

# Genetic distance not correlated with geographical distance: possible evidence for speciation in *Corallorhiza maculata*

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## Introduction

- *Corallorhiza maculata* is a parasitic plant that preys on fungi. It is non-photosynthetic and gathers nutrients by extracting carbon and nitrogen from the hyphae of mushrooms that enter its roots. Currently it is believed that there are six varieties of *C. maculata*, each of which associates with a different group of fungi.

- We are interested in the potential speciation of *C. maculata*.

- We made a phylogenetic tree by comparing the DNA sequence of the plant tissues taken from different populations found around the United States. These populations were in: Arizona, Colorado, Utah, New Mexico, and Wyoming.

- We chose specific regions of the DNA to compare. We amplified the internal transcribed spacer region and a chloroplast locus. Both of these regions were chosen because they are non-coding and highly variable.

- This project provides valuable insight into the evolutionary concepts of plants through association with their nutrient sources

## Methods

- Using the DNEasy extraction kit, we extracted DNA from fifteen tissue samples from the same species of Orchids, *C. maculata*, collected from different areas of the United States.

- We used PCR to amplify the internal transcribed spacer region in the DNA sequence using primers ITSIP with ITS4. We also amplified a chloroplast locus using the primers trnL with rpl32.

- We cleaned up my PCR reactions by removing unused PCR ingredients using ExoSAP. We then sent the DNA off to be sequenced by McLab in San Francisco California.

- Sequences were manually cleaned and aligned by eye using Bioedit software program.

- We built two maximum parsimony trees, one for each locus using the program MEGA 4.

## Results

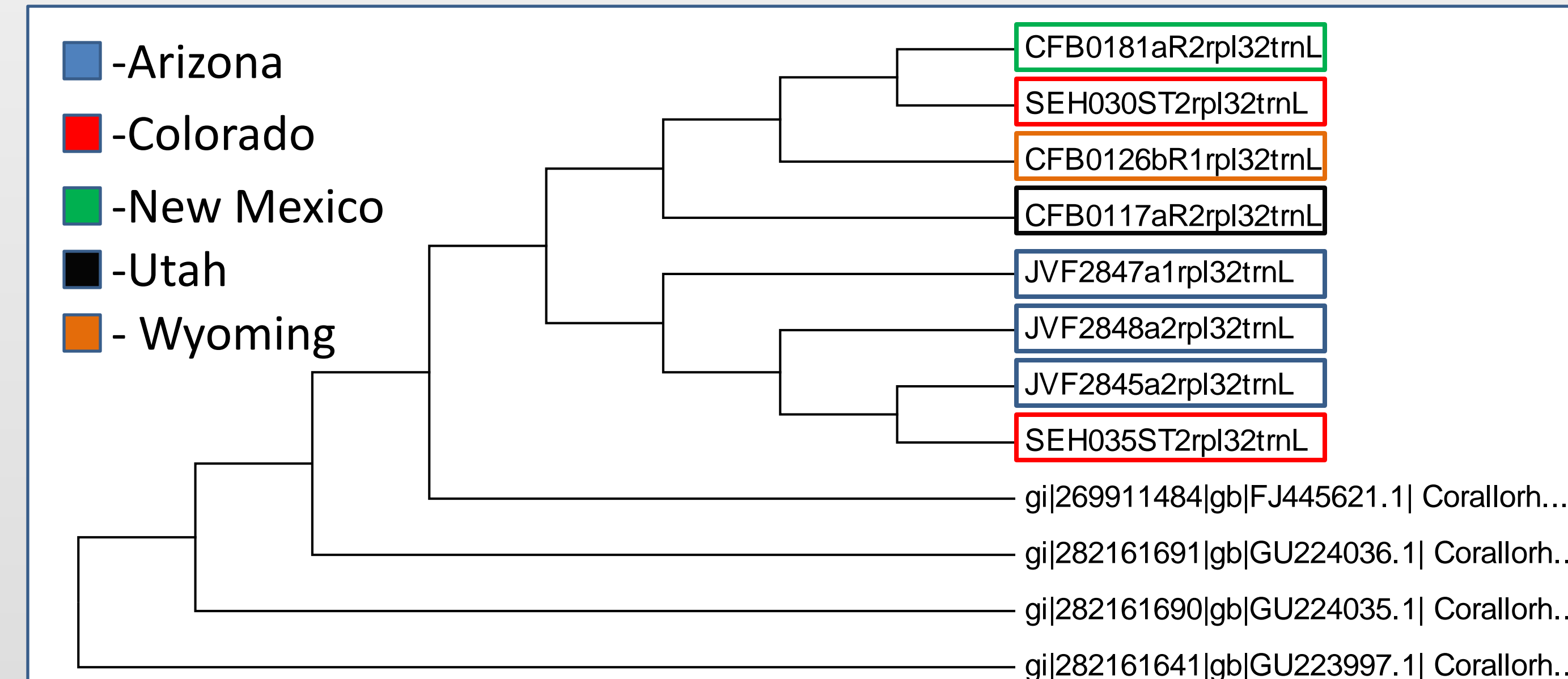


Figure 1. Maximum parsimony tree of chloroplast region

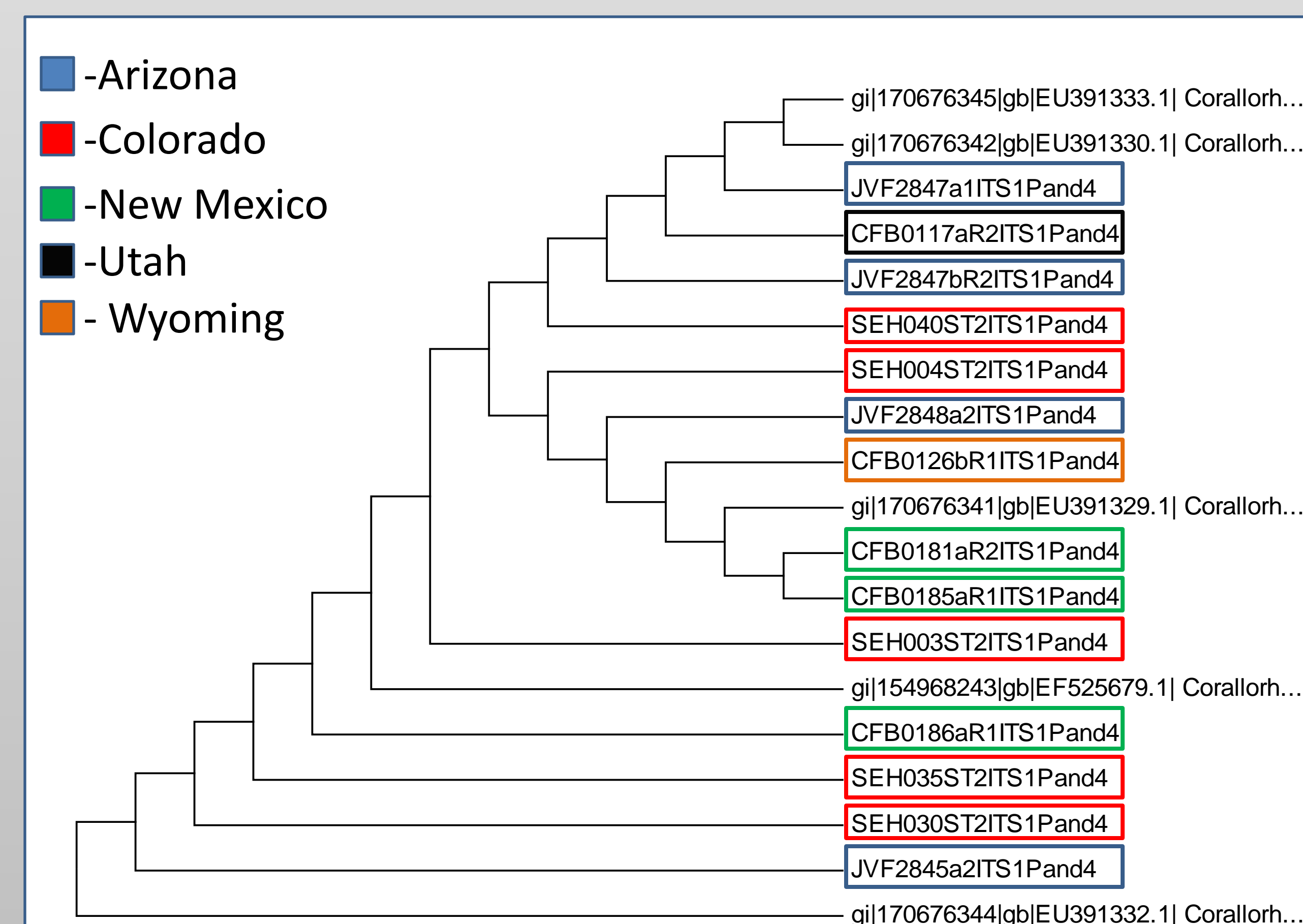


Figure 2. Maximum parsimony tree of internal transcribed spacer region

- By looking at the two phylogenetic trees, we were able to determine that several plants were genetically more related to plants in a different state, than to plants growing alongside them.

- In Figure 1. we saw a clade consisting of plants from Colorado and New Mexico, and another clade consisting of plants from Colorado and Arizona.

- In Figure 2. an individual from Arizona was closer in genetic makeup to another individual from Utah than to a different individual in Arizona



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## Conclusion

- After viewing our results, we believe that we are seeing some speciation in *C. maculata*.
- Many plants in a population are more closely related to others in a different state than their own population.
- This could be correlated to the specialization of the individuals to their fungal nutrient source.
- We could determine this by examining the fungal partners for individual plants in these locations.
- This project shows that there is significant structure within these populations which could imply that speciation is occurring.

