Diversity of mycorrhizal fungi in a temperate terrestrial orchid in ex situ and in situ environments

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Introduction

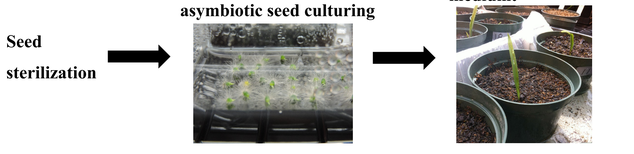
Variation in mycorrhizal diversity and specificity within the Orchidaceae is of special interest considering the intimate involvement of orchid mycorrhizal fungi (OMF) in germination and development of individuals. Yet, there remains a large gap in our understanding of OMF ecology especially when orchids are cultured ex situ for conservation applications. It seems that to minimize the potential for introducing undesirable mycorrhizal fungi along with the orchid plants, the most important step that researchers can include is to compare the fungi that form mycorrhizae with the orchid taxon of interest ex situ and in situ to inform conservation decisions.

Question and Hypothesis

Question: With which OMF do un-inoculated orchid seedlings form symbiosis when cultured ex situ in commercial potting mixture?
Hypothesis: Plants generated via asymbiotic germination and subsequent ex situ (ES) culture in plant potting mixture will have a different suite of mycorrhizal fungi within their roots in comparison to plants of the same species occurring in situ (IS) in their native habitat.

Materials and Methods

- *Study species*: We used the temperate terrestrial orchid taxon *Platanthera chapmanii* as a model species which has its disjunct populations restricted to southern Georgia, northern Florida, and southeastern Texas within the United States (Richards and Sharma 2014)
- *Study site*: We selected Longleaf pine savanna bog in southeast TX to collect seeds for ES plant culture, and for IS root collection
- *Ex-situ plant culture*



- *Root sampling*: Roots were sampled three times between 2012 and 2014 from ES (ES1, ES2 and ES3), and IS (IS1, IS2 and IS3) treatments during the reproductive phase of species

- Peloton inspection, DNA extraction and fungal DNA barcoding with nuclear ribosomal ITS (nrITS) locus
- Sanger sequencing, bioinformatic analyses with MEGA and biostatitical analyses in R

Results

- After quality filtering, 114 sequences clustered into 11 operational taxonomic units (OTUs) belonging to the fungal family Tulasnellaceae (Table 1). Shannon-Wiener (H) and Simpson diversity (D) indices were similar (p = 0.80 for both) for ES and IS OMF communities.

Table 1. Number of sequences representing 11 nuclear ribosomal internal transcribed spacer (nrITS) based fungal operational taxonomic units (OTUs) identified from the roots of 21 ex situ cultured (ES1, ES2, ES3), and 15 in situ occurring (IS1, IS2, IS3) plants of *Platanthera chapmanii*. Values in parentheses are the number of plants in which a specific OTU was documented.

OMF source	Ex situ (ES)			In situ (IS)		
	ES1	ES2	ES3	IS1	IS2	IS3
OTU						
T1						2 (1)
T2		1 (1)	13 (8)			7 (3)
T3					15 (5)	18 (5)
T4						1 (1)
T5	8 (1)					
T6		3 (1)				
T7			3 (2)			1 (1)
T8		21 (4)				
T9			1 (1)			
T10				6 (1)		8 (2)
T11		6 (2)				

- Beta diversity comparisons also showed similarity between ES and IS treatments based on unweighted (p = 0.20) and weighted (p = 0.10) Bray-Curtis dissimilarity index values (Fig. 1a and b).

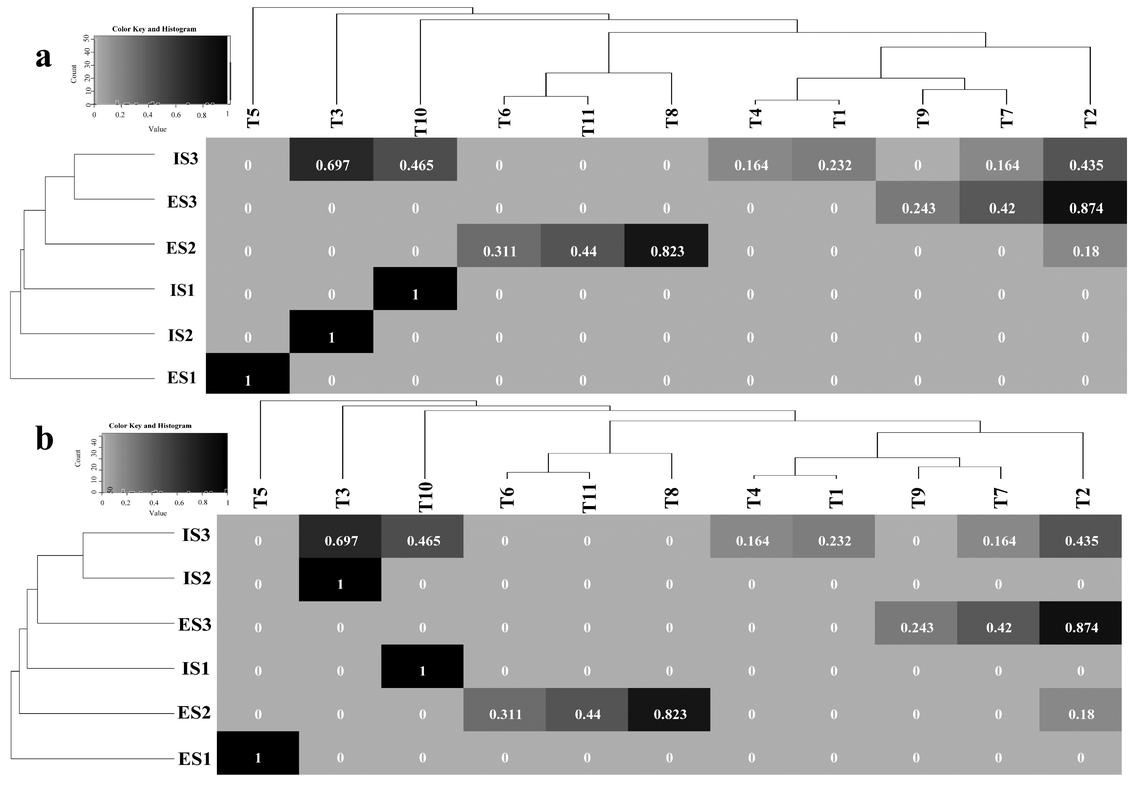


Fig. 1 Two-way hierarchical clustering of replicate sampling events based on 11 Operational Taxonomic Units (OTUs T1 to T11) identified from the nuclear ribosomal internal transcribed spacer (nrITS) sequences amplified from the roots of ex situ cultured (ES1, ES2, and ES3), and in situ occurring plants (IS1, IS2, and IS3) of *Platanthera chapmanii.* Bray-Curtis dissimilarity index was used to generate pairwise distances: **a)** a dendrogram based on OTU richness dissimilarities among the six sampling events, and **b)** a dendrogram based on OTU abundance dissimilarities among the same six sampling events. The grayscale shade bar provides the Bray-Curtis dissimilarity values.

- Bayesian and Maximum Likelihood (ML) phylograms clustered ES and IS derived fungal OTUs into the same clades (Fig. 2).

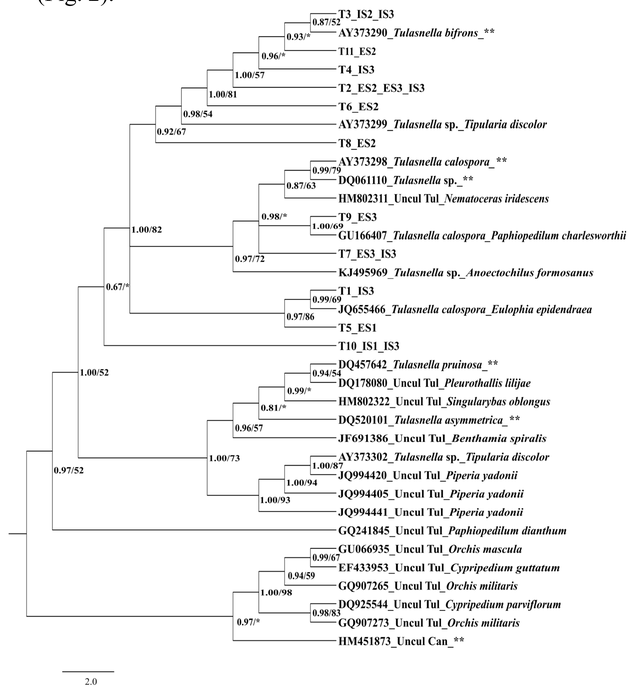


Fig 2. A combined mid-point rooted Bayesian and Maximum Likelihood (ML) tree of the fungal family Tulasnellaceae built with OMF OTUs from roots of *Platanthera chapmanii*. The first value among branch support values represents the Bayesian clade support, and the second value represents the ML bootstrap value.

Conclusions

Our results show that the lack of genetic separation of OTUs from ES and IS plants coupled with the overlap of OTUs across the two treatments underpin the specificity of *P. chapmanii* toward its preferred OMF regardless of the growing substrate and environment.

Acknowledgements

We gratefully acknowledge the support of :

- Texas Parks and Wildlife Department
- Joe Liggio and Pauline Singleton for assisting with root tissue collection permits, and Matt Richards (Atlanta Botanical Garden) for assisting with plant propagation

Literature Cited

Richards M, Sharma J (2014) Review of Conservation Efforts for *Platanthera chapmanii* in Texas and Georgia. The Native Orchid Conference Journal 11(1):1-11