

# Claytonia: Species Divergence of *C. scammaniana*, *C. sarmentosa*, and *C. arctica* sensu Porsild in Alaska

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## Introduction

*Claytonia*, commonly known as Spring Beauty, is a flower in the Portulacaceae family found in the alpine tundra of Alaska that is used as a subsistence food by the northern Alaska Natives. The primary objective of this project was to resolve the phylogenetic delineation between species of the genus *Claytonia*. A second objective was to be able to differentiate phylogenetically unique species in the Rhizomatosa core section. The goal of this study is to compare relationships among a subset of morphologically similar species throughout the state. Recently, Robin O'Quinn and Larry Hufford (2005) have studied *C. arctica*, *C. sarmentosa*, and *C. scammaniana* but their phylogenetic analysis produced an unresolved polytomy. This study also groups species in a phylogenetic tree using the ribosomal genes, specifically the ITS regions and the 5.8s coding region, as well as the chloroplast markers matK/trnK

## Materials and Methods

- Morphology was analyzed visually
- DNA was extracted using a Qiagen DNeasy extraction kit
- Polymerase Chain Reaction (PCR) amplified genes *matK*, *rps16*, and *trnSG* with the respective primers *matK*, *trnK*, *rps 16-f*, *rps 16-r*, *trnG*, and *trnS*.
- The PCR product was visualized under an ultraviolet light after gel electrophoresis and staining with ethidium bromide
- Phylogenetic trees were constructed using Maximum Parsimony and Maximum Likelihood RAxML ver. 7.2.7 (Stamatakis et al. 2008; Pfeiffer and Stamatakis 2010).

## Results

### Morphological Data-

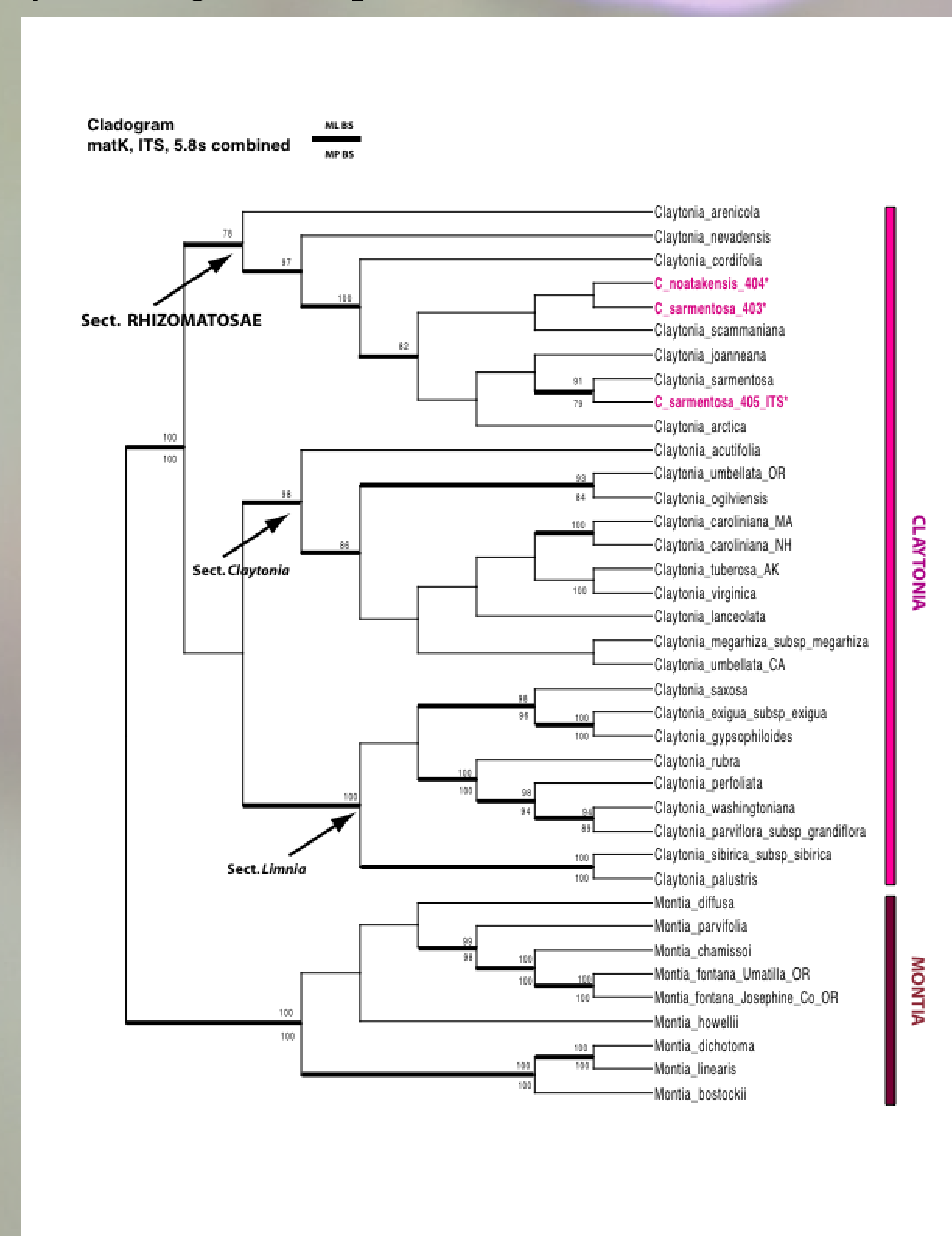
Three species of *Claytonia*, section Rhizomatosa (*C. arctica* sensu Porsild, *C. sarmentosa*, and *C. scammaniana*) can be distinguished based on a unique combination of characters (table 1).

**Table 1.** Distinguishing morphological characters of selected herbarium collections in *Claytonia* sect. Rhizomatosa

Species	I.D. Number	Basal Leaf Characteristics	Flower Characteristics
<i>C. arctica</i> sensu Porsild	V143814	Long petiole with linear leaf tips	Can have more than one flower per stem
<i>C. sarmentosa</i>	V142100	Short or lacking petiole with spatulate leaf tips	Can have more than one flower per stem
<i>C. scammaniana</i>	V86444	Short or lacking petiole with linear leaf tips	Strictly one-flowered

### Phylogenetic Analysis-

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. Maximum Parsimony (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 branches (Figure 1). The model of sequence evolution used under Maximum Likelihood (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 1.



**Figure 1.** Phylogenetic tree of *Claytonia* based on molecular sequences of nr ITS, 5.8s and cp *matK/trnK* showing well supported sect. Rhizomatosa, sect. Claytonia and sect. Limnia

- **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) **Rhizomatosa supported as a monophyletic in (ML BS = 78%)**

## Discussion

Evaluation of all molecular markers signify *Claytonia* and *Montia* each as a well-supported monophyletic group (Figure 1). O'Quinn and Hufford first hypothesized phylogenetic relationships in *Claytonia* based on molecular sequencing data in 2005. They established a revised classification system for *Claytonia* based on monophyletic clades. Each of the three sections revised by O'Quinn and Hufford were further supported in this study by high bootstrap support values (Figure 1). Within sect. Rhizomatosa relationships are still greatly unresolved. However, this study did find the putative new species *C. noatakensis* to be tentatively supported as sister to *C. sarmentosa* (Figure 1). Further molecular marker and samples are needed to resolve relationships within *Claytonia*, sect. Rhizomatosa entirely. Candidate genes such as the chloroplast *rps16* intron (Oxelmann et al., 1997) and the intergenic spacer (IGS) of *trnSG* (Hamilton 1999) have been used successfully in studies of closely related species and are currently being tested in the Ickert-Bond lab for their suitability to resolve relationships within sect. Rhizomatosa.

## Literature Cited

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