Using Morphology and Phylogeny to Distinguish between Three Species of *Eritrichium*

By Courtney-Jay Knowles. Mentor: Monte D. Garroutte. PI: Steffanie M. Ickert-Bond.

Introduction

Dr. David Murray began working on the treatment of *Eritrichium* for his Flora of North America In his data, there were little morphological differences between two of the *Eritrichium spp*., *E. chamissonis* and *E. aretioides.* Dr. Murray suggested that an effort was needed to be made to genetically distinguish between *Eritrichium* in Alaska. A colleague at the University of Salzburg, Austria, Dr. Andreas Tribsch, provided a genetic dataset comprising a number of chloroplast genes on several non-Alaskan *Eritrichium* species to Dr. Steffi Ickert-Bond for comparing to Alaska *Eritrichium*.

The objective of the project is to resolve the genetic differences between three species of Alaskan *Eritrichium*. Morphological differences were assessed. Phylogenetic differences based on sequence analysis for three chloroplast regions (trnS-trnG, ccmp3-trnR, and rps16 intron) were used to get the information needed to compare and contrast between species.

Materials and Methods

DNA extraction

Samples were extracted according to the manufacturer protocol, DNeasy Plant Mini Kit of the Qiagen Kit to isolate the DNA from silica preserved leaf tissue samples.

Polymerase Chain Reaction (PCR)

PCR amplification was done to select and amplify a specific sections of the chloroplast genome, gene1, gene2, and gene3. PCR conditions are provided in Table 1.

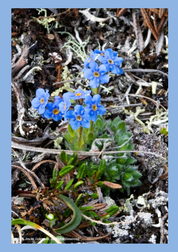
Sequence Analysis

Sequences were analyzed using CIPRES Science Gateway. Maximum Likelihood tree searches and ML bootstrapping were performed using RAxML ver. 7.2.7.

Table 2. Steps in PCR, gene used (forward and reverse), program name for each gene, times and temperatures of each step of PCR, and the number of cycles for each.

Steps	Rsp16 (Intron) PCR °C	Rsp16 PCR cycle min: secs	Ccmp3f PCR °C	Ccmp3f PCR min: secs	TrnSG PCR #1 °C	TrnSG PCR #1 min: secs	TrnSG PCR #2 °C	TrnSG PCR #2 mins: secs
Program Name	"Eri2"		"Eritrich"		"Eri3"		"TrnDT"	
Initial Denaturation	95˚C	2:30	95˚C	5:30	95˚C	5:00	94˚C	5:00
Cycle Denaturation	95˚Cp	0:30	95˚C	0:30	95˚C	0:45	94˚C	1:00
Primer Annealing	55˚C	1:00	51˚C	0:45	53˚C	1:00	54˚C	1:00
PCR Product Elongation	72˚C	1:30	72˚C	2:00	72˚C	1:00	72˚C	1:00
# of cycles	___	35	___	35	___	34	___	29
Final Elongation	72˚C	7:00	72˚C	7:00	72˚C	7:00	72˚C	10:00
Storage/Hold	4˚C	∞	4˚C	∞	4˚C	∞	4˚C	∞

E. splendens



E. aretioides

E. chamissonis

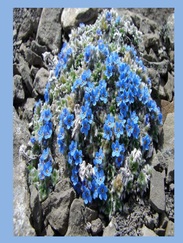
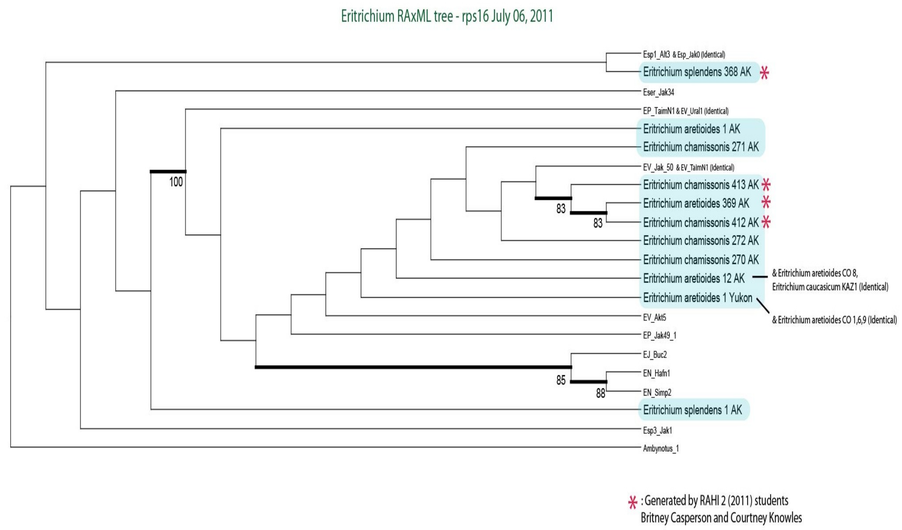


Figure 1. Phylogenetic placement of *E. splendens, E. aretioides and E. chamissonis* (*) in relation to other Alaskan species (highlighted) of *Eritrichium* as well as Russian samples.



Results

DNA from eight different species from Kodiak National Wildlife Refuge and Izembek National Wildlife Refuge were successfully extracted. The rps16 intron was successfully amplified for four specimens, including two species of *E. chamissonis* from Izembek National Wildlife Refuge (extraction #'s 412, 413), one *E. splendens* (extraction # 368) from Donnelley Dome and one *E. aretioides* (extraction #369) from Eagles Summit, Alaska.

Based on the maximum likelihood analysis of the rps16 sequence data of the three different *Eritrichium spp*. tree was produced (Figure 1 above). Overall, the phylogeny shows two main groups of species: A large clade containing the majority of species is highly supported as monophyletic (MLBS = 100%). Relationships within this clade are mostly unresolved, while two samples of *E. chamissonis* (extractions 412, 413) and *E. aretioides* (extraction 369) based on maximum MLSB values (82%). Interestingly, another species common in Alaska, *E. splendens* is shown to be distinct from this large clade containing *E. chamissonis* and *E. aretioides (s*ee Figure 1 above for more information).

Discussion and Conclusion

This is the first report of results from phylogenetic analysis of the rps16 intron data in *Eritrichium*. This marker has previously been shown to be fast evolving, allowing scientists to resolve closely related species (Oxelman et al., 1997). Although samples from Europe and Western Asia were included, our study focused on the specimens in Alaska.

A first attempt at resolving phylogenetic relationships in this genus shows some limitations in the data used. Overall resolution and tree structure is lacking. Nevertheless, the data strongly supports (MLBS = 100%) a single origin of the *E. chamissonis*/*E. aretioides* lineage that includes additional taxa from Western Asia and Europe (*E. villosum* and *E. nanum*). Interestingly the North American *E. splendens* appears to be the result of a different introduction into North America (Fig. 1) distinct from the larger highly supported clade including *E. chamissonis* and *E. aretioides.*

This study also suggested an Old World origin for *Cerastium alpinum* with at least two migrations to North America, similar to our preliminary findings in *Eritrichium*.

More widespread sampling throughout Alaska and adding even faster evolving genes or perhaps microsatellites will allow us to fully understand phylogenetic relationships within Alaskan *Eritrichium*.

Literature Cited

Oxelmann B., Liden M., and D. Berglund. 1997. Chloroplast rps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206:257-271.

Acknowledgements

PI: Steffi Ickert Bond. Mentor: Monte Garroutte. Lab Partner: Brittany Caspersen. RAHI Staff: Denise Wartes, Renee Pike, Greg Owens, TCs, Dr. Sue Hills, Dr. Cathy Catranis, Dr. Adrienne Orr. Funded and supported by the University of Alaska Fairbanks, Alaska BioPREP, Alaska INBRE and Rural Alaska Honors Institute, UAF.