toruloid aspect when growing into wood vessels and forming chains of two-celled chlamydo spores which, at maturity, protrude from the vessels. *Chlamydo spores* 7.4  $\times$  5.2  $\mu\text{m}$ , from light to dark brown, globose or subglobose.

Sexual morph not observed. Asexual morph with differentiated conidiogenesis not observed.

**Colony description.** Colonies growing on MEASW, reaching 28–29 mm diam after 28 days at 21 °C, from grey to dark green, floccose with irregular edges, reverse dark grey. Clear exudate often present. Growth on OASW reaching 27–40 mm diam at 24 °C and 14.5–26 mm diam at 15 °C; colonies on PDA attaining 38–45 mm diam and 19–29 mm diam at 24 °C and 15 °C, respectively.



**Figure 5.** *Neorousoella lignicola* sp. nov. 28-days-old colony at 21 °C on MEASW (A) and reverse (B); two-celled chlamydo spores inside wood vessels (C). Scale bar: 10  $\mu\text{m}$ .

*Rousoella margidorensis* sp. nov. E. Bovio, V. Prigione, A. Poli and G.C. Varese  
MYCOBANK: MB 832841

Figure 6

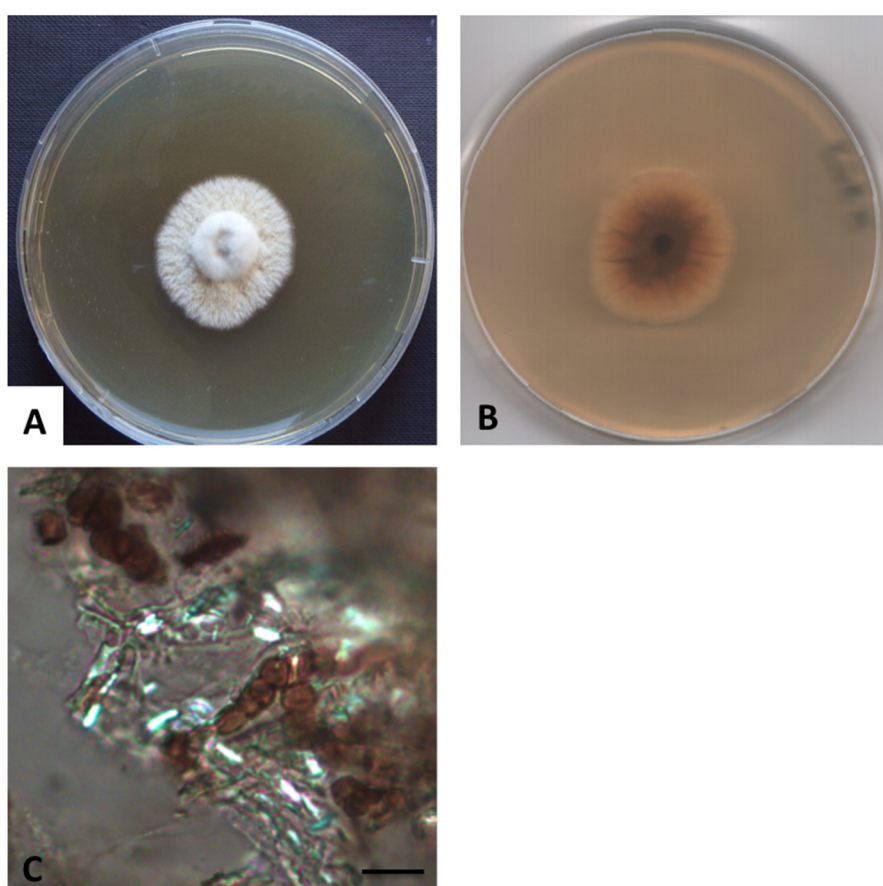
**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45'29"N, 10°18'24"E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5329 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at MUT.

**Etymology.** In reference to the area of origin, Margidore.

**Description.** Growing actively on *Pinus pinaster* wood. *Hyphae* approx. 2 µm wide, septate, brownish.

Sexual morph not observed. Asexual morph and differentiated conidiogenesis not observed.

**Colony description.** Colonies growing on MEASW, attaining 33–34 mm diam after 28 days at 21 °C; whitish, lighter to the edge, umbonate in the middle, reverse ochre. Caramel diffusible pigment produced. Growth on OASW reaching 45 mm diam at 24 °C and 27 mm diam at 15 °C; colonies on PDA attaining 45 mm diam and 23 mm diam at 24 °C and 15 °C, respectively.



**Figure 6.** *Roussoella margidorensis* sp. nov. 28-days-old colony at 21 °C on MEASW (A) and reverse (B); chlamydo spores (C). Scale bar: 10 µm.

*Roussoella mediterranea* sp. nov. A. Poli, E. Bovio, V. Prigione, and G.C. Varese

MYCOBANK: MB 832842

Figure 7

**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45'29"N, 10°18'24"E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5369 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at MUT.

**Additional material examined.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45'29"N, 10°18'24"E, from the brown alga *Padina pavonica*, March

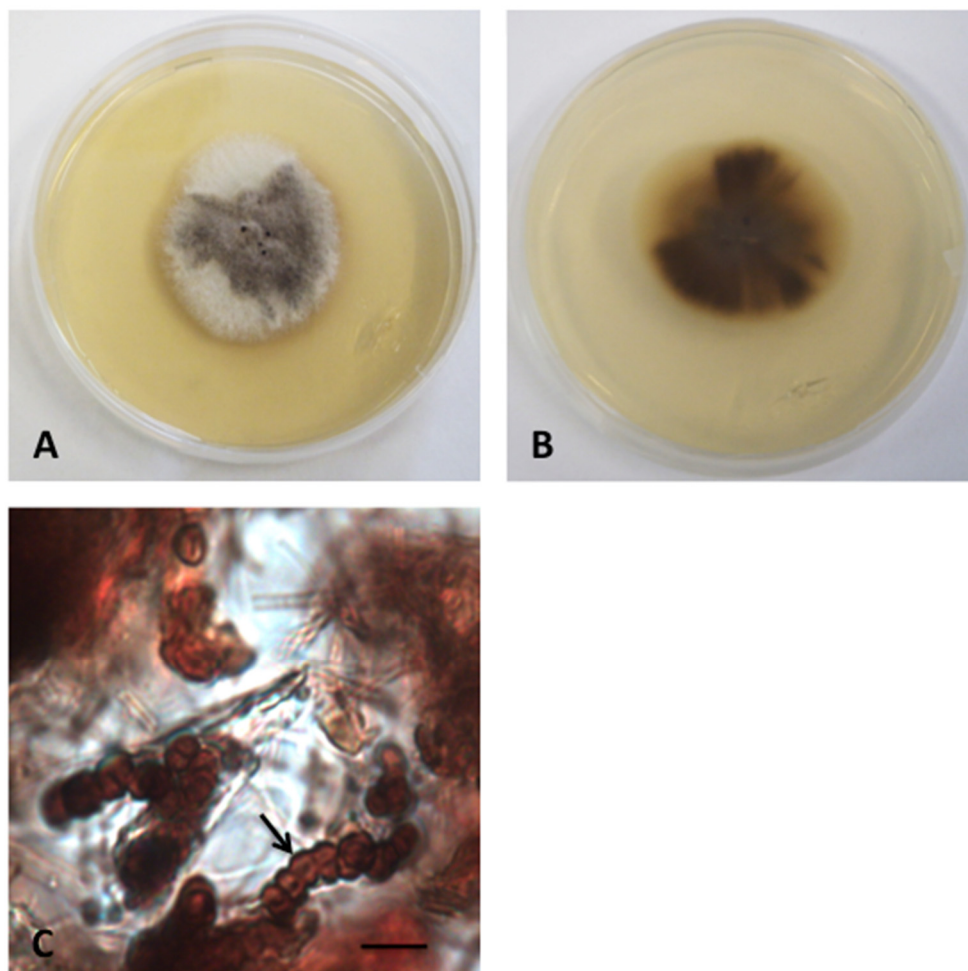
2010, R. Mussat-Sartor and N. Nurra, MUT 5306 (identical to MUT 5306 on the basis of micro- and minisatellite analyses)

**Etymology.** In reference to the geographical origin, Mediterranean Sea.

**Description in culture.** Growing actively on *Pinus pinaster* wood and poorly colonizing *Quercus ruber* cork. *Hyphae* 2.4  $\mu\text{m}$  wide, septate, dematiaceous. *Chlamydospores* 4.5  $\times$  5.7  $\mu\text{m}$ , from unicellular to 4-celled; branched chains of light to dark brown chlamydospores often present.

Sexual morph not observed. Asexual morph with differentiated conidiogenesis not observed.

**Colony description.** Colonies growing on MEASW, reaching 55 mm diam after 28 days at 21  $^{\circ}\text{C}$ , light grey, floccose, with umbonate area in the middle, reverse brown with lighter edges. Dark exudate present. Growth on OASW reaching 67–72 mm diam at 24  $^{\circ}\text{C}$  and 33–38 mm diam at 15  $^{\circ}\text{C}$ ; colonies on PDA attaining 69–76 mm diam and 32.5–39 mm diam at 24  $^{\circ}\text{C}$  and 15  $^{\circ}\text{C}$ , respectively.



**Figure 7.** *Roussoella mediterranea* sp. nov. 28-days-old colony at 21  $^{\circ}\text{C}$  on MEASW (A) and reverse (B); unicellular and multicellular chlamydospores indicated by a black arrow (C). Scale bar: 10  $\mu\text{m}$ .

*Roussoella padinae* sp. nov. V. Prigione, E. Bovio, A. Poli and G.C. Varese

MYCOBANK: MB 832843

Figure 8

**Type.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42 $^{\circ}$ 45'29"N, 10 $^{\circ}$ 18'24"E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5503 holotype, living culture permanently preserved in metabolically inactive state by deep-freezing at MUT.

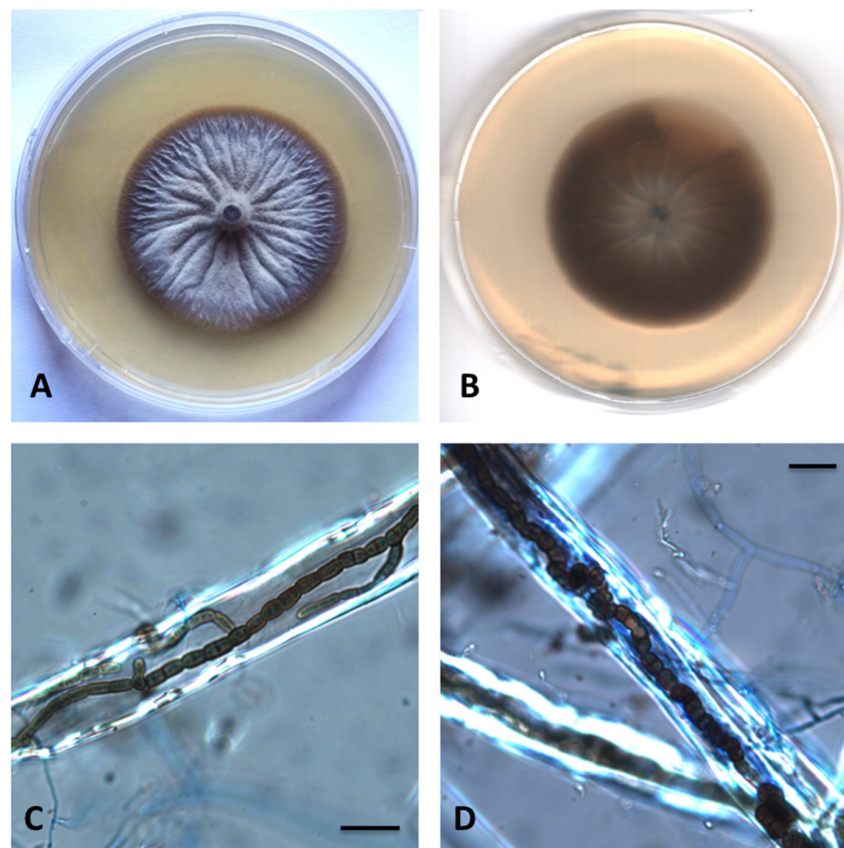
**Additional material examined.** Italy, Tuscany, Mediterranean Sea, Elba Island (LI), Margidore ISL, 14–15 m depth, UTM WGS84 42°45′29″N, 10°18′24″E, from the brown alga *Padina pavonica*, March 2010, R. Mussat-Sartor and N. Nurra, MUT 5341 and MUT 5345 (identical to MUT 5503 on the basis of micro- and minisatellite analyses)

**Etymology.** In reference to the original substratum, *Padina pavonica*.

**Description in culture.** Growing efficiently on *Quercus ruber* cork and poorly colonizing *Pinus pinaster* wood. *Hyphae* 3  $\mu\text{m}$  wide, septate, brownish, assuming toruloid aspect when growing into wood vessels and forming chains of two-celled chlamydospores which, at maturity, protrude from the vessels. *Chlamydospores* 5–7  $\times$  4  $\mu\text{m}$ , from light to dark brown, subglobose, ellipsoidal or cylindrical.

Sexual morph not observed. Asexual morph n with differentiated conidiogenesis not observed.

**Colony description.** Colonies growing on MEASW, reaching 53 mm diam after 28 days at 21 °C, from grey to dark green, floccose in the middle, with radial grooves, fimbriate edges, reverse brown. Growth on OASW reaching 57.5–65 mm diam at 24 °C and 30–35 mm diam at 15 °C; colonies on PDA attaining 60–69 mm diam and 30–34 mm diam at 24 °C and 15 °C, respectively.



**Figure 8.** *Roussoella padinae* sp. nov. 28-days-old colony at 21 °C on MEASW (A) and reverse (B); toruloid hyphae (C) and two-celled chlamydospores (D) inside wood vessels. Scale bars: 10  $\mu\text{m}$ .

#### 4. Discussion

The description of these new taxa was particularly challenging because neither asexual nor sexual reproductive structures developed in axenic conditions. Therefore, we were unable to describe the range of anatomical variations and diagnostic features among these newly recognized phylogenetic lineages. Indeed, strictly vegetative growth without sporulation is a common feature of many marine fungal strains [10,11,33]. Possibly, these organisms rely on hyphal fragmentation for their dispersal, or alternatively, the differentiation of reproductive structures may be obligatorily dependent on the peculiar environmental conditions under which they live (e.g., wet-dry cycles, high salinity,

low temperature, high pressure, etc.). During the study of these fungi, we tried to mimic the saline environment by using different culture media supplemented with natural sea water or sea salts. Although these culture methods were applied to induce sporulation, we observed that only media supplemented with sea water supported a measurable growth of vegetative mycelium (data not shown). The method introduced by Panebianco et al. [22] to induce sporulation by placing wood and cork specimens on the colony surface with their subsequent transfer into sea water, was only partially successful: out of seven species, three (*P. dematiacea*, *P. flabelliae*, *R. mediterranea*) developed chlamydospores in the mycelium above the wood surface, two (*N. lignicola*, *R. padinae*) gave rise to resting spores inside wood vessels. Most of the strains preferred to colonize *P. pinaster* wood rather than *Q. ruber* cork. These structures were interpreted as “chlamydospores” instead of “conidia” for the following reasons: (i) They were characterized by a very thick cell wall, a typical feature of resting spores; (ii) conidiogenous cells were never observed. Additional efforts to force the development of reproductive structures by using SNASW and pine needles, were also unsuccessful.

Both *R. padinae* and *N. lignicola* displayed a similar lignicolous behavior, growing and producing chlamydospores inside wooden vessels, although of different size and shape. The ability to form hyphae and to grow inside the wood vessels has been reported for a number of dark septate endophyte fungi in terrestrial environment [34] and, recently, for *Posidoniomyces atricolor* Vohník and Réblová, a marine endophyte that lives in association with the roots of *P. oceanica* [35]. By definition, endophytes live inside living plant tissues. To induce sporulation, sterilized specimens of dead wood were employed, therefore *R. padinae* and *N. lignicola* were inferred to be “lignicolous fungi” rather than “endophytes”. The observation of this growth characteristic in two different genera, may find its reason in an evolutionary adaptation to marine life in association with lignocellulosic matrices. Therefore, we can hypothesize their ecological role as saprobes involved in degrading organic matter.

Notwithstanding the lack of exhaustive descriptions of morphological features, the strongly supported phylogenetic and molecular analysis, conducted with five different genetic markers (nrSSU, nrITS, nrLSU, TEF1 $\alpha$  and RPB2) undoubtedly pointed out the differences among these species and their belonging to new taxa. This is also supported by the DNA diagnostic characters identified in the individual loci (Tables S1–S18). In particular, the present study introduces four new species of Roussoellaceae and three new species of Thyridariaceae. Indeed, only MUT 2452 and MUT 4893 were ascribable to the previously described *P. robiniae* (Figure 1). In the case of MUT 4884, the holotype of *P. dematiacea*, a novel genus was proposed since it formed a defined cluster with MUT 5310 and MUT 4419, well separated by the genera *Cycasicola*, *Liua* and *Thyridariella*.

Most of the Roussoellaceae and Thyridariaceae described to date are associated with terrestrial plants, especially bamboo and palm species [15,16]. In fact, only two species, *R. mangrovei* and *R. nitidula* have been retrieved from the marine environment ([www.marinefungi.org](http://www.marinefungi.org)). However, considering the present study, we can infer that these families are well represented in the sea, thus improving our knowledge on the largely unexplored fungal marine biodiversity.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-2818/12/4/144/s1>, Table S1: The eight variable sites detected in the nrITS region among *P. dematiacea* and its neighbor species, Table S2: The single variable site detected in the nrLSU region among *P. dematiacea* and its neighbor species, Table S3: The five variable sites detected in the nrSSU region among *P. dematiacea* and its neighbor species, Table S4: The six variable sites detected in the TEF1 $\alpha$  partial gene among *P. dematiacea* and its neighbor species, Table S5: The six variable sites detected in the nrITS region among *P. tyrrhenica*, *P. flabelliae* and their neighbor species, Table S6: The eight variable sites detected in the nrLSU region among *P. tyrrhenica*, *P. flabelliae* and their neighbor species, Table S7: The eight variable sites detected in the TEF1 $\alpha$  partial gene among *P. tyrrhenica*, *P. flabelliae* and their neighbor species, Table S8: The 33 variable sites detected in the RPB2 partial gene among *P. tyrrhenica*, *P. flabelliae* and their neighbor species, Table S9: The two variable sites detected in nrITS region among *R. mediterranea*, *R. padinae* and the neighbor species, Table S10: The single variable site detected in nrLSU region among *R. mediterranea*, *R. padinae*, and the neighbor species, Table S11: The six sites detected in the TEF1 $\alpha$  partial gene among *R. mediterranea*, *R. padinae* and the neighbor species, Table S12: The six sites detected in the RPB2 partial gene among *R. mediterranea*, *R. padinae* and the neighbor species, Table S13: The eight variable sites detected in the nrITS region among *N. lignicola* and its neighbor species, Table S14: The three variable sites detected in the nrLSU region among *N. lignicola* and its neighbor species, Table S15: The eight variable sites detected in the nrSSU region among *N. lignicola* and its neighbor species, Table S16: The ten sites detected in the TEF1 $\alpha$  partial gene among *N. lignicola* and its



neighbor species, Table S17: The three variable sites detected in the nrITS region among *R. margidoriensis* and its neighbor species, Table S18: The 29 variable sites detected in the TEF1 $\alpha$  partial gene among *R. margidoriensis* and its neighbor species

**Author Contributions:** Conceptualization, A.P., E.B., V.P., G.C.V.; methodology, A.P., E.B., V.P., G.C.V.; software, A.P.; validation, A.P., E.B., V.P., L.R., G.C.V.; formal analysis, A.P., E.B., V.P., L.R.; investigation, A.P., E.B., V.P., L.R., G.C.V.; resources, V.P., G.C.V.; data curation, A.P., V.P.; writing—original draft preparation, A.P., V.P.; writing—review and editing, A.P., E.B., V.P., G.C.V.; visualization, A.P., V.P.; supervision, V.P., G.C.V.; project administration, A.P., E.B., V.P., G.C.V.; funding acquisition, G.C.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Fondazione CRT, Torino, Italy and by the University of Torino (ex 60%).

**Acknowledgments:** The authors are grateful to Pelagosphaera s.c.r.l. for harvesting algal and seagrass samples.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Richards, T.A.; Jones, M.D.; Leonard, G.; Bass, D. Marine fungi: Their ecology and molecular diversity. *Ann. Rev. Mar. Sci.* **2012**, *4*, 495–522. [[CrossRef](#)] [[PubMed](#)]
- Amend, A.; Burgaud, G.; Cunliffe, M.; Edgcomb, V.P.; Ettinger, C.L.; Gutierrez, M.H.; Heitman, J.; Hom, E.F.Y.; Ianiri, G.; Jones, A.C.; et al. Fungi in the marine environment: Open questions and unsolved problems. *Mbio* **2019**, *10*, 15. [[CrossRef](#)] [[PubMed](#)]
- Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2019**, *36*, 122–173. [[CrossRef](#)] [[PubMed](#)]
- Jones, E.G.; Pang, K.-L. *Marine fungi and fungal-like organisms*; Jones, E.G., Pang, K.-L., Eds.; Walter de Gruyter: Berlin, Germany, 2012.
- Raghukumar, S. *Fungi in Coastal and Oceanic Marine Ecosystems: Marine Fungi*; Springer: Cham, Switzerland, 2017.
- Pang, K.L.; Overy, D.P.; Jones, E.B.G.; Calado, M.D.; Burgaud, G.; Walker, A.K.; Johnson, J.A.; Kerr, R.G.; Cha, H.J.; Bills, G.F. ‘Marine fungi’ and ‘marine-derived fungi’ in natural product chemistry research: Toward a new consensual definition. *Fungal Biol. Rev.* **2016**, *30*, 163–175. [[CrossRef](#)]
- Jones, E.B.G.; Pang, K.-L.; Abdel-Wahab, M.A.; Scholz, B.; Hyde, K.D.; Boekhout, T.; Ebel, R.; Rateb, M.E.; Henderson, L.; Sakayaroj, J.; et al. An online resource for marine fungi. *Fungal Divers.* **2019**, *96*, 347–433. [[CrossRef](#)]
- Jones, E.B.G. Fifty years of marine mycology. *Fungal Divers.* **2011**, *50*, 73–112. [[CrossRef](#)]
- Jones, E.B.G.; Suetrong, S.; Sakayaroj, J.; Bahkali, A.H.; Abdel-Wahab, M.A.; Boekhout, T.; Pang, K.L. Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. *Fungal Divers.* **2015**, *73*, 1–72. [[CrossRef](#)]
- Garzoli, L.; Poli, A.; Prigione, V.; Gnavi, G.; Varese, G.C. Peacock’s tail with a fungal cocktail: First assessment of the mycobiota associated with the brown alga *Padina pavonica*. *Fungal Ecol.* **2018**, *35*, 87–97. [[CrossRef](#)]
- Gnavi, G.; Garzoli, L.; Polil, A.; Prigione, V.; Burgaud, G.; Varese, G.C. The culturable mycobiota of *Flabellia petiolata*: First survey of marine fungi associated to a Mediterranean green alga. *PLoS ONE* **2017**, *12*. [[CrossRef](#)]
- Panno, L.; Bruno, M.; Voyron, S.; Anastasi, A.; Gnavi, G.; Miserere, L.; Varese, G.C. Diversity, ecological role and potential biotechnological applications of marine fungi associated to the seagrass *Posidonia oceanica*. *New Biotechnol.* **2013**, *30*, 685–694. [[CrossRef](#)]
- Bovio, E.; Garzoli, L.; Poli, A.; Prigione, V.; Firsova, D.; McCormack, G.; Varese, G. The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. spongiae*. *Fungal Syst. Evol.* **2018**, *1*, 141–167. [[CrossRef](#)]
- Liu, J.-K.; Phookamsak, R.; Dai, D.-Q.; Tanaka, K.; Jones, E.; Xu, J.-C.; Chukeatirote, E.; Hyde, K.D. Roussoellaceae, a new pleosporalean family to accommodate the genera *Neorousoella* gen. nov., *Rousoella* and *Rousoellopsis*. *Phytotaxa* **2014**, *181*, 1–33. [[CrossRef](#)]
- Jaklitsch, W.M.; Voglmayr, H. Hidden diversity in *Thyridaria* and a new circumscription of the Thyridariaceae. *Stud. Mycol.* **2016**, *85*, 35–64. [[CrossRef](#)]
- Tibpromma, S.; Hyde, K.D.; Jeewon, R.; Maharachchikumbura, S.S.N.; Liu, J.K.; Bhat, D.J.; Jones, E.B.G.; McKenzie, E.H.C.; Camporesi, E.; Bulgakov, T.S.; et al. Fungal diversity notes 491–602: Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Divers.* **2017**, *83*, 1–261. [[CrossRef](#)]

17. Devadatha, B.; Sarma, V.V.; Jeewon, R.; Wanasinghe, D.N.; Hyde, K.D.; Jones, E.B.G. *Thyridariella*, a novel marine fungal genus from India: Morphological characterization and phylogeny inferred from multigene DNA sequence analyses. *Mycol. Prog.* **2018**, *17*, 791–804. [[CrossRef](#)]
18. Jayasiri, S.; Hyde, K.; Jones, E.; McKenzie, E.; Jeewon, R.; Phillips, A.; Bhat, D.; Wanasinghe, D.; Liu, J.; Lu, Y.; et al. Diversity, morphology and molecular phylogeny of Dothideomycetes on decaying wild seed pods and fruits. *Mycosphere* **2019**, *10*, 1–186. [[CrossRef](#)]
19. Phookamsak, R.; Hyde, K.D.; Jeewon, R.; Bhat, D.J.; Jones, E.B.G.; Maharachchikumbura, S.S.N.; Raspe, O.; Karunarathna, S.C.; Wanasinghe, D.N.; Hongsanan, S.; et al. Fungal diversity notes 929–1035: Taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal Divers.* **2019**, *95*, 1–273. [[CrossRef](#)]
20. Jiang, H.B.; Hyde, K.D.; Jayawardena, R.S.; Doilom, M.; Xu, J.C.; Phookamsak, R. Taxonomic and phylogenetic characterizations reveal two new species and two new records of *Rousoella* (Rousoellaceae, Pleosporales) from Yunnan, China. *Mycol. Prog.* **2019**, *18*, 577–591. [[CrossRef](#)]
21. Dai, D.Q.; Phookamsak, R.; Wijayawardene, N.N.; Li, W.J.; Bhat, D.J.; Xu, J.C.; Taylor, J.E.; Hyde, K.D.; Chukeatirote, E. Bambusicolous fungi. *Fungal Divers.* **2017**, *82*, 1–105. [[CrossRef](#)]
22. Panebianco, C.; Tam, W.Y.; Jones, E.B.G. The effect of pre-inoculation of balsa wood by selected marine fungi and their effect on subsequent colonisation in the sea. *Fungal Divers.* **2002**, *10*, 77–88.
23. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A guide to methods and applications*; Academic Press: New York, NY, USA, 1990; Volume 18, pp. 315–322.
24. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [[CrossRef](#)]
25. Stielow, J.; Lévesque, C.; Seifert, K.; Meyer, W.; Iriny, L.; Smits, D.; Renfurm, R.; Verkley, G.; Groenewald, M.; Chaduli, D. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* **2015**, *35*, 242–263. [[CrossRef](#)]
26. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Mol. Biol. Evol.* **1999**, *16*, 1799–1808. [[CrossRef](#)]
27. Vaidya, G.; Lohman, D.J.; Meier, R. SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **2011**, *27*, 171–180. [[CrossRef](#)]
28. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [[CrossRef](#)]
29. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [[CrossRef](#)]
30. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)]
31. Lieckfeldt, E.; Meyer, W.; Borner, T. Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. *J. Basic Microbiol.* **1993**, *33*, 413–426. [[CrossRef](#)]
32. Poli, A.; Lazzari, A.; Prigione, V.; Voyron, S.; Spadaro, D.; Varese, G.C. Influence of plant genotype on the cultivable fungi associated to tomato rhizosphere and roots in different soils. *Fungal Biol.* **2016**, *120*, 862–872. [[CrossRef](#)]
33. Poli, A.; Vizzini, A.; Prigione, V.; Varese, G.C. Basidiomycota isolated from the Mediterranean Sea-Phylogeny and putative ecological roles. *Fungal Ecol.* **2018**, *36*, 51–62. [[CrossRef](#)]
34. Oses, R.; Valenzuela, S.; Freer, J.; Sanfuentes, E.; Rodriguez, J. Fungal endophytes in xylem of healthy Chilean trees and their possible role in early wood decay. *Fungal Divers.* **2008**, *33*, 77–86.
35. Vohnik, M.; Borovce, O.; Kolarikova, Z.; Sudova, R.; Reblova, M. Extensive sampling and high-throughput sequencing reveal *Posidoniomyces atricolor* gen. et sp. nov. (Aigialaceae, Pleosporales) as the dominant root mycobiont of the dominant Mediterranean seagrass *Posidonia oceanica*. *Myckeys* **2019**, *55*, 59–86. [[CrossRef](#)] [[PubMed](#)]

