Metabarcoding reveals diverse endophytic fungal communities in *Vaccinium myrtillus* plant organs and suggests systemic distribution of some ericoid mycorrhizal and DSE fungi

Authors:

Stefania Daghino[1], Elena Martino[2], Samuele Voyron[1,2], Silvia Perotto[2,*]

[1] Institute for Sustainable Plant Protection, CNR, Strada delle Cacce 73, 10135 Torino and V. le Mattioli 25, 10125 Torino, Italy.

[2] Department of Life Science and Systems Biology, University of Torino, V. le Mattioli 25, 10125, Torino, Italy.

Figure S1. Alpha-diversity of the fungal communities associated with the different plant organs. The alpha-diversity associated with each organ has been calculated according to a) Chao1 and b) Shannon diversity metrics with following parameters: data input: filtered, experimental factor: organ, taxonomic level: feature, statistical method: Mann-Whitney/Kruskal-Wallis.

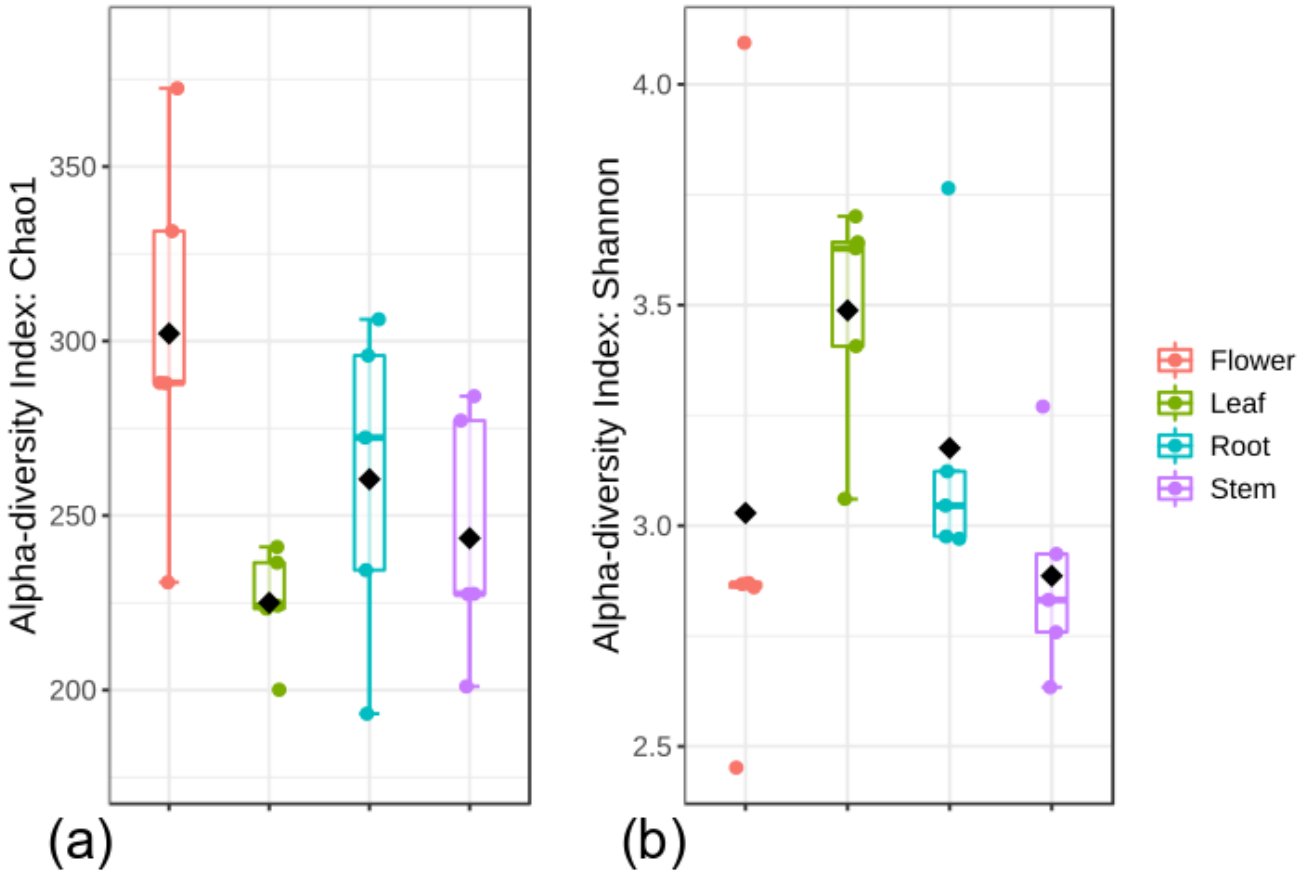

Figure S2. Fungal phyla associated with different plant organs. (a) relative abundance of phyla, (b) phyla with statistically different abundance among the organs (Pairwise Wilcoxon rank sum test/Kruskal-Wallis with BH-adjusted p-val<0.05).

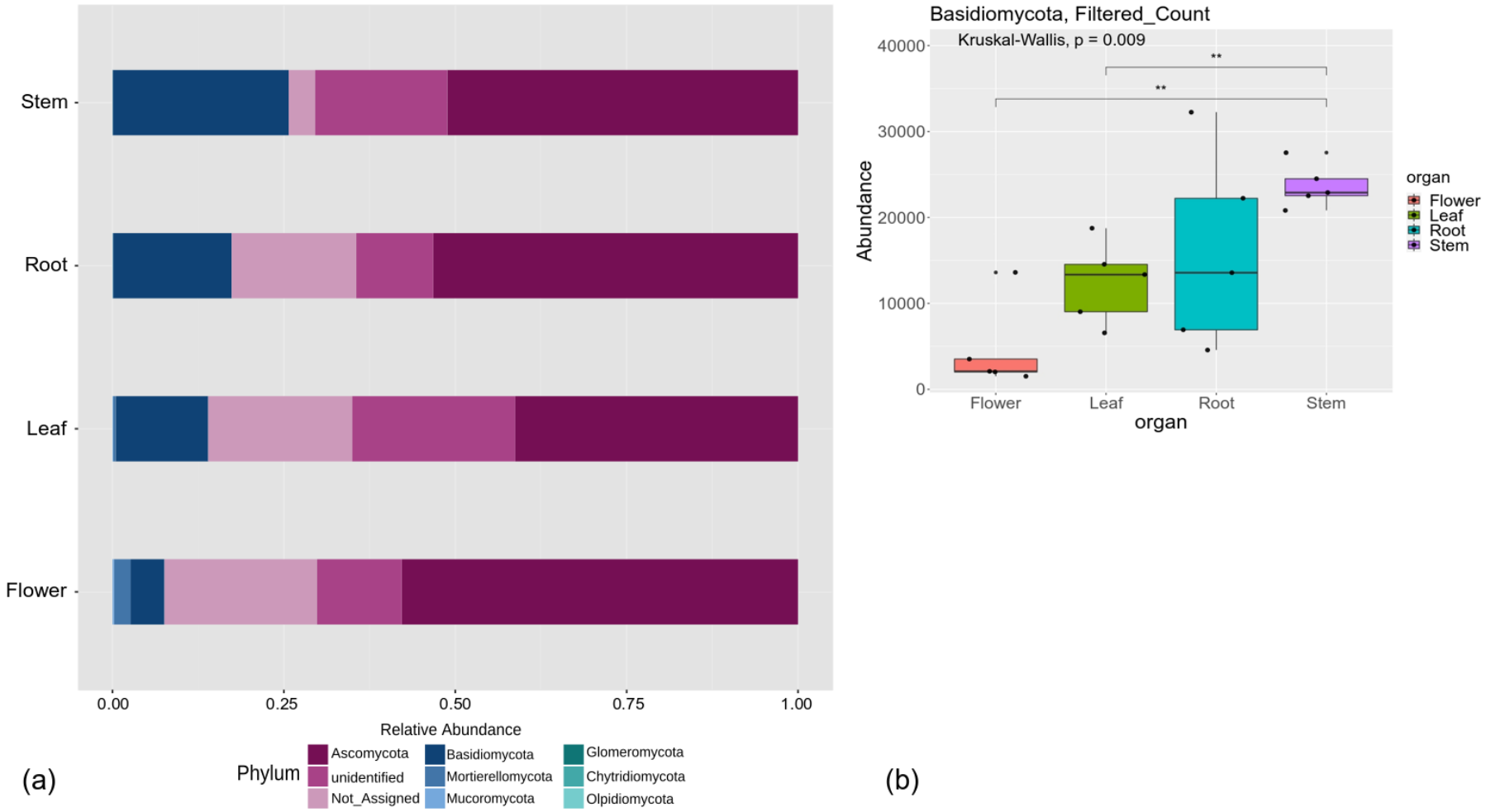

Figure S3. Fungal genera associated with different plant organs. (a) relative abundance of genera, (b) genus with statistically different abundance among the organs (Pairwise Wilcoxon rank sum test/Kruskal-Wallis with BH-adjusted p-val<0.05).

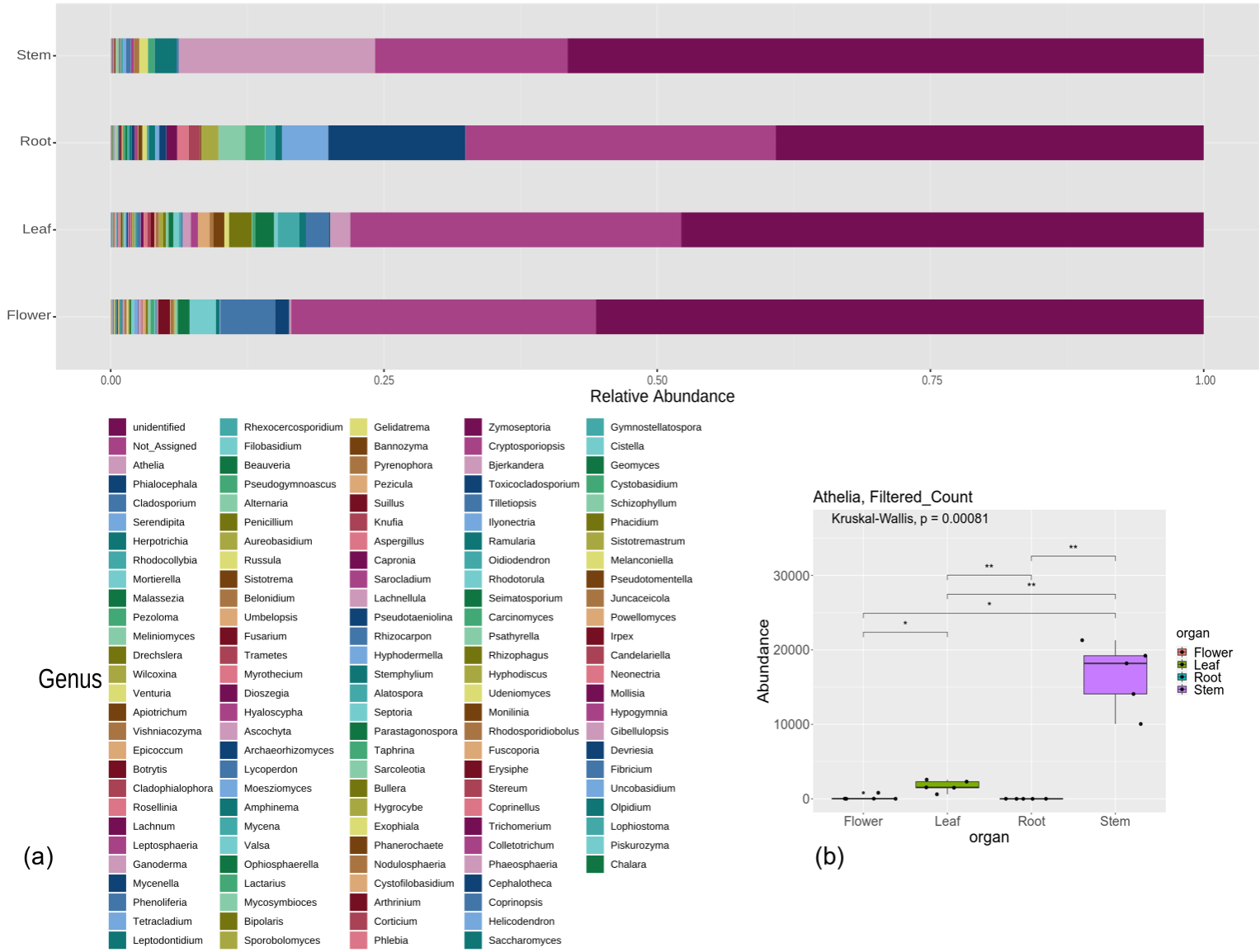

Figure S4. Correlation network analysis, based on Pearson's correlation, which determines whether linear relationships exist between two taxa. Each node represents a class (a) or a genus (b). The edges represent correlations between taxa pairs (red= positive; blue= negative correlation). The nodes are colored on the basis of the relative abundance in different organs (lilac: roots; green: flowers; orange: leafs; magenta: stems).

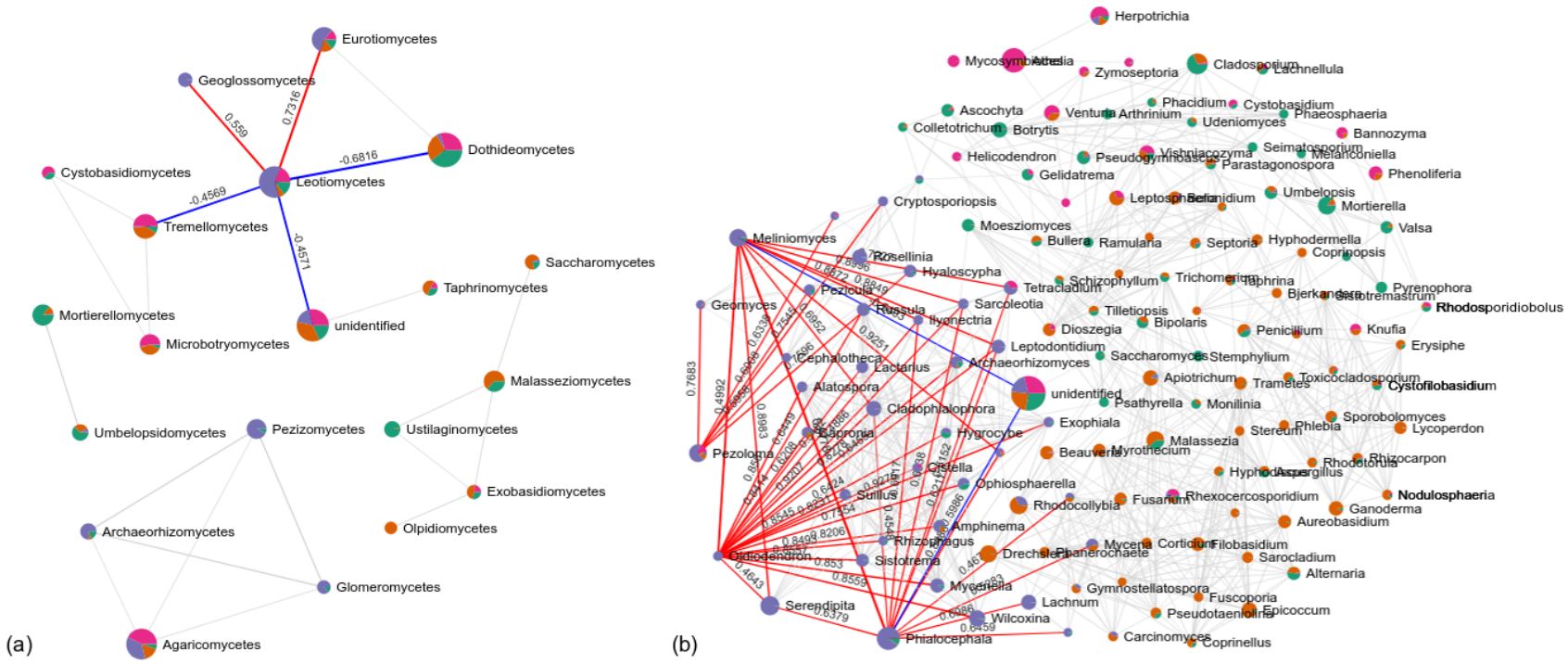

Figure S5. OTU presence in the different samples and fungal genera in the core OTUs. a) Venn diagram of the OTUs from the different plant organs; b) relative abundance of the genera within the 214 core OTUs.

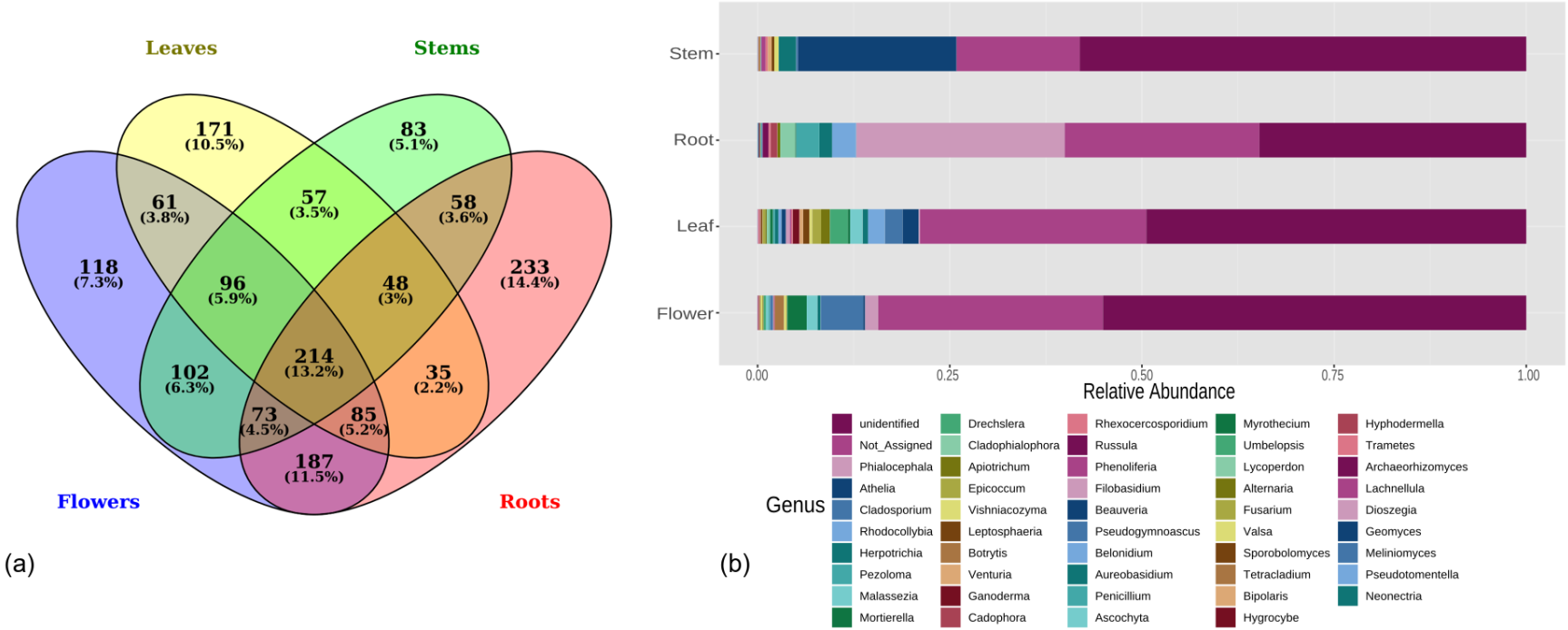

Figure S6. Phylogenetic analysis of the isolates and OTUs attributed to *Oidiodendron spp.* ITS2 sequences from the isolates attributed by morphological observation to *Oidiodendron spp.* and of the OTUs attributed to the *Oidiodendron* genus by matching with the Unite database have been aligned. The Bayesian consensus tree is shown with posterior probabilities (pp). Bootstrap support (bs) for maximum likelihood analyses is given if >50%. These values are reported close to branches separated by hyphen (pp-bs). Taxa names are maintained as in GenBank (accession numbers included), followed by the isolate. The OTUs found in this paper are indicated by their respective number. The color code indicates the *V. myrtillus* organ in which the OTU has been detected, only if represented by at least 10 reads. OTUs represented by less than 10 reads in one organ have not been indicated. Orange indicates the fungi isolated from roots in this paper.

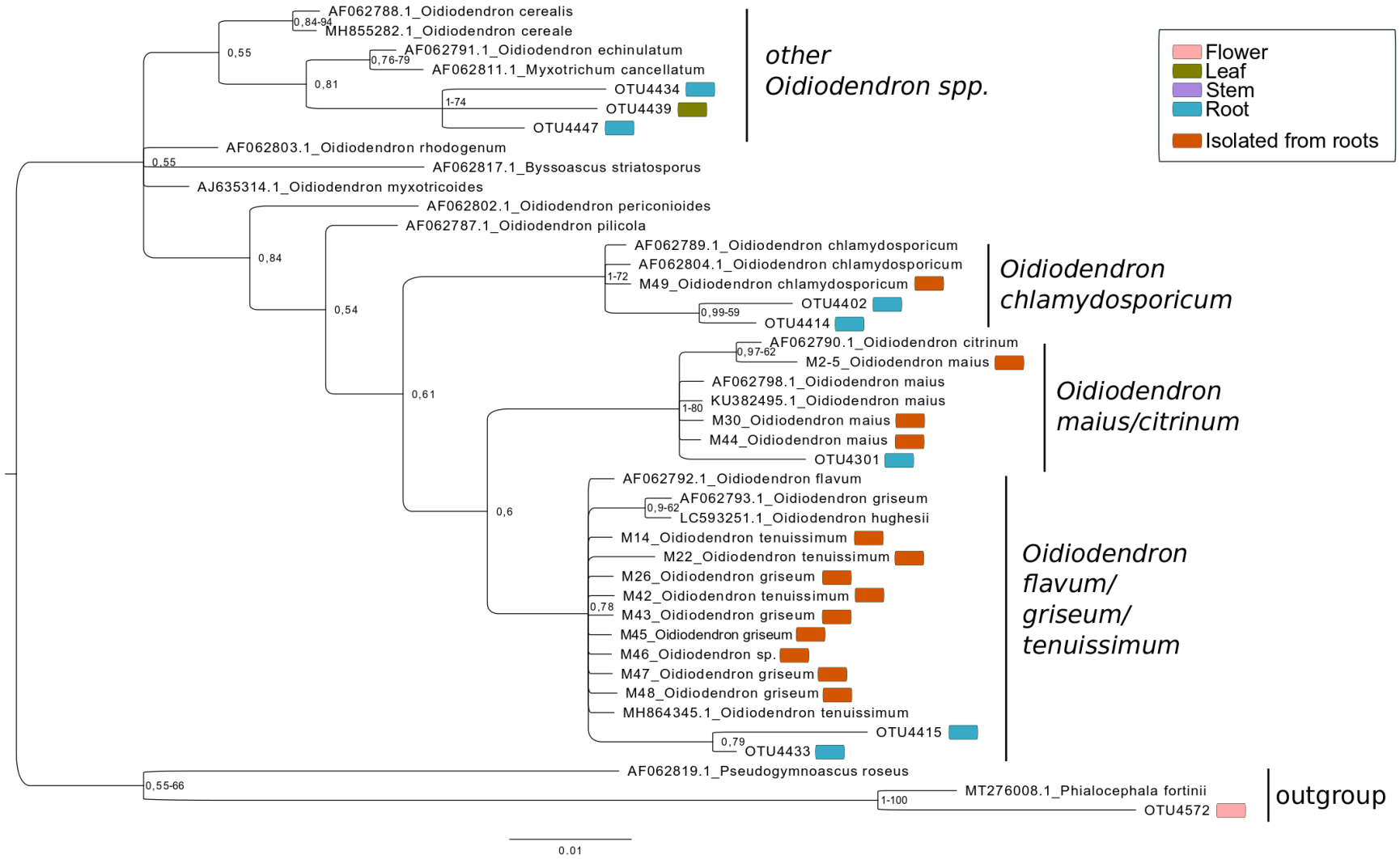

Figure S7. Phylogenetic analysis of the isolates and OTUs attributed to the *Rhizoscyphus ericae* aggregate. ITS2 sequences from the isolates attributed by morphological observation to *C. luteo-olivacea* and of the OTUs attributed to *Cadophora spp*. and to other species in the REA by matching with the Unite database, have been aligned. The Bayesian consensus tree is shown with posterior probabilities (pp). Bootstrap support (bs) for maximum likelihood analyses is given if >50%. These values are reported close to branches separated by hyphen (pp-bs). Taxa names are maintained as in GenBank (accession numbers included), followed by the isolate. The OTUs found in this paper are indicated by their respective number. The color code indicates the *V. myrtillus* organ in which the OTU has been detected, only if represented by at least 10 reads. OTUs represented by less than 10 reads in one organ have not been indicated. Orange indicates the fungi isolated from roots in this paper.

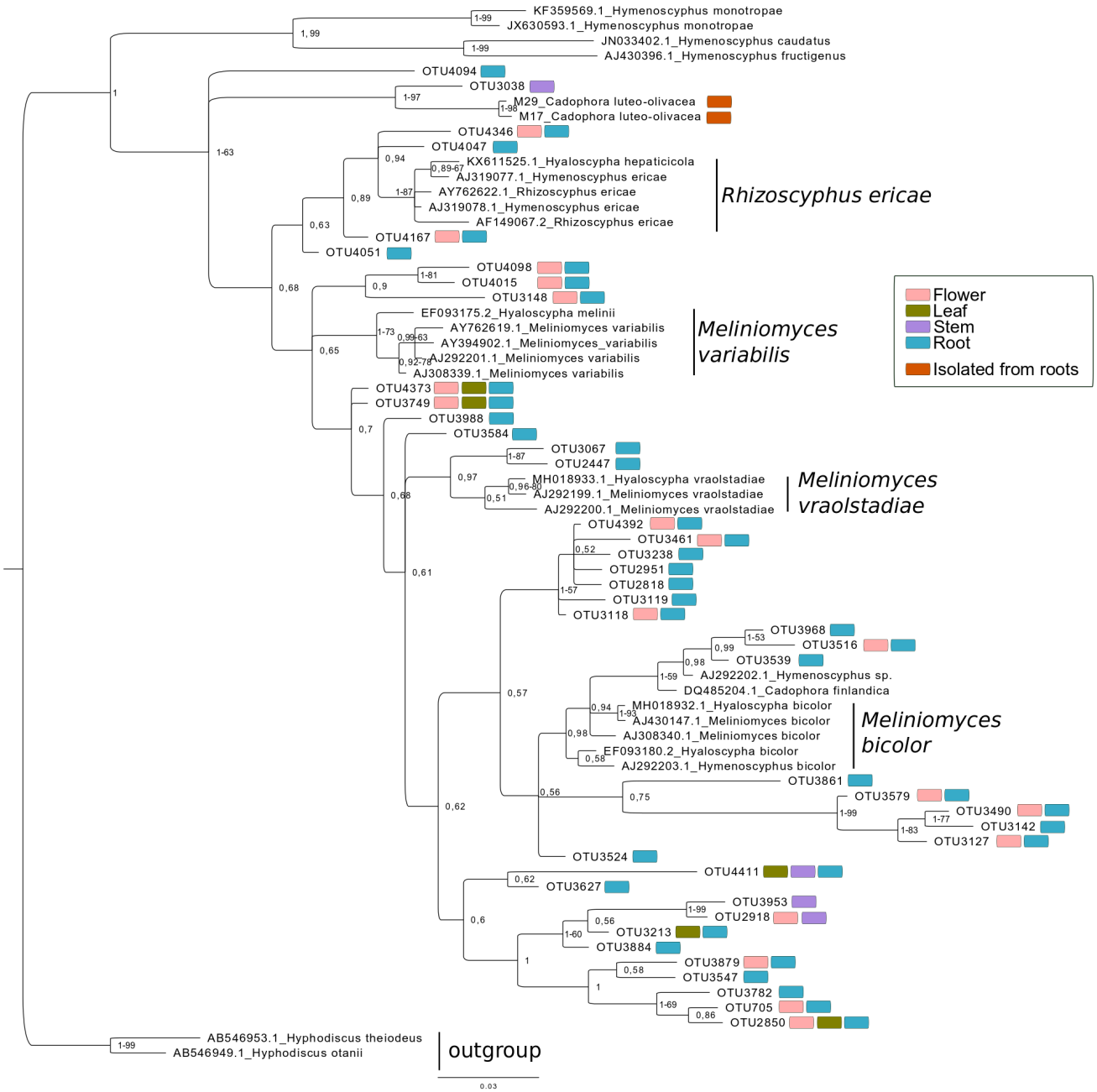

Figure S8. Detection of Helotiales isolates in the plant *in vitro*. Amplification of the ITS2 regions from the above ground organs of *V. myrtillus* (Vm) plants grown *in vitro* in the presence of 3 *Helotiales* isolated in this work (M17, M30, M42, respectively attributed to *C. luteo-olivacea*, *O. maius* and *O. tenuissimum*) and an *O. maius* strain from a previous work (OmMUT1381[70]). ITS9F/ITS4ngs primers have been used in the PCR, expected to amplify the fungal ITS2 region. Samples: 1) Vm-OmMUT1381; 2) Vm-M17; 3) Vm-M30; 4) Vm-M42; 5) Vm; 6) Basidiomicetes sp. genomic DNA; 7) OmMUT1381 genomic DNA; 8) OmMUT1348[70] genomic DNA; 9) empty negative control. The amplicons indicated by red rectangles (a) and (b) have been separately eluted from gel and Sanger-sequenced (see Supplementary Table S3).

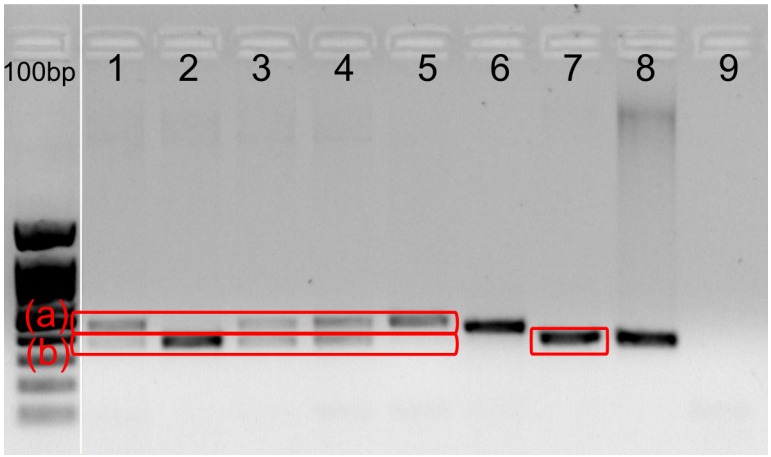

Table S2. Identification of *V. myrtillus* root-isolated strains by molecular analysis of the ITS2 region (second column), identification of the Helotiales strains by morpholgical (third column) and phylogenetic (fourth column) analysis. NA=not analysed. MUT= Mycotheca Universitatis Taurinensis.

Morphotype ID	Taxa assigned by molecular analysis	Taxa assigned by micromorphology observation	Taxa assigned by phylogenetic analysis	GenBank Accession number	MUT code for deposited strains
M2-5	*Oidiodendron sp.*	*Oidiodendron maius*	*Oidiodendron maius*	OL333436	6402
M3	*Penicillium sp.*	*NA*	*NA*		
M4	Fungi sp.	*NA*	*NA*		
M6	Mucoromycota	*NA*	*NA*		
M7	Mucoromycota	*NA*	*NA*		
M8A	Mucoromycota	*NA*	*NA*		
M8B	*Penicillium sp.*	*NA*	*NA*		
M9	Mucoromycota	*NA*	*NA*		
M10	Mucoromycota	*NA*	*NA*		
M11	*Penicillium sp.*	*NA*	*NA*		
M12	*Penicillium sp.*	*NA*	*NA*		
M13	*Penicillium sp.*	*NA*	*NA*		
M14	*Oidiodendron sp.*	*Oidiodendron tenuissimum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333437	
M17	*Cadophora sp.*	*Cadophora luteo-olivacea*	*Rhizoscyphus ericae aggregate*	OL333438	6464
M19	*Penicillium sp.*	*NA*	*NA*		
M20	*Penicillium sp.*	*NA*	*NA*		
M21B	*Umbelopsis sp.*	*NA*	*NA*		
M22	*Oidiodendron sp.*	*Oidiodendron tenuissimum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333439	
M23	Mucoromycota	*NA*	*NA*		
M24	*Penicillium sp.*	*NA*	*NA*		
M25	*Penicillium sp.*	*NA*	*NA*		
M26	*Oidiodendron sp.*	*Oidiodendron griseum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333440	
M28	*Pleotrichocladium opacum*	*NA*	*NA*		
M29	*Cadophora sp.*	*Cadophora luteo-olivacea*	*Rhizoscyphus ericae aggregate*	OL333441	
M30	*Oidiodendron sp.*	*Oidiodendron maius*	*Oidiodendron maius*	OL333434	6400
M37	*Apiotrichum porosum*	*NA*	*NA*		
M39	*Penicillium sp.*	*NA*	*NA*		
M40	*Penicillium sp.*	*NA*	*NA*		
M42	*Oidiodendron sp.*	*Oidiodendron tenuissimum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333442	6465

M43	*Oidiodendron sp.*	*Oidiodendron griseum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333443	
M44	*Oidiodendron sp.*	*Oidiodendron maius*	*Oidiodendron maius*	OL333435	6401
M45-2	*Oidiodendron sp.*	*Oidiodendron griseum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333444	
M46	*Oidiodendron sp.*	Oidiodendron sp.	*Oidiodendron tenuissimum, flavus, griseum group*	OL333445	
M47	*Oidiodendron sp.*	*Oidiodendron griseum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333446	
M48	*Oidiodendron sp.*	*Oidiodendron griseum*	*Oidiodendron tenuissimum, flavus, griseum group*	OL333448	6466
M49	*Oidiodendron sp.*	*Oidiodendron chlamydosporicum*	*Oidiodendron chlamydosporicum*	OL333447	6467
M50	*Penicillium sp.*	*NA*	*NA*		

Table S3. Identification of the amplicons obtained by PCR of the above-ground organs of *in vitro* colonized *V. myrtillus* (see Supplementary Fig. S8).

Amplicon ID	BlastN first match	BlastN identification	Unite first match (accession number, e-value)	Phylogenetic analysis	Morphotype ID	Reference
1a	Angiosperm environmental sample clone (FJ554198.1, e-val=0)	*Vaccinium sp.*	--	--	--	
1b	*Oidiodendron maius (MT278164.1, e-val= 7e-117)*	*Oidiodendron maius*	*Oidiodendron maius (MH860825, 2e-119)*	*O.maius*	OmZn	70
2b	*Cadophora sp. (MN365799.1, e-val= 6e-144)*	*Cadophora sp.*	Fungi (MN151924, 2e-146)	*Cadophora*	M17	this paper
3a	ND		--	--	--	
3b	Uncultured Oidiodendron clone (KY684480.1, e-val= 4-e140)	*Oidiodendron maius*	*Oidiodendron maius (KY910207, 9e-143)*	*O.maius*	M30	this paper
4a	*Vaccinium myrtillus (KM209458.1, e-val=6e-164)*	*Vaccinium myrtillus*	--	--	--	
4b	*Oidiodendron griseum (MH865155.1, e-val= 6e-118)*	*Oidiodendron sp.*	*Oidiodendron griseum (MH865155, 1e-120)*	*O.griseum/ O.tenuissimum*	M42	this paper
5a	*Vaccinium myrtillus (KM209458.1, e-val=7e-163)*	*Vaccinium myrtillus*	--	--	--	
7b	*Oidiodendron maius CBS 926.73 (MH860824.1, e-val= 1e-139)*	*Oidiodendron maius*	*Oidiodendron maius (MH860825, 3e-142)*	*O.maius*	OmZn	70

Table S4. GenBank accession numbers and taxa used in the phylogenetic analysis. References: 29 and 68.

Accession number	**Taxon description**
AB546949.1	*Hyphodiscus otanii*
AB546953.1	*Hyphodiscus theiodeus*
AF062787.1	*Oidiodendron pilicola*
AF062788.1	*Oidiodendron cerealis*
AF062789.1	*Oidiodendron chlamydosporicum*
AF062790.1	*Oidiodendron citrinum*
AF062791.1	*Oidiodendron echinulatum*
AF062792.1	*Oidiodendron flavum*
AF062793.1	*Oidiodendron griseum*
AF062798.1	*Oidiodendron maius*
AF062802.1	*Oidiodendron periconioides*
AF062803.1	*Oidiodendron rhodogenum*
AF062804.1	*Oidiodendron chlamydosporicum*
AF062811.1	*Myxotrichum cancellatum*
AF062817.1	*Byssoascus striatosporus*
AF062819.1	*Pseudogymnoascus roseus*
AF149067.2	*Rhizoscyphus ericae*
AJ292199.1	*Meliniomyces vraolstadiae*
AJ292200.1	*Meliniomyces vraolstadiae*
AJ292201.1	*Meliniomyces variabilis*
AJ292202.1	*Hymenoscyphus sp.*
AJ292203.1	*Hymenoscyphus bicolor*
AJ308339.1	*Meliniomyces variabilis*
AJ308340.1	*Meliniomyces bicolor*
AJ319077.1	*Hymenoscyphus ericae*
AJ319078.1	*Hymenoscyphus ericae*
AJ430147.1	*Meliniomyces bicolor*
AJ430396.1	*Hymenoscyphus fructigenus*
AJ635314.1	*Oidiodendron myxotrichoides*
AY394902.1	*Meliniomyces variabilis*
AY762619.1	*Meliniomyces variabilis*
AY762622.1	*Rhizoscyphus ericae*
DQ485204.1	*Cadophora finlandica*
EF093175.2	*Hyaloscypha melinii*
EF093180.2	*Hyaloscypha bicolor*
JN033402.1	*Hymenoscyphus caudatus*
JX630593.1	*Hymenoscyphus monotropae*
KF359569.1	*Hymenoscyphus monotropae*
KU382495.1	*Oidiodendron maius*
KX611525.1	*Hyaloscypha hepaticicola*
LC593251.1	*Oidiodendron hughesii*
MH018932.1	*Hyaloscypha bicolor*
MH018933.1	*Hyaloscypha vraolstadiae*
MH855282.1	*Oidiodendron cereale*
MH864345.1	*Oidiodendron tenuissimum*
MT276008.1	*Phialocephala fortinii*