

Are root mycorrhizal communities an indicator of flowering in populations of a rare North American plant?

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Introduction

The Orchidaceae family is considered as one of the largest families of angiosperms. All orchid species exhibit obligatory symbiotic association with orchid mycorrhizal fungi (OMF) (Pandey *et al*). The OMF communities can vary among individual plants of a species and among populations which can potentially shape the demographic patterns of species by affecting host plant and population responses. *Platanthera (Piperia) cooperi* is a rare perennial orchid species endemic to the California Floristic Province that exhibits large demographic variation. Some individuals within a single population, and sometime entire populations, do not flower. The objective of this study was to understand the role of root associated OMF communities in the phenological transition of plants from vegetative to flowering stage.

Questions and Hypotheses

- Do flowering and non-flowering individuals of *P. cooperi* host distinct mycorrhizal fungal communities within their roots?
- Do populations with distinct population dynamics host distinct mycorrhizal fungal communities?
- We hypothesized that OMF communities will be distinct between flowering and non-flowering individuals; and, flowering populations will also host distinct OMF communities in comparison to non-flowering populations. Further, root OMF assemblages will be distinct between small and large populations.

Materials and Methods

The model species in this study was a perennial orchid species, *P. cooperi*, which occurs in the coastal scrub habitat within the California Floristic Province, USA.



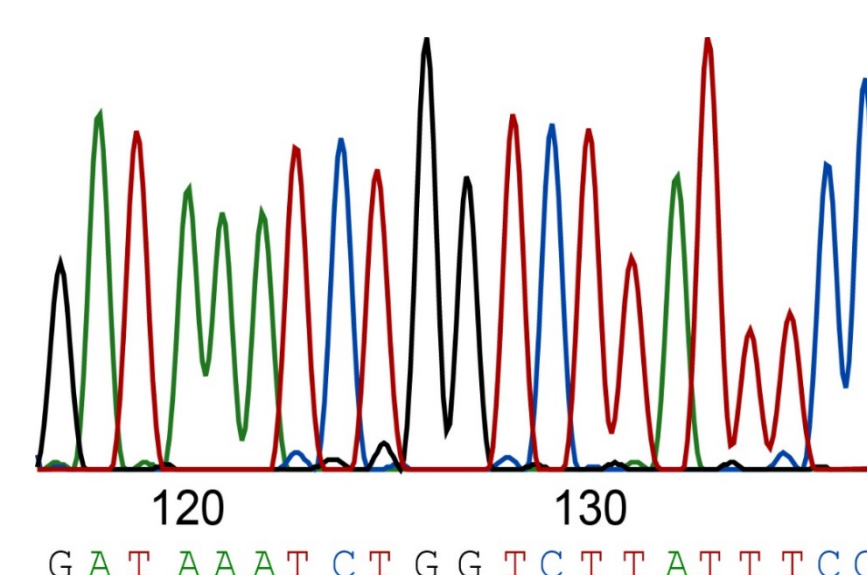
Sampling sites in California: two in peninsular Point Loma (PLF, PLE), San Diego County, and two on Santa Catalina Island (SCE, SCW), Los Angeles County.



Roots were collected from flowering and non-flowering plants from different populations of *P. cooperi* across four years.



The roots were surface sterilized, and DNA was isolated and barcoded with nuclear ribosomal ITS (nrITS) locus.



The amplified DNA was sequenced with Sanger sequencing, and analyzed with MEGA and R studio.

Results

- We generated 86 sequences from the roots of 56 individuals. Of these, 62 sequences were identified as belonging to Tulasnellaceae fungal family, 17 were identified as Ceratobasidiaceae and 7 were identified as Hymenochaetaceae. After clustering sequences with a 97% similarity threshold, fourteen operational taxonomic units (OTUs) were recovered, of which seven belonged to the Tulasnellaceae (Table 1), four belonged to the Ceratobasidiaceae (Table 1) and three belonged to the Hymenochaetaceae (Table 1).
- The permutational multivariate analysis of variance (PERMANOVA) test revealed significant differences in OMF communities in response to interactions of phenology, population, and sampling year ($p < 0.05$ for all).

Site	Phenology	Operational Taxonomic Unit (OTU)													
		Tulasnellaceae							Ceratobasidiaceae			Hymenochaetaceae			
		0	8	2	3	11	12	6	7	1	13	10	4	5	9
PLE	F				1 (1)										
	NF			11 (5)	2 (1)	1 (1)						3 (1)	1 (1)		
PLF	F	1 (1)			4 (4)			8 (7)	1 (1)						
	V				3 (3)			1 (1)							
SCE	NF				2 (1)			3 (2)	3 (2)						
	F		2 (1)					6 (5)							
SCW	V							3 (3)			1 (1)				
	NF			2 (1)				6 (4)							
	F							3 (2)							
	NF							3 (2)		9 (6)			4 (4)	2 (2)	

F = Flowering; NF = Non-flowering; V = vegetative at the time of collection.

Table 1. Distribution of Operational Taxonomic Units in flowering (F), and non-flowering (NF) individuals among four different populations of *P. cooperi*. Numbers enclosed inside parentheses represent the number of individual sampled plants.

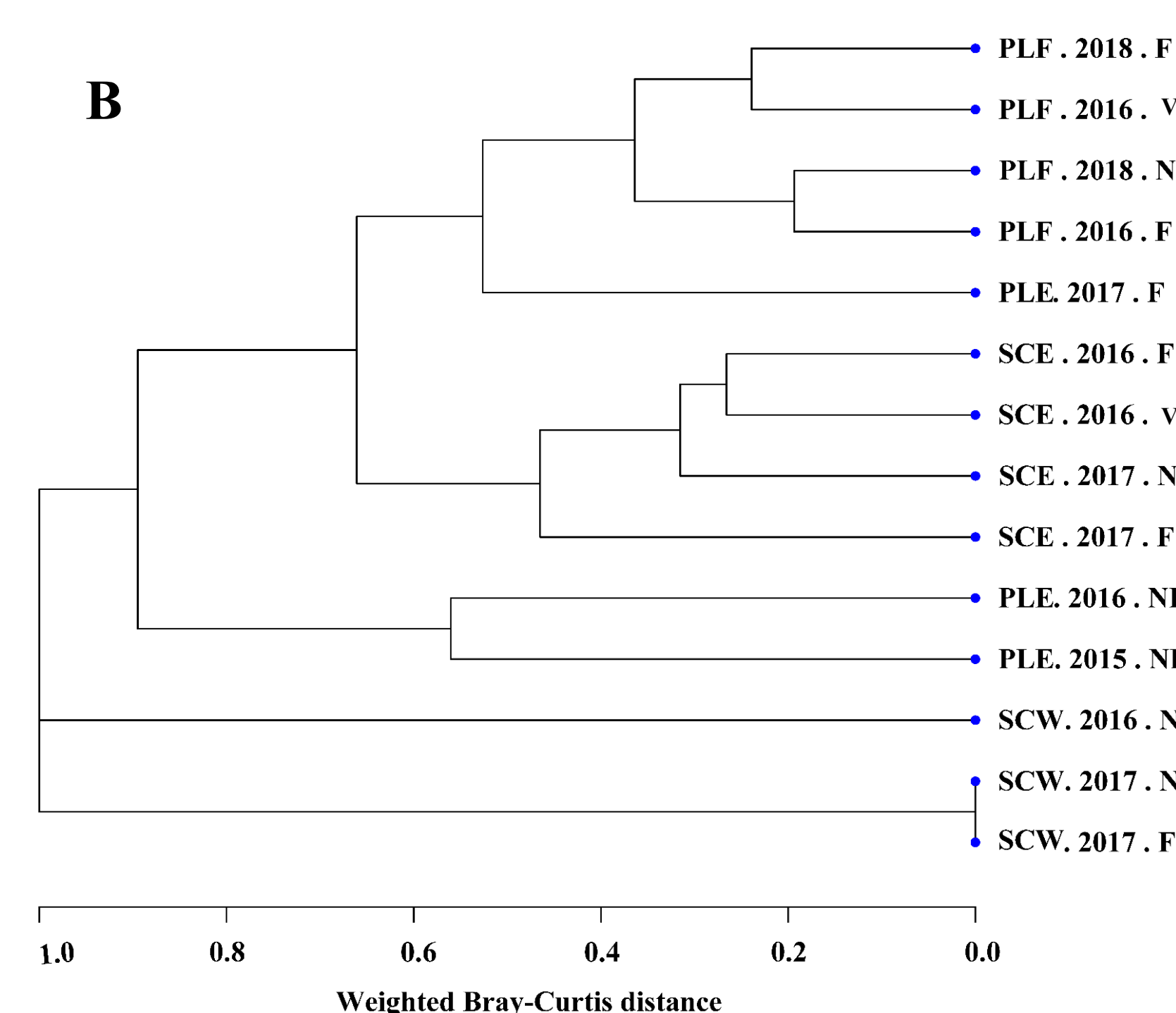
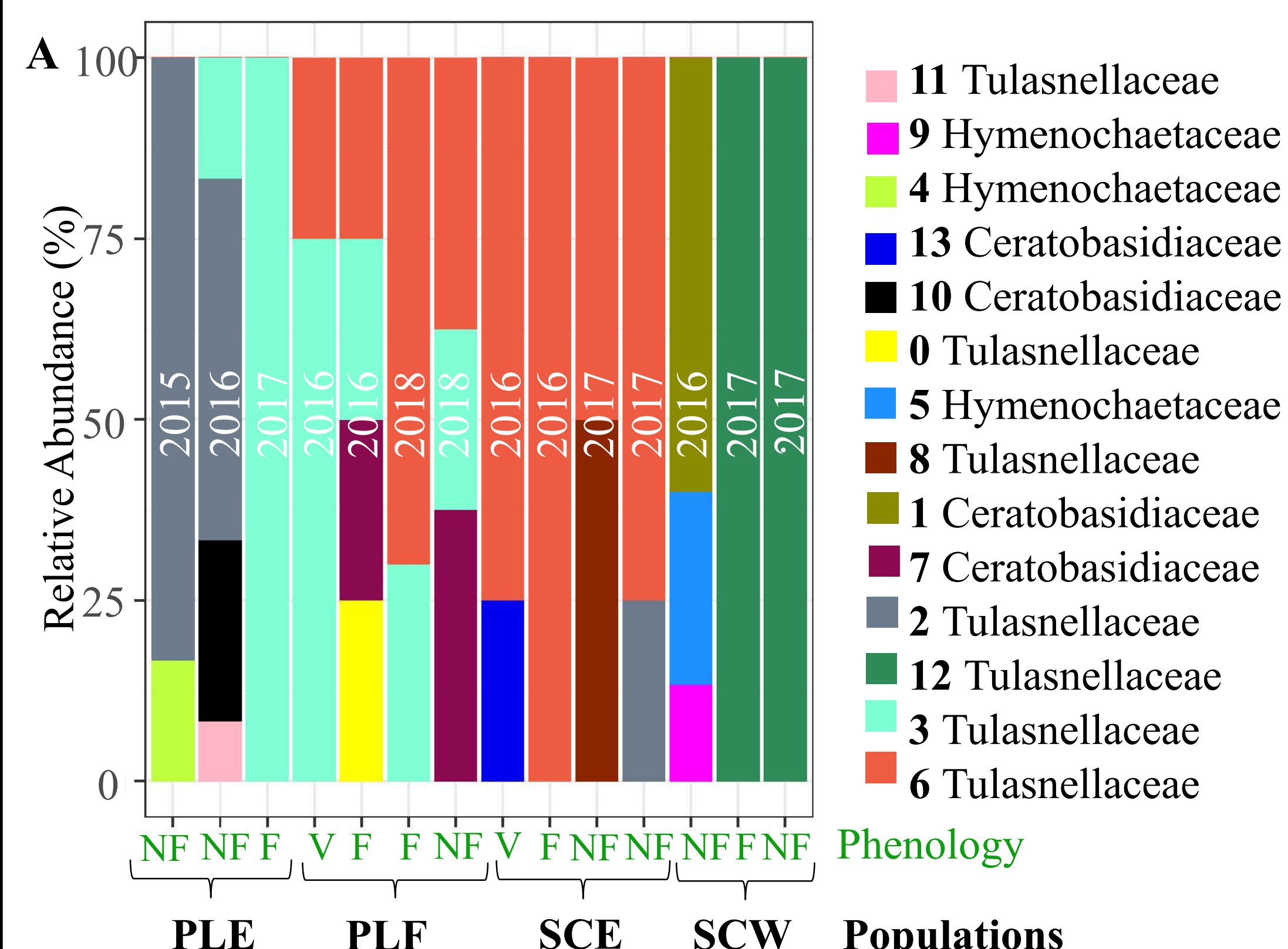


Figure 1. Distribution of Operational Taxonomic Units (OTUs). **A)** Relative abundance of Tulasnellaceae, Ceratobasidiaceae and Hymenochaetaceae OTUs identified from roots of flowering (F), and non-flowering (NF) individuals among four populations of *P. cooperi*. **B)** Hierarchical clustering of F and NF individuals sampled across four populations and four years.

- Differences observed in OMF communities between flowering and non-flowering individuals were represented by the interactions between sampled populations and years. The individuals hosted by non-flowering populations (SCW in 2016 and PLE in 2015 and 2016) showed high dissimilarity in OMF communities by assembling OTUs belonging to three fungal families.
- The large populations of *P. cooperi* (PLF and SCE) showed small differences in OMF communities hosted by flowering and non-flowering individuals in comparison to small populations (PLE and SCW) (Fig. 1 A and B).
- Further, the distribution of specific OTUs depended on the population demography. The large populations showed overlapping OMF communities, whereas small populations exhibited distinct OMF communities from large populations and from each other (Fig. 1 A and B).

Results

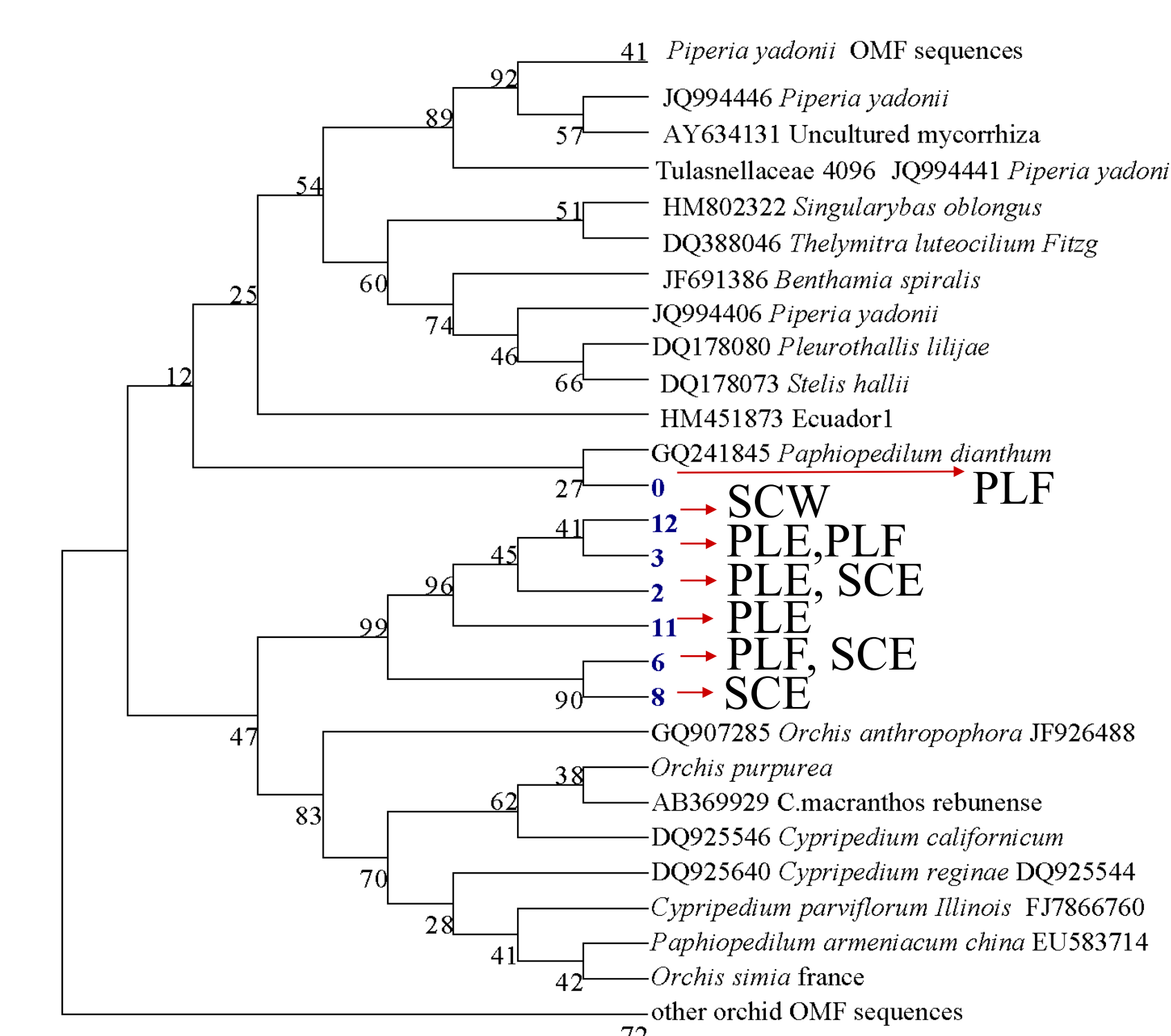


Figure 2. Maximum likelihood phylogram of Tulasnellaceae OTUs generated with general time reversible model and 1000 bootstrap replicates.

- The Tulasnellaceae OTUs recovered from *P. cooperi* roots clustered in a narrow clade showing high specificity of the orchid toward its mycorrhizal fungi. The OTUs identified exclusively from large populations clustered separately from other Tulasnellaceae OTUs.

Conclusions

- The differences in OMF communities in flowering and non-flowering individuals were dictated by the spatial and temporal demography of the host population.
- The distribution of OTUs in flowering and non-flowering individuals in larger populations of *P. cooperi* (PLF and SCE) is more uniform across years than in the smaller populations (PLE and SCW).
- Within Tulasnellaceae, flowering and non-flowering plants associated with phylogenetically similar OTUs.

Literature Cited

- Pandey M, Sharma J, Lee D, Yadon V. (2013). A narrowly endemic photosynthetic orchid is non-specific in its mycorrhizal associations. *Molecular Ecology*. [cited 2018 Mar 18; 22 (8): 2341 – 2354. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.12249> doi: 10.1111/mec.12249

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