

# Differentiation of *Claytonia scammaniana* from Other Alaskan species of *Claytonia*

By: Robert J. Doering, Stephany Jeffers, and Stefanie Ickert-Bond, Museum of the North, University of Alaska Fairbanks, Fairbanks, AK

## Introduction

*Claytonia scammaniana* (Spring Beauty, Montiaceae) is a perennial, naturally growing in Alaska. Botanists in Alaska have long been interested in this species to better understand the differences between the four closely related species of *Claytonia* in Alaska. Robin O'Quinn and Larry Hufford (2005) published a study regarding the Montiaceae, subfamily Montieae including *Claytonia* and *Montia*. They used molecular and phylogenetic methods to determine how related the genera of Montieae, and the species within each genus are. The same molecular and phylogenetic methods they used were embodied in the research in this project. The O'Quinn & Hufford study (2005) classified the ~40 species of *Claytonia* into three sections (sect. *Claytonia*, sect. *Limnia*, and sect. *Rhizomatosae*). The Beringian sect. *Rhizomatosae* was of particular interest to our study, as it contains most of the Alaskan species. In this study we focused on differentiating *C. scammaniana* from other closely related *Claytonia* species using phylogenetic and molecular methods.

## Materials and Methods

- Samples used were *C. scammaniana*, *C. sarmentosa*, *C. eschscholtzii*, and *C. porsildii*.
- DNA extraction was done for each sample using a DNeasy kit (Qiagen, Valencia, CA)
- PCR amplification was done for genes matK (including the trnK intron), trnD, trnSG, rps16, and the ribosomal ITS region
- PCR amplicons were purified, using a PCR Purification kit including the microfuge and vacuum® protocol (Qiagen, Valencia, CA)
- Purified PCR products were sequenced using API 3130XL Genetic Analyzer (Foster City, CA) located in the Institute of Arctic Biology DNA Core Lab, UAF, Fairbanks, AK.
- Sequenced DNA was examined using Cyprus software.
- Phylogenetic analyses used Maximum Parsimony (MP) and a model-based approach using Maximum Likelihood (ML) available on the CIPRES Science Gateway

## Results

In the phylogenetic analysis, the overall length of the aligned matrix that used chloroplast trnK/matK, and the nuclear ITS, and 5.8s region was 2037 bp. Three samples yielded good sequence data for both the chloroplast trnK intron, the matK gene and the nuclear ribosomal ITS and 5.8s region. MP analysis of the combined matrix produced 616 MP trees with a tree length of 810 steps. Maximum parsimony clade support (MP BS) is shown above the branches (Figure 1). The model of sequence evolution used under ML was determined to be a General Time Reversible (GTR) + Gamma model and the final ML optimization Likelihood was -8207.730650. Maximum Likelihood bootstrap support values (ML BS) from rapid bootstrapping are shown above branches in Figure 2.

- *Montia* and *Claytonia* = highly supported monophyletic sister genera (ML BS= 100 %):
  - Within *Claytonia* three sections are supported (Figure 1)
    - 1) *Limnia* highly supported (ML BS = 100%) as monophyletic;
    - 2) *Claytonia* highly supported as monophyletic (ML BS = 98%);
    - 3) *Rhizomatosae* supported as a monophyletic in (ML BS = 78%)
  - The phylogram (Figure 2) of the combined matrix shows:
    - 1) *Limnia* has accumulated the most nucleotide differences per site
    - 2) *Claytonia* and *Rhizomatosae* sit on comparatively shorter branches
    - 3) *Rhizomatosae*; *C. arenicola* and *C. nevadensis* are very distinct vs the clade of *C. cordifolia*, *C. noatakensis*, *C. sarmentosa*, *C. joanneana*, and *C. artica*
    - 4) Two UAF accessions of *C. sarmentosa* have identical sequences

## Discussion and Conclusion

Analyses of all molecular markers indicate *Claytonia* and *Montia* each as a well-supported monophyletic group. (Figs. 1-2). Phylogenetic relationships in *Claytonia* were first hypothesized by O'Quinn and Hufford based on molecular sequencing data in 2005. They established a revised classification system for *Claytonia* based on monophyletic clades. Each of these three sections are further supported in our study by high bootstrap support values (Figs. 1-2). Within sect. *Rhizomatosae* relationships are largely unresolved. Interestingly, we find the putative new species, *Claytonia noatakensis* to be tentatively supported as a sister to *Claytonia sarmentosa* (Fig. 1). Regarding *C. scammaniana*, the results are inconclusive. Only one sample was used, and the bootstrap value showed little support in distinguishing *C. scammaniana* from the other *Claytonia* species. Further, more thorough research is required. Additional molecular marker and samples are needed to fully resolve relationships within *Claytonia*, sect. *Rhizomatosae*. Candidate genes such as the chloroplast rps16 intron (Oxelman et al., 1997) and the intergenic spacer (IGS) of trnSG (Hamilton 1999) have been successfully used in studies of closely related species and are currently being tested in the Ickert-Bond lab for their suitability to resolve relationships within sect. *Rhizomatosae*.

## Literature Cited

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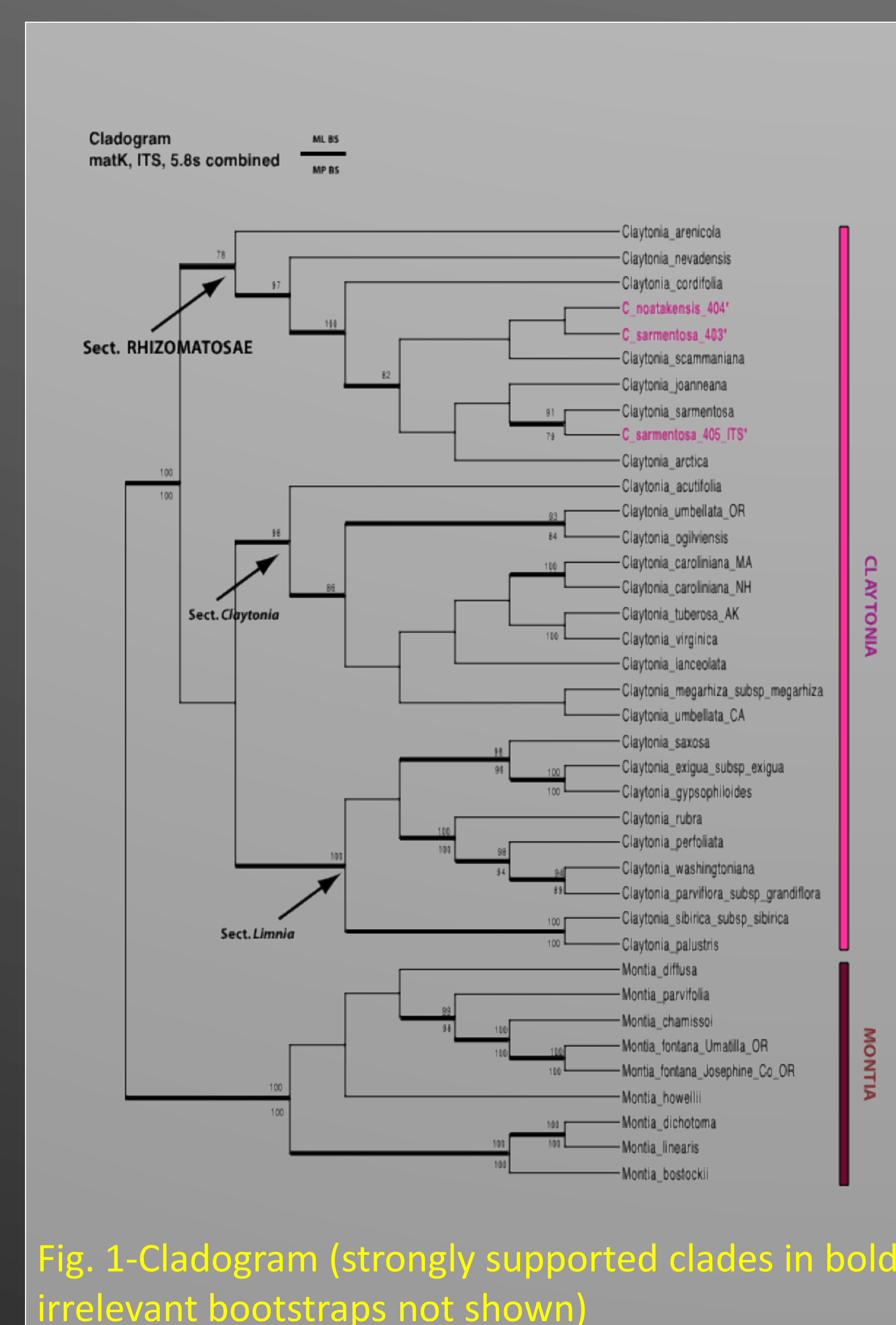


Fig. 1-Cladogram (strongly supported clades in bold, irrelevant bootstraps not shown)



Fig. 2-Phylogram (strongly supported clades in bold, irrelevant bootstraps not shown)

