

PHYLOGENY, BIOGEOGRAPHY AND EVOLUTION OF PERENNATION STRUCTURES
IN MONTIEAE (PORTULACACEAE)

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of ROBIN LEA O'QUINN find it satisfactory and recommend that it be accepted.

Chair

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PHYLOGENY, BIOGEOGRAPHY AND EVOLUTION OF PERENNATION
STRUCTURES IN MONTIEAE (PORTULACACEAE)

Abstract

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Phylogeny reconstructions based on analyses of DNA sequence data from nuclear ribosomal and chloroplast markers were used to revise the taxonomy of Montieae (Portulacaceae), infer biogeographic diversification of perennial clades in *Claytonia*, and test hypotheses of morphological homology. Phylogenetic results resolved two major clades that correspond to *Claytonia* and *Montia* as previously circumscribed. In each genus, we recognize three subclades as sections. In *Claytonia*, section *Limnia*, all annuals except for the perennials *C. sibirica* and *C. palustris*; section *Rhizomatosae*, high elevation or high latitude perennials with rhizomatous or caudicose growth habits, except for the annual *C. arenicola* and section *Claytonia*, species with spheric or obconic underground perennation structures. In *Montia* pollen characteristics provide morphological synapomorphies for sections *Montiastrum* and *Montia*. Geographical distribution distinguishes *Australiensis*, except for *M. howellii*. Biogeographical analyses suggest a western North American origin for Montieae. Perennial clades of *Claytonia* have largely congruent distributions but different biogeographical histories. Biogeographical analyses reconstruct a single vicariance event in section *Rhizomatosae* that separates a western North American grade from a high latitude clade, which is consistent with hypotheses of Miocene cordilleran migration

followed by isolation and speciation in high latitude refugia during the Pleistocene. Lack of phylogenetic resolution in *Claytonia* sect. *Claytonia* limits biogeographical inferences for this clade; however, we infer a widespread North American distribution prior to the Pleistocene and subsequent multiple vicariance events between Beringia and North America. Perennation structures in *Claytonia* sect. *Claytonia* differ primarily in whether the primary taproot is incorporated. *Claytonia megarhiza* perennation structures are predominantly root, but include also shoot; whereas, those of *C. lanceolata* and *C. tuberosa* are exclusively shoot, and *C. virginica* and *C. umbellata* are predominantly shoot but often retain a portion of primary taproot. Perennation structures among *Claytonia* sect. *Claytonia* are strictly not structural homologues because of these variations in composition. In the *C. sibirica* species complex there are three morphologically and ecologically distinct taxa, one morphotype exhibits leaf modifications that result in bulb formation and this habit differs from other members of the complex. We hypothesize that this modification has resulted from selection for serpentine endemism.

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DEDICATION

For my beautiful sister and friend, Becky Lynne O'Quinn

INTRODUCTION

Understanding evolutionary patterns of diversification requires a clear understanding of evolutionary relationships. In the following studies, I have used the tribe Montieae (Portulacaceae), a small group of herbaceous annuals and perennials of varied habitats with a diverse distribution and a tremendous degree of vegetative variation, to explore aspects of biogeographical and morphological diversity within a phylogenetic context.

Taxonomic treatments have varied widely for Montieae and a consensus on generic circumscription is lacking. Family level phylogenetic analyses that have included members of Montieae have not addressed relationships within the tribe, however they have consistently shown Montieae to be monophyletic (Carolin 1987; Hershkovitz 1993; Hershkovitz and Zimmer 1997, 2000; Applequist and Wallace 2001). Previous taxonomic treatments have followed one of three approaches to generic circumscription, they have: 1) broadly circumscribed *Claytonia* and recognized a monotypic *Montia*, represented by *M. fontana*, 2) narrowed the circumscription of *Claytonia* and expanded *Montia* and, more rarely, 3) recognized a narrow *Claytonia*, a monotypic *Montia* and several segregate genera. More recent treatments have followed the second approach. Under this generic circumscription, *Claytonia* form a basal rosette of foliage leaves and have a single pair of opposite leaves on their aerial axes. In contrast, *Montia* do not form a basal rosette and have multiple leaves on their aerial axes. In *Claytonia*, sectional delimitations are based on life form and emphasize types of perennation structures. I use a molecular, cladistic approach to identify relationships among monophyletic clades and sister species in Montieae with the goal of testing previous hypotheses for generic and sectional circumscriptions and revising the taxonomy to better reflect our understanding of evolution.

Montieae has its greatest species richness in western North America, but extends also into eastern North America, northeastern Asia, South and Central America and Australasia. *Montia*, has a worldwide distribution, however, much of that distribution is continentally marginal and consists of the single taxon *M. fontana*, whereas most of the species diversity is in western North America, South and Central America and Australasia. In contrast, *Claytonia*, has an exclusively Northern Hemisphere distribution, extending only as far south as the Guatemalan highlands and as far northeast as $\sim 90^\circ$ east longitude. Previous phylogenetic hypotheses have nested Montieae in an Americas/ Australasian clade (Carolin 1987; Hershkovitz 1993; Hershkovitz and Zimmer 1997, 2000; Applequist and Wallace 2001). Given the distribution of *Montia*, relative to *Claytonia*, it is reasonable to hypothesize a southern hemisphere origin for Montieae, however, Applequist and Wallace (2001) reconstructed a western North American origin for Montieae. Regarding the origin and distribution of *Claytonia*, Swanson (1966) hypothesized a northern origin. However, in none of the preceding analyses was taxon sampling in Montieae sufficient to answer either the origin of the tribe or the directions of geographic radiations.

Taxon distribution data together with robust phylogenetic hypotheses provide a powerful tool for investigating the biogeographic diversification of perennial clades in *Claytonia*, in which two well-supported perennial clades have trans-Beringian distributions. This pattern of distribution, with taxon development at both high and low latitudes, allows us to test proposed hypotheses for the origin and diversification of the high latitude Beringian flora. For example, early biogeographer's suggested that alpine cordillera served as migration routes from southern floras in western North America and northeastern Asia. More recently, Hultén (1937) hypothesized that large tracks of unglaciated area could have served as high latitude refugia.

Murray (1981, 1995) expanded that hypothesis by suggesting that migration routes between Beringia and Asia were open for longer intervals during the Pleistocene, potentially biasing the composition of the Beringian Flora. Murray (1981,1995) also hypothesized that colonizations to and from Beringia from coastal refugia during interglacials, as well as glacial and post-glacial *in situ* evolution may have contributed to the origin and evolution of the Beringian flora. Using phylogenetic reconstructions derived from analyses of molecular data in combination with model-based methods for ancestral areas reconstruction I infer the ancestral area for Montieae and the biogeographic diversification of perennial clades in *Claytonia*.

Montieae exhibits a tremendous degree of morphological variation, especially with respect to specializations for perennation and vegetative reproduction. Within a phylogenetic framework we can detect patterns of morphological variation in Montieae and identify morphologically diverse clades, which pose special problems for homology assessment. Accurate homology assessment is an essential step in evolutionary studies because it is the process for identifying the evolutionary transformations that lead to morphological diversification.

Phylogeny reconstructions indicate that *Claytonia* sect. *Claytonia* is morphologically more diverse than has been previously hypothesized and includes taxa with both globose and elongate underground perennation structures. Previous interpretations of the underground structures in sect. *Claytonia* have varied widely, and perennation structures have been identified as both shoots and roots. A closer inspection of the diverse perennation forms found in sect. *Claytonia* reveals a grade from the strongly globose structures of *C. virginica* and *C. lanceolata* to the more obconic forms of *C. megarhiza* and *C. acutifolia*. I address the structural identity of

perennation structures using the distinction between roots and shoots to provide explicit criteria for characterizing homologies among perennation structures.

The *Claytonia sibirica* species complex (Miller et al 1984) exhibits shoot morphological variation among its three members. In this group, one of the three morphotypes has a unique leaf modification, which has been described as bulbiferous and is presumed to be involved in perennation. *Claytonia sibirica* var. *sibirica* is a common understory taxon of coastal and mesic forests that ranges from northern Santa Cruz county, California, to the Commander islands at the far western tip of the Aleutian chain. It occurs also in disjunct populations in the inland northwest. *Claytonia sibirica* var. *bulbillifera* is geographically localized to the Klamath Region of southern Oregon and Northern California. This variety associates frequently with serpentine substrates, and tolerates drier, sunnier habitats than var. *sibirica*. The sister taxon to the *sibirica* varieties, *Claytonia palustris*, is uncommon and narrowly endemic to two small montane regions at either end of the Sierra Nevada, as well as a small population in Siskiyou county, California. *C. palustris* is unique in preferring persistently wet, sunny habitats and in being strongly stoloniferous. All three taxa differ significantly, both ecologically and morphologically, but the occurrence of a bulbiferous form is particularly interesting because it is the only perennial taxa in this species complex to exhibit this modification. Bulbs are associated with perennation, because they provide a source of stored nutrients for renewed growth, when access to soil nutrients is limiting, or where nutrient mobility is compromised. In this study I address the morphological identity of structures described as bulbs and bulbiferous and hypothesize the origins of these specializations. I present a particular bias towards understanding the unique morphological specializations in *Claytonia sibirica* var. *bulbillifera*, however I arrive at my inferences by

comparatively examining *C. sibirica* var. *sibirica*, whose shoot systems are the least specialized, and *C. palustris*, which is the sister taxon to the *C. sibirica* varieties.

Literature Cited

- APPLEQUIST, W. and R. S. WALLACE. 2001. Phylogeny of the Portulacaceous cohort based on *ndhF* sequence data. *Systematic Botany* 26: 406-419.
- CAROLIN, R. 1987. A review of the family Portulacaceae. *Australian Journal of Botany* 35: 383-412.
- HERSHKOVITZ, M. A. 1993. Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on phylogeny of the portulacaceous alliance. *Annals of the Missouri Botanical Garden* 80: 333-365.
- and E. A. ZIMMER. 1997. On the evolutionary origin of the cacti. *Taxon* 46: 217-232.
- and —. 2000. Ribosomal DNA evidence and disjunctions of western American Portulacaceae. *Molecular Phylogenetics and Evolution* 15: 419-439.
- HULTÉN, E. 1937. *Outline of the history of arctic and boreal biota during the Quarternary period*. Stockholm: Bokforlags Aktiebolaget Thule.
- MILLER, J. M., K. L. CHAMBERS and C. E. FELLOWS. 1984. Cytogeographic patterns and relationships in the *Claytonia sibirica* complex (Portulacaceae). *Systematic Botany* 9:266-271.
- MURRAY, D. F. 1981. The role of arctic refugia in the evolution of the arctic vascular flora--a Beringian perspective. Pp. 11-20 in *Evolution today*, Proceedings of the Second International Congress of Systematics and Evolutionary Biology. eds. G. G. E. Scudder and J. L. Reveal. Pittsburgh: Hunt Institute for Botanical Documentation.

- . 1995. Causes of arctic plant diversity: origin and evolution. Pp. 21-43 in *Arctic and alpine biodiversity: patterns, causes and ecosystems consequences*, eds. F. S. I. Chapin and C. Körner. Berlin: Springer-Verlag.
- SWANSON, J. R. 1966. A synopsis of relationships in Montioideae (Portulacaceae). *Brittonia* 18: 229-240.

ATTRIBUTION

Each of the chapters included as a part of this dissertation are presented in the format that they have been, or will be, submitted to peer reviewed journals and therefore include as co-authors the people who have made a significant contribution to the work. In all cases I performed all of the laboratory work and data analyses and assumed the role of first author in writing the text of each manuscript. This work would not have been possible without the guidance and assistance of Larry Hufford. Larry has mentored me in the development of research ideas prior to and throughout the course of this work and has provided expertise in the areas of phylogenetics and plant morphology.

CHAPTER ONE

Molecular Systematics of Montieae (Portulacaceae): Implications for Taxonomy, Biogeography and Ecology

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ABSTRACT. DNA sequence data from nuclear ribosomal internal transcribed spacer, including the 5.8s coding region, and plastid *trnK/matK* were used to reconstruct the phylogeny of Montieae (Portulacaceae), and a revised classification for the group based on monophyly is proposed. Montieae consists of the sister clades *Claytonia* and *Montia*. In *Claytonia*, there is strong support for the following clades: section *Limnia*, all annuals except the perennials *C. sibirica* and *C. palustris*; section *Rhizomatosae*, high elevation or high latitude perennials with rhizomatous or caudicose growth habits; and section *Claytonia*, species with spheric or obconic underground perennation structures. All analyses recover clades of *Montia* circumscribed as sections *Montiastrum*, *Australiensis*, and *Montia*. Heenan's Australasian *Neopaxia* is placed robustly in *Montia* section *Australiensis*, a clade limited primarily to Australia and New Zealand. DIVA and MacClade reconstruct the ancestral area for Montieae as western North America. Beringian taxa of *Claytonia* section *Rhizomatosae* are derived from low latitude western North American ancestors adapted to persistently wet, alpine habitats. Although we infer that section *Claytonia* had multiple shifts to northern high latitudes and potentially to eastern North America from western North America, relationships among clades in this section have limited support. Habitat reconstructions show that the plesiomorphic condition for moisture regime in section *Claytonia* is largely maintained over shifts in elevation/latitude.

The portulacaceous tribe Montieae has been circumscribed on the basis of shared floral characteristics (Gray 1887; Holm 1905, 1913; McNeill 1974, 1975) that include a disepalous calyx, pentamerous corolla, and a three or six ovulate, tricarpellate gynoecium, but none of these states are synapomorphic. Phylogenetic studies (Carolin 1987; Hershkovitz 1993; Hershkovitz and Zimmer 1997, 2000; Applequist and Wallace 2001) provide support for a monophyletic Montieae that shares these floral states. Within Montieae there has been considerable uncertainty about taxonomic circumscriptions and relationships, which have been hypothesized largely on the basis of vegetative morphology, including growth habit, perennation specializations (Gray 1887; Greene 1891; Swanson 1966), and palynology (Nilsson 1967). However, the homologies of many vegetative characters, especially specializations for perennation, remain unclear (Sharsmith 1938; Chambers 1963) and this has diminished the robustness of some evolutionary hypotheses.

Generic circumscriptions in Montieae have varied (Table 1). All Montieae have been circumscribed as *Claytonia* by a few workers (Davis 1951, 1966; Boivin 1967), but most treatments have recognized the two genera, *Montia* and *Claytonia* (Bentham and Hooker 1862; Gray 1887; Pax 1889; Greene 1891; Howell 1893; Gray and Robinson 1897; Holm 1905, 1913; von Poellnitz 1932; Pax and Hoffman 1934; Swanson 1966; McNeill 1974, 1975; Chambers 1993a, b; Miller 2003). *Claytonia* has been commonly circumscribed to include those species that have a single pair of opposite, cauline leaves (Greene 1891; Swanson 1966; McNeill 1975; Chambers 1993a; Miller 2003), and *Montia* as those species that have more than one pair of cauline leaves. Some workers have recognized additional genera. Rydberg (1906, 1917, 1932) circumscribed *Crunocallis*, *Limnalsine*, *Limnia*, *Montiastrum*, and *Naiocrene*. Nilsson (1966a,

b, 1967, 1970, 1971a, b) recognized all of Rydberg's genera except *Limnia*, and described the new segregates *Maxia*, *Mona*, and *Neopaxia*. Yurtsev (1972) recognized the perennial members of Nilsson's (1971a) *Montiastrum* as *Claytoniella*. Although most workers have not applied these segregate genera, Heenan's (1999) revision of Australasian Montieae recognized and included several new species of *Neopaxia*. Significantly, with the exception of McNeill's (1975) phenetic analyses, few robust hypotheses have been available for determining the composition of clades or sister species relationships within the tribe and no cladistic methods have been applied to taxonomic problems in Montieae. Our objective is to use molecular phylogenetics to hypothesize relationships in Montieae and to propose a revised taxonomy that reflects monophyletic groups.

We also use our phylogenetic results to infer an ancestral area for Montieae. Phylogenetic studies of Portulacaceae sensu lato have identified a western Americas/Australia clade and an eastern Americas/Africa clade (Carolin 1987; Hershkovitz 1993; Hershkovitz and Zimmer 1997, 2000; Applequist and Wallace 2001). Those analyses have consistently placed Montieae in the western Americas/Australia clade. Montieae have their greatest species richness in North America, but also have representatives in South and Central America, northeastern Asia, New Zealand, and Australia. Based on a broad analysis of Portulacaceae, Applequist and Wallace (2001) hypothesized a North American origin for Montieae, which we seek to test with our greater sampling in the tribe.

We are especially interested in the geographic and habitat diversification of perennial claytonias. Several perennial claytonias are high latitude endemics and understanding their origins can contribute to hypotheses on the diversification of northern high latitude floras. Earlier hypotheses have derived the postglacial floras of the northern high latitudes from the

alpine cordilleran floras in eastern Asia and western North America (Murray 1981, 1995; Graham 1999) or from high latitude refugia (Hulten 1937; Murray 1981). Murray (1981, 1995) emphasized these alternatives and introduced additional considerations, such as Asian migrants to high latitude refugia during the Pleistocene, interglacial colonizations from coastal refugia, and glacial and postglacial in-situ evolution of new species. Colonization of high latitude ecosystems from alpine regions south of the glacial maximum or persistence in high latitude refugia may imply that the ancestors of the modern high northern latitude flora were ecologically adapted for the post-glacial environment (Bliss 1971; Murray 1981). Dispersal from some lower latitude environments inhabited by Montieae could have required ecological transformation. We test for ecological persistence (ecological niche conservatism) versus transformation (ecological evolution) in the evolution of northern high latitude claytonias.

Both nuclear ribosomal and plastid markers are analyzed under different optimality criteria to assess phylogenetic signal in the data. Robust, consistent results are used to revise the taxonomy of Montieae to reflect monophyly. Alternative phylogenetic topologies are used to infer the ancestral area of Montieae and hypothesize biogeographic and ecological transformations in the evolution of perennial *Claytonia*.

MATERIALS AND METHODS

Taxon Sampling. We sampled 52 accessions of Montieae (Appendix 1). This included representatives of all eight North American *Montia* recognized by Miller (2003), the single Siberian endemic, *M. vassilievii*, and six of the eight Australasian species of *Neopaxia* sensu Heenan (1999). From *Claytonia*, we sampled 24 of the 26 North American species recognized by Miller (2003) and the single Siberian endemic, *C. joanneana*. We were unable to sample three montias from Guatemala (*M. calcicola* Standley & Steyerf.), Colombia (*M. meredensis*

Friedrich.), and Venezuela (*M. biapiculata* Lourt.) and two Rocky Mountain *Claytonia* (*C. multiscapa* Rydb. and *C. rosea* Rydb.).

We sampled multiple accessions of several taxa to test for intraspecific sequence variation in the internal transcribed spacer regions of nuclear ribosomal DNA (ITS). When multiple accessions yielded identical sequences, one was selected at random for inclusion in the analyses to reduce redundancy and computation time. Identical intraspecific ITS sequences were found in *C. perfoliata*, *C. cordifolia*, *C. joanneana*, *C. sarmentosa*, *C. scamanianna*, *C. lanceolata*, *Montia chammissoi*, *M. linearis*, and *M. parvifolia*. *Claytonia gypsophiloides* and both subspecies of *C. exigua* (sensu Miller and Chambers 1993) had identical ITS sequences, but are maintained in the analyses because of their differing *trnK/matK* sequences. When intraspecific sequence variability was found, all accessions were included in the analyses.

Outgroup selection was based on results from phylogenetic analyses of Portulacaceae s.l. (Hershkovitz and Zimmer 2000; Applequist and Wallace 2001). The outgroups *Calandrinia ciliata*, *C. affinis* Gillies ex Arn., *Cistanthe tweedyi* (A. Gray) Hershkovitz, *C. laxiflora* (Phillipi) D. I. Ford, *Lewisia columbiana*, and *L. rediviva* were used for preliminary analyses of ITS, but outgroup deletion experiments (not shown) demonstrated that the number and combination of outgroups had no effect on the ingroup topology. Thus, to reduce computation time for the final analyses presented here, only three outgroups were used.

PCR Amplification and DNA Sequencing. Total DNA was extracted from field collected and silica dried or herbarium material using the standard CTAB protocol of Doyle and Doyle (1987). We amplified and sequenced ITS, including the 5.8S gene, and the *trnK* intron and 5' end of the *matK* coding region (*trnK/matK*) from the plastid genome to provide phylogenetic characters. We used the polymerase chain reaction (PCR) to amplify ITS using the primer pair

Nnc18S10 and C26A (Soltis et al. 1997). For amplification of double-stranded ITS rDNA fragments, our PCR mix contained 20mM Tris HCL pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.01% Tween-20, 150μM dNTPs, 0.5μM forward and reverse primers, DMSO, 0.2μL, Taq polymerase (Promega®, Madison, Wisconsin, USA), ~20μg genomic DNA and water for a 25μL total reaction volume. We used a “touchdown” PCR profile of 4 min at 95°C, 5 cycles of 1min at 94°C, 1min at 53°C and 2 min at 72°C, decreasing the annealing temperature by one degree each cycle, followed by 35 cycles with a 48°C annealing temperature, and a final 72°C extension of 5 min.

The primer pair *trnK*-3914F and *matK*-1470R (Johnson and Soltis 1994) was used to amplify *trnK/matK*. The PCR mix used to amplify this fragment was the same as the ITS mix, except DMSO was excluded and the total reaction volume was 50μL. The PCR profile for *trnK/matK* was 3 min. at 95°C, 5 cycles of 1min at 94°C, 1min at 48°C and 3 min at 72°C for 35 cycles, with a final extension of 15 min at 72°C.

Double stranded PCR amplification products were cleaned using polyethylene glycol. Cleaned PCR products were direct sequenced using ABI Big Dye terminators and visualized on an ABI 377 automated sequencer. For ITS, the PCR primers were used to cycle sequence. To cycle sequence the ~1300 bp of *trnK/matK*, two internal primers, 360F (5'CGGGAAAGGCTTCTCCACG3') and 670R (5'GGAATTTCCACAATGACTGC3'), were designed to use in conjunction with the PCR primers.

Phylogenetic Analyses. Datasets were aligned manually in Se-AI (Rambaut 1996). Character homology was equivocal for 29 *trnK/matK* characters, and these were excluded from analyses. We conducted separate parsimony analyses on aligned DNA sequence matrices for three data sets: full ITS, reduced ITS and *trnK/matK* (TreeBASE study accession number S1180,

matrix accession numbers M2040-M2042). The reduced ITS dataset matches the taxa of the *trnK/matK* dataset and was constructed to conduct maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses on a combined data set that includes the same set of taxa for both markers (TreeBASE matrices M2043, M2044). We were unable to match taxon sampling between the full ITS and *trnK/matK* datasets because amplification of the longer *trnK/matK* proved problematic for several taxa sampled from herbarium specimens.

MP and ML analyses used PAUP*4.0 (Swofford 2002). The heuristic searches used random taxon addition to obtain starting trees and the tree bisection-reconnection (TBR) branch swapping option. Searches were replicated 1,000 times for MP and 100 times for ML. Nucleotide characters were equally weighted and unordered. Indels did not provide any phylogenetic information and were treated as missing data for both ITS datasets. The *trnK/matK* data had seven indels that were phylogenetically informative, and these were coded as presence-absence characters in a nucleotide plus indels data set used only for the MP analyses. Clade support for MP and ML analyses was assessed using the non-parametric bootstrap (Felsenstein 1985) implemented in PAUP*4.0 (Swofford 2002) using 1,000 pseudoreplicates for MP and 100 for ML. Bootstrap analyses applied random taxon addition and TBR branch swapping. We assessed branch decay (Bremer 1988; Donoghue et al. 1992) for the parsimony results using AutoDecay (Eriksson 1999) and PAUP*4.0 (Swofford 2002).

Modeltest (Posada and Crandall 1988) was used to estimate the best substitution model for the combined data set. Modeltest uses two approaches for model estimation, a hierarchical likelihood ratio test (HLR) and the Akaike information criterion (AIC), to assess which of 56 models best fits the data. A GTR + I + Γ model was estimated for the combined ITS and

trnK/matK data under both HLR and AIC criteria and was applied to ML analyses with the parameter estimates derived from HLR test in Modeltest.

Mr. Bayes ver. 3.0 (Hulsenbeck and Ronquist 2001) was used for BI analyses. The reduced ITS and *trnK/matK* data sets were assigned to separate partitions. For both data partitions we used the following likelihood model: six substitution rates (Nst = 6), rates followed a gamma distribution with four categories (rates = gamma; ngamma = 4), and no sites were assumed invariable (= GTR + Γ). Starting model parameters were assigned uniform prior probabilities and estimated as part of the analysis, but unlinked between data partitions, allowing them to vary independently. We conducted five replicate BI analyses to assess mixing between Markov chains and convergence of likelihood scores across chains and across separate analyses. Each analysis ran four chains for five million generations starting from a random tree. Trees and parameters were saved every 100 generations, producing 50,000 trees. Ten thousand trees were discarded as the ‘burn-in’ (i.e., trees sampled before the chains had reached stationarity). Thus, 40,000 trees were used to establish the posterior probability distribution from which clade probabilities were drawn. PAUP* (Swofford 2002) was used to generate a 50% majority rule consensus tree and posterior probabilities (PP) were averaged across independent runs (Fig. 2C).

Constraint Analyses. We used two constraint topologies to examine alternative phylogenetic hypotheses. Each constraint forced the monophyly of only one node. One constraint forced the monophyly of *C. acutifolia*, *C. arctica*, *C. megarhiza*, and *C. joanneana*, which formed section *Caudicosae* sensu Swanson (1966) and McNeill (1975). The second constraint forced the monophyly of the Beringian members of section *Claytonia* from our own analyses, which included *C. tuberosa*, *C. ogilviensis*, *C. acutifolia*, and *C. megarhiza*. We applied these constraints to searches using the combined reduced ITS and *trnK/matK* dataset in

parsimony analyses. We conducted full heuristic searches for the most parsimonious cladograms under each of the topology constraints (all analyses swapped to completion), permitting us to compare the lengths of constrained topologies to that of the most parsimonious topologies from the unconstrained analyses.

Biogeographic and Habitat Analyses. To assess the ancestral area for Montieae, we analyzed geographic distributions using DIVA (Ronquist 1996) and MacClade 4.0 (Maddison and Maddison 2000). Sampled taxa were coded for one or more of the following geographic areas, which encompass the distribution of Montieae: 1) Beringia – for taxa that occur exclusively or predominantly above 55° north latitude, 2) western North America – for taxa south of 55° north latitude, north of Mexico, and west of the Rocky Mountains, 3) eastern North America – for taxa east of the Rocky Mountains, 4) South America, 5) Australasia/New Zealand, and 6) Europe. MacClade optimizations for ancestral area used the strict consensus tree from the combined MP analysis and the 50% majority rule consensus tree from our BI analysis. DIVA analyses required fully bifurcated topologies (Ronquist 1996, 1997), therefore we randomly selected two fully resolved trees, one MP tree and one BI tree, from our combined data analyses. DIVA reconstructions were implemented as exact searches without restriction on the number of areas allowed per node.

MacClade 4.0 (Maddison and Maddison 2000) and DIVA (Ronquist 1996) were used also to examine biogeographic and habitat transformations in the perennial claytonias. The resolved MP and BI trees from our combined data analyses described above were used in both MacClade and DIVA reconstructions in all subsequent analyses. Although MacClade disallows polymorphic state assignments at interior nodes (in contrast to DIVA) it can reconstruct different

nodal states under either ACCTRAN (homoplasies maximized as reversals), or DELTRAN (homoplasies maximized as parallelisms); therefore, both options were applied.

Perennial claytonias were coded for geographic distribution and habitat characters. Distribution states are described above. Two habitat characters were coded: (1) moisture (persistently wet [PW] or seasonally dry [SD]) and (2) elevation/latitude (high [H] or low [L]). Taxa coded as PW spend their active growth cycle in saturated moisture conditions (i.e., in streambeds, stream margins or in snowbank meltwater); whereas, taxa coded as SD experience drying during their active growth cycle. Taxa were coded as H if they occur at elevations $\geq 1,500$ meters in elevation or $\geq 55^\circ$ north latitude and as L if they occur at elevations $< 1,500$ meters or $< 55^\circ$ north latitude. State assignments for taxa (Table 2) were based on field observations, herbarium specimen data and floristic treatments (Anderson 1959; Cody 1996; Henry 1915; Hultén 1928, 1968; Polunin 1959; Chambers 1993a; Tolmachev and Yurtsev 1996; Miller 2003).

RESULTS

Parsimony Analyses. The aligned, full ITS data set of 52 taxa has 661 characters, including 207 that are parsimony informative. MP analysis of this data set swapped to completion and produced 420 most parsimonious trees of 663 steps (consistency index [C. I.] = 0.5767, retention index [R. I.] = 0.8364, re-scaled consistency index [R. C.] = 0.5298; Fig. 1A). The reduced ITS data set of 40 taxa has 658 total characters, including 198 that are parsimony informative. This analysis swapped to completion and produced 28 most parsimonious trees of 610 steps (C. I. = 0.5879, R. I. = 0.7951 and R. C. = 0.5110; Fig. 1B). The aligned *trnK/matK* data set of 40 taxa has 1,365 characters, including 179 that are parsimony informative. This data set swapped to completion, resulting in 12 equally parsimonious trees of 437 steps (C. I. = 0.7200, R. I. = 0.8694 and R. C. = 0.7023; Fig. 1C). The data set (= combined data) of 40 taxa

that combines the reduced ITS and *trnK/matK* sequences has 2,023 characters (plus seven indels), including 377 characters that are parsimony informative. Analysis of the combined data swapped to completion, producing 56 equally parsimonious trees of 1,063 steps (C. I. = 0.6229, R. I. = 0.8131, R. C. = 0.5693; Fig. 2A).

MP analyses constrained to force the monophyly of section *Caudicosae* (*C. acutifolia*, *C. megarhiza*, *C. joanneana* and *C. arctica*) resulted in 22 trees of 1,105 steps (42 steps longer than our unconstrained MP trees). Topology constraints that forced the monophyly of *C. acutifolia*, *C. tuberosa*, *C. ogilviensis* and *C. megarhiza*, the Beringian members of sect. *Claytonia*, resulted in 115 trees of 1,072 steps (eight steps longer than our unconstrained MP trees).

Maximum Likelihood and Bayesian Inference. ML and BI used the same combined data set as the parsimony analyses, but the seven indel characters were removed. The single ML tree had a $-\ln$ likelihood = 9109.71 (Fig. 2B). The five independent BI analyses resulted in identical 50% majority rule consensus topologies (Fig. 2C). Posterior probabilities varied minimally (1-2%) among the independent analyses, from which we infer that the chains reached stationarity and both mixing and convergence were achieved. Averaged PP values from the independent analyses are shown on the 50% majority rule consensus tree (Fig. 2C).

Ancestral Area Reconstruction. DIVA (Ronquist 1996) and MacClade (Maddison and Maddison 2000) reconstruct the ancestral area for Montieae as western North America. MacClade reconstructions for the basal nodes were the same for the MP and BI 50% majority rule consensus trees (data not shown). Likewise, the MP and BI topologies we tested using DIVA had identical character states at the basal nodes, despite differing topologies within sect. *Claytonia* (Fig. 3A, B). The MP tree (Fig. 3A) resulted in a single optimal reconstruction

requiring 17 vicariance/dispersal events. The BI tree (Fig. 3B) had three alternative optimal reconstructions requiring 18 vicariance/dispersal events.

Biogeography of Perennial Claytonia. DIVA (Ronquist 1996) reconstructed six vicariance/dispersal events for the MP tree (Fig. 3A). In sect. *Rhizomatosae*, a single vicariance event separates the Beringian taxa from their lower latitude relatives (node 5). In sect. *Claytonia* there are four vicariance events. (1) Vicariance separates the Beringian *C. acutifolia* from the rest of sect. *Claytonia* in western North America (node 9). (2) Vicariance establishes a western North American/eastern North American disjunction (node 12). (3) Vicariance separates eastern North American and Beringian taxa (node 14). (4) Vicariance separates western North American and Beringian taxa (node 16). Dispersal from western North America to Beringia occurs in *C. megarhiza*.

For the BI tree, DIVA (Ronquist 1996) reconstructed three equally optimal alternatives that required six vicariance/dispersals (Fig. 3B). Transformations in sect. *Rhizomatosae* are identical to those of the MP tree. In contrast, reconstructions differ for sect. *Claytonia* in which there are two or three independent vicariance events between western North America and Beringia (nodes 12, 14, and 16) and one between eastern North America and Beringia (node 13). One or two vicariance events can be inferred between western North America and eastern North America (nodes 10 and 12), or alternatively between a more widespread ancestor and western North America and Beringia or eastern North America (node 12). *Claytonia megarhiza* at northern latitudes is reconstructed as a dispersal event from western North America.

MacClade 4.0 (Maddison and Maddison 2000) reconstructions of geographic transitions among perennial claytonias were identical under ACCTRAN and DELTRAN for the MP tree (Fig. 3C). In sect. *Claytonia*, these include two independent dispersal events from western North

America to Beringia (nodes 9 and 16), one dispersal from western North America to eastern North America (node 12) and one dispersal from eastern North America to Beringia (node 14). In sect. *Rhizomatosae* a single dispersal from western North America to Beringia is inferred (node 5). For the BI tree (Figs. 3D, E), ACCTRAN resolved states for all nodes, but DELTRAN reconstructed as equivocal the branch for *C. virginica* + *C. tuberosa* (Fig. 3E, node 12). The DELTRAN reconstruction indicates dispersal at that node was either from Beringia to eastern North America or vice versa. The BI tree (Fig. 3D, E) has two or three independent dispersals to Beringia from western North America in sect. *Claytonia* (nodes 12, 14, and 16) and one in sect. *Rhizomatosae* (node 5). In sect. *Claytonia*, dispersals to eastern North America were either both from western North America (nodes 10 and 13) or one was from Beringia (node 12).

Habitat. We optimized character states for moisture and elevation/latitude on the same MP and BI trees used for biogeography reconstructions to explore the implications of alternative taxon placements for understanding habitat transitions in the evolution of perennial claytonias. Reconstructions on the MP and BI trees (Table 3) were conducted using both MacClade (Maddison and Maddison 2000) and DIVA (Ronquist 1996).

DISCUSSION

Comparisons Among Phylogenetic Analyses. The *trnK/matK* data resulted in more resolved, robust trees with less homoplasy, as measured by C. I., R. I., and R. C., than ITS (Fig. 1B, C). For the combined data, ML and BI analyses resolved more clades than MP (Fig. 2), but all produced largely congruent topologies with a well supported Montieae (BS 87-100%, PP 100%) in which monophyletic *Claytonia* (BS 95-100%, PP 100%) and *Montia* (BS 47-97%, PP 100%) are sisters. *Claytonia* includes a strongly supported clade of annuals and the perennials *C. sibirica* and *C. palustris* (= sect. *Limnia*; BS 81-100%, PP 100%), a clade of high latitude

species with rhizomatous or caudicose growth habits (= sect. *Rhizomatosae*; BS 98-100%, PP 100%), and a clade of species with spherical or obconic underground perennation structures (= sect. *Claytonia*; BS 87-100%, PP 100%). In *Montia*, all analyses recover three clades, which we designate as sections *Montiastrum* (BS 100%, PP 100%), *Australiensis* (BS 98-100%, PP 100%), and *Montia* (BS 53-100%, PP 100%).

The *trnK/matK* and ITS data produce largely congruent topologies, although there are several cases in *Claytonia* where sister taxon relationships differ among analyses and two cases in *Montia* where incongruences are observed. In *Claytonia* all of the variably placed branches collapse in trees one step longer and support values are generally weak, which leads us to conclude that the apparent incongruences are more likely the result of low signal in the data rather than chloroplast capture or paralogous ITS sequences.

In *Montia*, *M. howellii* is sister to *Montiastrum* (BS 69%) in the ITS results, but sister to *Australiensis* in the *trnK/matK* and all combined data results (BS 98%). In this case, chloroplast capture is a possible, but probably unlikely source of conflict given the considerable geographic distance between these two taxa (although see sect. *Australiensis* below). *Montia diffusa* is sister to all other *Montia* with ITS, but there is no support for this placement. In all other analyses *M. diffusa* is sister to sect. *Montia* (MP-BS 57-93%, ML-BS 91%, BI p. p. 1.0), nevertheless we cannot rule out chloroplast capture or ITS paralogy.

Taxonomy of Montieae. Taxonomic treatments of Montieae have differed in the circumscription and ranking of taxa. Workers have had contrasting emphases on particular suites of characters, leading some to describe several segregate genera from broadly circumscribed *Claytonia* and *Montia* (Rydberg 1906, 1932; Nilsson 1966a, b, 1967, 1970, 1971a, b; Yurtsev 1972; Heenan 1999). In order to provide a more robust classification for Montieae, we adopt

monophyly as a primary criterion for taxon delimitation. Given the arbitrariness of taxonomic ranking, we opt to treat conservatively the two major, well supported clades recovered in Montieae as *Claytonia* and *Montia* (Figs. 1A, 2A; Table 1), corresponding to those genera as circumscribed by Swanson (1966) and McNeill (1975). Both *Claytonia* and *Montia* were recognized in the earliest treatments of the tribe (Bentham and Hooker 1862; Gray 1887; Pax 1889; Gray and Robinson 1897; Holm 1905, 1913) and most subsequent authors have also treated Montieae as consisting of these two genera. In contrast, Rydberg (1906, 1917, 1932) and Nilsson (1966a, b, 1967, 1970, 1971a, b) offered taxonomies that included segregate genera. Our subgeneric taxonomies for *Claytonia* and *Montia* also follow conservatively the sections used by other workers to distinguish major groups; we apply these existing sectional names for well supported monophyletic clades (Figs. 1A, 2A; Table 1).

Claytonia. The monophyletic *Claytonia* recovered in our analyses has the morphological synapomorphy of a shoot that forms initially a basal rosette of leaves and inflorescence axes that have two expanded, cauline leaves in opposite positions that do not subtend flowers. Contrary to Swanson's (1966) suggestion that *Claytonia* are unbranched, we find that shoot systems produce branches that are inflorescences as well as those that are vegetative.

We recovered well supported clades that correspond generally to the sections *Limnia*, *Claytonia*, and *Rhizomatosae* (Fig. 2A; Table 1) of Swanson (1966) and McNeill (1975). However, our analyses do not recover a clade that corresponds to sect. *Caudicosae* as delimited by those authors. Instead, taxa assigned previously to sect. *Caudicosae* are distributed among the clades corresponding to sections *Claytonia*, *Rhizomatosae*, and *Limnia*, and trees constrained to force the monophyly of sect. *Caudicosae* are considerably longer (42 steps) than our MP trees from unconstrained analysis.

SECT. *CLAYTONIA*. This clade consists of perennials that have specialized subterranean perennating structures. As circumscribed by Swanson (1966) and McNeill (1975), sect. *Claytonia* s. s. was limited to taxa with spherical or sub-spherical perennation structures. In contrast, our results demonstrate that a monophyletic sect. *Claytonia* is more inclusive, having also taxa with obconic perennating structures, notably *C. megarhiza* and *C. acutifolia*, which were assigned previously to sect. *Caudicosae* by Swanson (1966) and McNeill (1975). Doyle (1983) found that *C. megarhiza* and *C. acutifolia* share with all other sampled members of sect. *Claytonia* a common diploid *C. virginica* flavonoid type (Race III).

Among the taxa that have spherical underground perennating structures, Shelly et al. (1998) hypothesized that *C. virginica* and *C. tuberosa* belonged to a narrow-leaved complex, including also *C. multiscapa* and *C. rosea*, and Stewart and Wiens (1971) identified a potential relationship between *C. caroliniana* and *C. lanceolata* based on their ecological similarities. Although *C. caroliniana* and *C. virginica* are the only members of sect. *Claytonia* found in eastern North America, Doyle (1983) suggested that the two species had been long isolated and that many of their flavonoid, karyotypic and morphological similarities were convergences, however, our results suggest they may be instead be synapomorphies. Our MP results for ITS and the combined data provide weak support for the clades *C. virginica* + *C. caroliniana* and *C. lanceolata* + *C. tuberosa* (Figs. 1A, B; 2A), however, ML and BI results of the combined data provide weak support for a *C. virginica* + *C. tuberosa* clade (Figs. 2B, C).

Two sampled populations from different geographic regions attributed to *C. umbellata* did not form a monophyletic group. The Oregon population is sister to the Yukon endemic *C. ogilviensis* (BS 91%; PP 100%; Figs. 1B; 2A-C), whereas the California population is weakly placed as sister to the widespread *C. megarhiza*. McNeill (1972) noted the great similarity of *C.*

ogilviensis and *C. umbellata*, but distinguished them by petal size and color differences. However, Chambers (1993a) reported petal size variation in *C. umbellata* that encompasses the range that McNeill (1972) reported for *C. ogilviensis*. Petal color in the Oregon population of *C. umbellata* is darker than in more southern populations of the species on examined herbarium specimens, which may be synapomorphic with the dark color of *C. ogilviensis* petals. Further investigation of character variation and phylogeography of *C. umbellata*, *C. megarhiza*, and *C. ogilviensis* is warranted.

SECT. *RHIZOMATOSAE*. In our results this clade includes species McNeill (1975) assigned to sect. *Rhizomatosae*, as well as *C. arctica* and *C. joanneana*, which he had assigned to sect. *Caudicosae*. The sampled *C. porsildii*, included only for ITS (Fig.1A), also forms part of sect. *Rhizomatosae*. Section *Rhizomatose* was initially circumscribed by Gray (1887, p. 280) to include only *C. sarmentosa* and *C. cordifolia*, which he recognized as sharing “creeping or little-thickened rootstocks.” *Claytonia nevadensis* and *C. scammaniana*, which McNeill (1975) placed also in sect. *Rhizomatosae*, have similar shoot systems. In contrast, *C. joanneana* has caudices that are thicker and appear to be longer-lived than the strictly rhizomatous forms. Herbarium specimens of *C. arctica* have greater variability in shoots, some are similar to robust *C. joanneana* and others are like slightly thickened *C. sarmentosa*. The emphasis placed on vegetative characters in previous taxonomic studies may explain why the broader *Rhizomatosae* assemblage including *C. joanneana* and *C. arctica* has gone unrecognized.

Section *Rhizomatosae* has a strongly supported monophyletic group limited to high northern latitudes that consists of *C. scammaniana*, *C. sarmentosa*, *C. joanneana*, and *C. arctica*, although relationships among these species are largely unresolved (Figs. 1A-C; 2A-C). We discuss below the geographic origin of this clade, which may be a recent radiation (Fig. 4).

Our parsimony results (Figs. 1A-C; 2A) placed *C. arenicola* in a polytomy with the *Limnia*, *Claytonia*, and *Rhizomatosae* clades, but its placement as sister to the core members of sect. *Rhizomatosae* was resolved by ML (Fig. 2B; BS 83%) and BI (Fig. 2C; PP 100%). We provisionally include it as part of sect. *Rhizomatosae* (Table 1). Our results differ considerably from previous taxonomic treatments of *C. arenicola*. The annual habit of this taxon is unique in our sect. *Rhizomatosae* and was emphasized by Swanson (1966), McNeill (1975), and Miller and Chambers (1977) in their inclusion of *C. arenicola* in sect. *Limnia*. Unlike sect. *Limnia* (including the perennials *C. sibirica* and *C. palustris*), in which flowers have three ovules, *C. arenicola* has six ovules per ovary, a condition it shares with the perennial members of sect. *Rhizomatosae*. Based on our ML and BI topologies we infer that the annual habit evolved independently in *C. arenicola* and the annuals of sect. *Limnia*.

SECT. *LIMNIA*. This strongly supported clade corresponds to McNeill's (1975) sect. *Limnia* minus *C. arenicola*. Our results recover three major clades in the section, including (1) *C. exigua* + *C. gypsophiloides* + *C. saxosa*, (2) the *C. perfoliata* complex, and (3) *C. palustris* + *C. sibirica*.

Fellows (1975) initially allied *C. exigua* and *C. gypsophiloides* and, subsequently, Miller and Chambers (1977) used similarities in seed coat morphology and a base chromosome number of $X = 8$ to predict that *C. saxosa* was also allied to these two species. Our data provide strong support for this association. ITS sequences for *C. gypsophiloides* and all subspecies of *C. exigua* were identical (however *trnK/matK* sequences differed) and our phylogenetic results place *C. saxosa* as the sister of *C. exigua* + *C. gypsophiloides* in all analyses.

Miller and Chambers (1993) described the *C. perfoliata* complex as a polyploid assemblage centered on three diploids, *C. perfoliata*, *C. rubra*, and *C. parviflora*, plus their

polyploid derivatives, which they delimited as subspecies. Several of these subspecies were not sampled for our analyses, but we recovered a strongly supported clade (BS 99-100%) consisting of *C. perfoliata*, *C. parviflora*, *C. rubra*, and *C. washingtoniana*. *Claytonia washingtoniana* has been hypothesized to be a fertile hybrid between *C. perfoliata* and *C. sibirica* (Fellows 1971; Chambers 1993a), thus its placement in this clade requires further investigation.

The phenetic analyses of McNeill (1975) grouped *C. sibirica* and *C. palustris*, which is consistent with our results. This clade is sister to the rest of sect. *Limnia* in the ML and BI results (Fig. 2B, C), but MP analyses (Figs. 1A-C, 2A) failed to resolve relationships among major clades of the section. *Claytonia palustris* and *C. sibirica* are largely perennial taxa, although many individuals in populations of the latter are facultatively annuals.

Montia. We recover a monophyletic *Montia* that corresponds to the genus as circumscribed by Swanson (1966) and McNeill (1975). Rydberg (1906, 1917, 1932) and Nilsson (1966a, b, 1967, 1970, 1971a, b), in contrast, recognized several genera in this group, some of which correspond to clades supported by our results. We recognize three major clades in *Montia* as the sections *Montia*, *Montiastrum*, and *Australiensis* (Figs. 1A, 2A). *Montia* share pantocolpate pollen (Nilsson 1967), which may be synapomorphic for the genus. Tricolpate pollen are characteristic of *Claytonia* and *Lewisia*, which appears to be the sister of Montieae; thus, we hypothesize that tricolpate pollen is plesiomorphic for Montieae. We add the caveat, however, that the sister of *Lewisia* + Montieae are clades of *Calandrina* (Hershkovitz 1993; Hershkovitz and Zimmer 2000; Applequist and Wallace 2001) in which tricolpate, pantocolpate, and pantoporate pollen are found (Nilsson 1967).

SECT. MONTIA. Our results recover a clade that consists of *M. fontana*, *M. chamissoi*, *M. parvifolia*, and *M. diffusa*. This assemblage has not been recognized in previous taxonomic

treatments; however, these taxa share the *Montia*-type pollen characterized by Nilsson (1967), and they were considered by him to be more closely related to each other than to *Montiastrum*. The presumed synapomorphic character this pollen type provides is aperture membranes that have usually only one row of projections, but variations of this pollen type were used by Nilsson (1967) to characterize genera.

Taxa of our sect. *Montia* have been recognized as parts of disparate sections or segregate genera by earlier workers. For example, section *Montia* (or the genus *Montia* s. s.) has been treated as the polymorphic taxon *M. fontana* or this species and various segregates (e.g., Gray 1887; Pax and Hoffman 1934; Nilsson 1966 a, b, 1967; Miller 2003; Table 1). Swanson (1966), however, included *M. chamissoi* with *M. fontana* in his sect. *Montia*. *Montia chamissoi*, placed in our results as sister to *M. fontana*, was treated by Gray (1887) and Pax and Hoffmann (1934) as part of sect. *Alsinastrum*, but not by Rydberg (1906) or Nilsson (1970). In addition, we find *M. parvifolia* and *M. diffusa* form part of a monophyletic sect. *Montia* despite alternative treatments by some other workers (Table 1).

Montia calcicola, *M. meridensis*, and *M. biapiculata* were not available to sample for our analyses but may be members of our expanded sect. *Montia*. For example, Nilsson (1970) placed *M. calcicola* in *Crunocallis* and *M. meridensis* was originally placed in sect. *Alsinastrum* by Friedrich (1954). Nilsson (1966a) later removed *M. meridensis* to the genus *Mona* because of its intectate pollen that differs from that of taxa of sect. *Montia* as well as all other members of the genus (Nilsson 1966a, 1967). Lourteig (1991) recognized the Colombian endemic *M. biapiculata* based primarily on its geographic isolation, but found it difficult to assign to a particular section. We withhold a sectional assignment for this taxon until it is included in phylogenetic analyses (Table 1).

SECT. *MONTIASTRUM*. Gray (1887) first recognized sect. *Montiastrum* as consisting of *M. diffusa*, *M. linearis*, *M. dichotoma*, and *M. howellii*. Rydberg (1917) segregated the group as the genus *Montiastrum*, and this was followed by Nilsson (1971a). In contrast to Rydberg's treatment (1917, 1932), Nilsson included *M. vassilievii*, but removed *M. howellii* from *Montiastrum*. McNeill and Findlay (1971) elucidated the pollen morphology of *M. bostockii* and placed it in sect. *Montiastrum* with *M. vassilievii*, *M. dichotoma*, and *M. linearis*. In his *Montiastrum*, Nilsson (1971) suggested that the annuals *M. linearis* and *M. dichotoma* were derived relative to the perennials *M. vassilievii* and *M. bostockii*. Yurtsev (1972) segregated the perennial members from Nilsson's (1971) *Montiastrum* as the genus *Claytoniella*. Our results place the annuals as sister species (BS 100%), and they are sister to the perennial *M. bostockii*. We sampled *M. vassilievii* only for ITS, and in the MP analyses of that marker it was placed in a polytomy with *M. bostockii* and *M. dichotoma* + *M. linearis* (Fig. 1A). Perennials and annuals differ in habit, shoot architecture and floral morphology. The perennials have plagiotropic, little-branched shoots and flowers with five stamens, whereas the annuals have erect, highly branched shoots and flowers with (one-) three stamens. *Montia bostockii* and *M. vassilievii* differ from one another primarily in overall size, with *M. bostockii* being considerably larger than *M. vassilievii*. These two taxa are disjunct between east and west Beringia. *Montia vassilievii* is locally endemic to the Anadyr Basin of northeastern Siberia (west Beringia) and Wrangel Island, and *M. bostockii* is distributed in Central Alaska, southwest Yukon Territories (east Beringia), as well as a disjunct population at Toolik Lake in Arctic Alaska.

Pollen that are tholate, having wart-like projections of the pollen wall between the apertures (Nilsson 1967; McNeill and Findlay 1971), are synapomorphic for sect. *Montiastrum*. The pollen wall of the wart-like tholi is atectate (lacking columellae). At germination, clusters of

adjacent tholi fracture, producing a cap that falls off to permit the emergence of the pollen tube (Nilsson 1967).

SECT. *AUSTRALIENSIS*. Geography has provided the primary means to distinguish *M. australasica*, as the only member of Montieae in Australia and taxa on New Zealand, from the rest of *Montia*. Taxonomic treatments have varied for the New Zealand populations, which have been considered part of *M. australasica* (Nilsson 1966a), as *M. calycina* (Pax and Hoffman 1934), or more recently as seven independent species (Heenan 1999). Our analysis of ITS sequences found *M. australasica* to be nested among accessions sampled from New Zealand (Fig. 1A). We sampled six of the New Zealand species recognized by Heenan (1999), but only five are included in our full ITS analysis. Two taxa had identical sequences, and PCR amplifications for *trnK/matK* were unsuccessful for several sampled herbarium specimens. Species from Australia and New Zealand were treated as *Neopaxia* by Nilsson (1966a) and Heenan (1999). We advocate their inclusion in the monophyletic *Montia* that we circumscribe on the basis of pantocolpate pollen. Synapomorphic for these Australian and New Zealand taxa are aperture membranes that have two or three rows of small projections and a chromosome number of $2n = 96$ (Nilsson 1967). Heenan (1999) suggested that *Neopaxia* were distinguished by highly branched shoot systems with creeping stems; however, this habit is also characteristic of *M. howellii*, the South American species, including *M. fontana* (sect. *Montia*), and numerous others. We suggest that highly branched, repent shoot systems are more likely plesiomorphic for *Montia* and have been modified in several cases.

Our results demonstrate that *M. howellii* forms a well-supported monophyletic group with the Australian and New Zealand taxa (Figs. 2A-C), and we recommend its inclusion in sect. *Australiensis*. Unlike most other *Montia*, *M. howellii* has pollen apertures that have smooth

membranes; the rows of projections that help to distinguish among the clades have been lost in this species. The disjunction of *M. howellii* from its sister Australasian *Montia* is notable. Miller (2003) depicted the distribution of *M. howellii* as continuous from southern Vancouver to northern California, whereas Nilsson (1971b) described the distribution as disjunct between northern California and the mouth of the Columbia River. We have found that herbarium specimens match Nilsson's rather than Miller's depiction of its distribution. Nilsson emphasized that populations of *M. howellii* are localized around the ports of Eureka, California, and Portland, Oregon, although populations occur also in Corvallis, Oregon. The majority of collections for this taxon are from the type locality at Sauvie's Island, which is situated at the confluence of the Willamette and Columbia rivers in the shipping channel for the port of Portland. We hypothesize that *M. howellii* is a recent introduction to western North America deposited in the ballast of ships from New Zealand.

Biogeography. Previous phylogenetic analyses of the portulacaceous alliance suggest Montieae are nested in a western Americas/Australian clade (HersHKovitz 1993; HersHKovitz and Zimmer 1997, 2000; Applequist and Wallace 2001). Our results agree with Applequist and Wallace (2001), indicating that the most basal nodes of Montieae are western North American, where a fairly rich grade of extant species remains (Figs. 3A, B). Caution is necessary, however, because we were unable to sample Central and South American *Montia*, and Applequist and Wallace (2001) sampled sparsely from the Australian calandrinias.

Ancestral area reconstruction suggests that both *Claytonia* and *Montia* have a western North American origin (Fig. 3A, B), but these two monophyletic groups have very different distributions. Whereas *Montia* occur in both the northern and southern hemispheres, *Claytonia*

are exclusively northern. We have focused our biogeographic study on perennial clades of *Claytonia* (Fig. 4A, B).

In western America, perennial *Claytonia* of sects. *Claytonia* and *Rhizomatosae* extend from northern Mexico to northeastern Asia and in eastern American from New Foundland to Texas (Fig. 4A, B). Both sections include several endemic Beringian taxa. There have been several hypotheses to explain the origin of post-Pleistocene Beringian floras, including: (1) dispersal from high latitude refugia north of the glaciers in both Asia and North America, (2) dispersal from alpine or montane habitats of either Asia or North America, south of the glaciers, (3) dispersal from coastal refugia, and (4) the retention (and in situ evolution) of taxa in Beringian refugia (Hultén 1937; Murray 1981, 1995). Our results resolve few clades in sect. *Claytonia* (Figs. 1A-C, 2A-C), and we reconstruct considerably different biogeographic patterns from sampled MP and BI topologies (Figs. 3A-E). Although we infer multiple origins for Beringian taxa, reconstructions for dispersal and vicariance are inconclusive and constraint analyses that force the monophyly of the Beringian members result in trees only eight steps longer than our shortest MP trees. In the better resolved sect. *Rhizomatosae* a northward migration from low latitudes in western North America to Beringia produced a northern clade and a southern grade. All taxa of the northern clade are found in both eastern and western Beringia, except *C. joanneana*, which is restricted to western Beringia (Siberia). This pattern of migration from low latitudes in western North America through Beringia to northeastern Asia contrasts with the presumed predominant direction of migration (Murray 1981), although various taxa from North America have been hypothesized to have dispersed to Asia via Beringia (Hong 1983; Schultheis and Donoghue 2004).

The Beringian endemics of *Claytonia* have a distribution that fits Thorne's (1972) Beringian-Arctic disjunction. These plants occur on the Arctic shores on either side of the Bering Sea in North America and Eurasia, but do not extend far west or east of their Beringian center (e.g., they are not North ampho-Atlantic; Thorne 1972). This geographic pattern was suggested by Hultén (1937) to characterize taxa that survived the Pleistocene glaciations in northern refugia, such as Alaska–Yukon, northern Beringia and northeastern Siberia. Thorne (1972) suggested that Beringian-Arctic disjuncts might also have included formerly more circum-Arctic groups whose ranges were reduced by glaciation in eastern North America, Greenland and the European Arctic. This later explanation may account for the occurrence of eastern North American species of perennial *Claytonia* (i.e., *C. carolinina* and *C. virginica*) whose ranges are not entirely consistent with a Beringian–Arctic disjunction (Fig. 5A).

Hultén (1937) associated elements of the postglacial flora of Beringia with particular patterns of migration from Pleistocene refugia. He suggested that *C. arctica*, *C. acutifolia*, and *C. tuberosa* survived in a northern Beringian coastal refugium; *C. sarmentosa* in a southern Beringian refugium; and that *C. joanneana* was associated with a more continental western Beringian refugium. Based on our results, we infer that at least two clades of *Claytonia*, corresponding to sects. *Claytonia* and *Rhizomatosae*, would have been widespread in Beringia before the Pleistocene if Hultén's (1937) proposals accurately characterize the origins of current distributions.

The Beringian species of sects. *Claytonia* and *Rhizomatosae* have very little sequence variation in either of the sampled markers. This reflects either extremely low rates of sequence evolution or recent radiation. A recent radiation hypothesis is consistent with our proposal that two clades of perennial claytonias were widespread in Beringia prior to the Pleistocene

glaciations and that the isolation of populations in multiple refugia has promoted allopatric speciation.

Habitat. Ricklefs and Latham (1992), Peterson (1999), and Wiens (2004) have all argued that ecological niche conservatism over evolutionary time has played a key role in speciation. Niche fidelity combined with limited niche availability in a changing landscape acts to fragment widespread taxa (lineage splitting), leading to reproductive isolation, local adaptation and ultimately speciation (Ricklefs and Latham 1992; Peterson 1999; Wiens 2004). This hypothesis makes a prediction whose signature should be discernable in a phylogenetic framework: habitat preferences should be conserved across nodes, at least over short time scales, despite historical changes in ecosystems.

Habitat fidelity among perennial claytonias may reflect key functional traits. In sect. *Claytonia*, reconstructions indicate that a plesiomorphic seasonally dry moisture state is conserved across speciation events, even when there are accompanying shifts in elevation or latitude (Table 3). In two independent cases, however, a latitudinal shift from low to high is accompanied by a shift in the moisture regime from seasonally dry to persistently wet (*C. tuberosa* and *C. acutifolia*; Table 4; Figs. 3A, B). The Beringian endemics in sect. *Rhizomatosae* are derived from lower latitude western North American ancestors. The southern grade of this section includes species that are found in alpine to cool montane environments of the American west. Habitat character state reconstructions indicate that a shift to a persistently wet moisture regime and higher elevation habitats precede the origin of the high latitude clade. This result supports a hypothesis that pre-adapted taxa from southern alpine regions in western North America diversified in arctic habitats. Thus, our results are consistent with a hypothesis of niche conservatism across speciation in clades of perennial *Claytonia*.

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LITERATURE CITED

- ANDERSON, J. P. 1959. *Flora of Alaska and adjacent parts of Canada*. Ames: Iowa State University Press.
- APPLEQUIST, W. and R. S. WALLACE. 2001. Phylogeny of the Portulacaceae cohort based on *ndhF* sequence data. *Systematic Botany* 26: 406-419.
- BENTHAM, G. A. and J. D. HOOKER. 1862. Order XXIII Portulacaceae. Pp. 155-159 in *Genera Plantarum*. London: Reeve and Co.
- BLISS, L. C. 1971. Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics* 2: 405-437.
- BOIVIN, B. 1967. Énumération des plantes du Canada. III. Herbidées, 1^o partie: Digitae: Dimerae, Liberae. *Naturaliste Canadien* 93: 583-646.
- BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogeny reconstruction. *Evolution* 42: 795-803.

- CAROLIN, R. 1987. A review of the family Portulacaceae. *Australian Journal of Botany* 35: 383-412.
- CHAMBERS, K. L. 1963. *Claytonia nevadensis* in Oregon. *Leaflets of Western Botany* 10: 1-8.
- . 1993a. *Claytonia* L. (Portulacaceae). Pp. 898-900. in *The Jepson manual: higher plants of California*, ed. J. C. Hickman. Berkeley: University of California Press.
- . 1993b. *Montia* L. (Portulacaceae). Pp. 904. in *The Jepson manual: higher plants of California*, ed. J. C. Hickman. Berkeley: University of California Press.
- CODY, W. J. 1996. *Flora of the Yukon Territory*. Ontario, Ottawa: NRC Research Press.
- DAVIS, R. J. 1951. Nomenclatural recombinations in Idaho plants. *Madroño* 11: 143-144.
- . 1966. The North American perennial species of *Claytonia*. *Brittonia* 18: 285-303.
- DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, and J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 79: 249-265.
- DOYLE, J. J. 1983. Flavonoid races of *Claytonia virginica* (Portulacaceae). *American Journal of Botany* 70: 1085-1092.
- and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.
- ERIKSSON, T. 1999. AutoDecay, version 4.0 (program distributed by the author). Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm.
- FELLOWS, C. E. 1971. A cytotaxonomic study of the origin of *Claytonia washingtoniana*. M. S. thesis. Arcata, California: Humboldt State College.
- . 1975. Chromosome counts and a new combination in *Claytonia* sect. *Limnia* (Portulacaceae). *Madroño* 23: 296-297.

- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- FRIEDRICH, H.C. 1954. Neufunde aus Venezuela. *Mitteilungen der Botanischen Staatssammlung München* 9-10: 456-459.
- GRAHAM, A. 1999. *Late Cretaceous and Cenozoic history of North American vegetation, north of Mexico*. New York: Oxford University Press, Inc.
- GRAY, A. 1887. Contributions to American Botany. XV. 1. Revision of some polypetalous genera and orders precursory to the Flora of North America. *Proceedings of the American Academy of Arts and Sciences* 22: 270-314.
- and B. L. ROBINSON. 1897. *Claytonia* and *Montia*. Pp. 270-277 in *Synoptical flora of North America. Vol. 1 part 1*, ed. B. L. Robinson. New York: American Book Company.
- GREENE, E. L. 1891. *Flora Franciscana*. San Francisco: Curbey & Co.
- HEENAN, P. B. 1999. A taxonomic revision of *Neopaxia* Ö. Nilss. (Portulacaceae). *New Zealand Journal of Botany* 37: 213-234.
- HENRY, J. K. 1915. *Flora of southern British Columbia and Vancouver Island*. Toronto: W. J. Gage & Co., Limited.
- HERSHKOVITZ, M. A. 1993. Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on phylogeny of the portulacaceous alliance. *Annals of the Missouri Botanical Garden* 80: 333-365.
- and E. A. ZIMMER. 1997. On the evolutionary origin of the cacti. *Taxon* 46: 217-232.
- and —. 2000. Ribosomal DNA evidence and disjunctions of western American Portulacaceae. *Molecular Phylogenetics and Evolution* 15: 419-439.

- HOLM, T. 1905. *Claytonia* Gronov. A morphological and anatomical study. *Memoirs of the National Academy of Sciences* 10: 27-37.
- . 1913. Types of *Claytonia* Gronov. *Mindeskript for Japetus Steenstrup* 21: 1-11.
- HONG, D. 1983. The distribution of Scrophulariaceae in the Holarctic with special reference to the floristic relationships between eastern Asia and eastern North America. *Annals of the Missouri Botanical Garden* 70: 701-712.
- HOWELL, T. 1893. A rearrangement of American Portulacaceae. *Erythea* 1: 29-41.
- HULSENBECK, J. P. and F. RONQUIST. 2001. Mr. Bayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.
- HULTÉN, E. 1968. *Flora of Alaska and neighboring territories*. Stanford: Stanford University Press.
- . 1937. *Outline of the history of arctic and boreal biota during the Quarternary period*. Stockholm: Bokforlags Aktiebolaget Thule.
- . 1928. *Flora of Kamchatka and the adjacent islands*. Uppsala: Alquist & Wiksells Boktryckeri A. B.
- JOHNSON, L. A. and D. E. SOLTIS. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. s. *Systematic Botany* 19: 143-156.
- LOURTEIG, A. 1991. El género *Montia* (Portulacaceae) en el Hemisferio Austral. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales Correspondiente de la Española*. 18: 41-48.
- MADDISON, W. P. and D. R. MADDISON. 2000. *MacClade: analysis of phylogeny and character evolution*. Sunderland: Sinauer Associates.

- MCNEILL, J. 1972. New taxa of *Claytonia* section *Claytonia* (Portulacaceae). *Canadian Journal of Botany* 50: 1895-1898.
- . 1974. Synopsis of a revised classification of the Portulacaceae. *Taxon* 23: 725-728.
- . 1975. A generic revision of Portulacaceae tribe Montieae using techniques of numerical taxonomy. *Canadian Journal of Botany* 53: 789-809.
- and J. N. FINDLAY. 1971. The systematic position of *Claytonia bostockii*. *Canadian Journal of Botany* 49: 713-715.
- MILLER, J. M. 2003. *Claytonia* L. and *Montia* L. (Portulacaceae). Pp. 465-475; 485-488 in *Flora of North America north of Mexico, Volume 4 Magnoliophyta: Caryophyllidae, part 1*, eds. Flora of North America Committee. New York: Oxford University Press.
- and K. L. CHAMBERS. 1977. Chromosome numbers and relationships of *Claytonia saxosa* and *C. arenicola* (Portulacaceae). *Madroño*: 62-63.
- and —. 1993. Nomenclatural changes and new taxa in *Claytonia* (Portulacaceae). *Novon* 3: 268-273.
- MURRAY, D. F. 1981. The role of arctic refugia in the evolution of the arctic vascular flora--a Beringian perspective. Pp. 11-20 in *Evolution today*, Proceedings of the Second International Congress of Systematics and Evolutionary Biology. eds. G. G. E. Scudder and J. L. Reveal. Pittsburgh: Hunt Institute for Botanical Documentation.
- . 1995. Causes of arctic plant diversity: origin and evolution. Pp. 21-43 in *Arctic and alpine biodiversity: patterns, causes and ecosystems consequences*, eds. F. S. I. Chapin and C. Korner. Berlin: Springer-Verlag.
- NILSSON, Ö. 1966a. Studies in *Montia* L. and *Claytonia* L. and allied genera: I. Two new genera, *Mona* and *Paxia*. *Botaniska Notiser* 119: 265-285.

- . 1966b. Studies in *Montia* L. and *Claytonia* L. and allied genera II. Some chromosome numbers. *Botaniska Notiser* 119: 464-468.
- . 1967. Studies in *Montia* L. and *Claytonia* L. and allied genera III. Pollen Morphology. *Grana Palynologica* 7: 279-363.
- . 1970. Studies in *Montia* L. and *Claytonia* L. and allied genera IV. The genus *Crunocallis* Rydb. *Botaniska Notiser* 123: 119-148.
- . 1971a. Studies in *Montia* L. and *Claytonia* L. and allied genera V. The genus *Montiastrum* (Gray) Rydb. *Botaniska Notiser* 124: 87-121.
- . 1971b. Studies in *Montia* L. and *Claytonia* L. and allied genera VI. The genera *Limnalsine* Rydb. and *Maxia* Ö Nilss. *Botaniska Notiser* 124: 187-207.
- PAX, F. 1889. Portulacaceae. Pp. 51-60 in *Die natürlichen Pflanzenfamilien. Vol. 3, part 1b*, eds. A. Engler and K. Prantl. Leipzig: Wilhelm Englemann.
- and K. HOFFMAN. 1934. Portulacaceae. Pp. 234-262 in *Die natürlichen Pflanzenfamilien Vol. 16c*, eds. A. Engler & H. Harms. Leipzig: Wilhelm Englemann.
- PETERSON, A. T. 1999. Conservatism of ecological niches in evolutionary time. *Science* 285: 1265-1267.
- POLUNIN, N. 1959. *Circumpolar arctic flora*. London: Oxford University Press.
- POSADA, D. and K. A. CRANDALL. 1988. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- RAMBAUT, A. 1996. Se-Al Sequence alignment editor. Version 1.0 alpha 1. Distributed on the web at <http://evolve.zoo.ox.ac.uk/Se-Al/Se-Al.html>.

- RICKLEFS, R. E. and R. E. LATHAM. 1992. Intercontinental correlation of geographical ranges suggests stasis in ecological traits of relict genera of temperate perennial plants. *The American Naturalist* 139: 1305-1321.
- RONQUIST, F. 1996. DIVA, version 1.1. Computer program and manual available by anonymous FTP from Uppsala University (<http://ftp.uu.se>).
- . 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* 46: 195-203.
- RYDBERG, P. A. 1906. Studies on the Rocky Mountain flora. XVI. *Bulletin of the Torrey Botanical Club* 33: 137-161.
- . 1917. *Flora of the Rocky Mountains and adjacent plains*. New York: New York Botanical Garden.
- . 1932. Portulacaceae. Pp. 270-336 in *North American flora*. New York: New York Botanical Garden.
- SCHULTHEIS, L. M. and M. J. DONOGHUE. 2004. Molecular phylogeny and biogeography of *Ribes* (Grossulariaceae), with an emphasis on Gooseberries (subg. *Grossularia*). *Systematic Botany* 29: 77-96.
- SHARSMITH, C. W. 1938. On the identity of *Claytonia nevadensis* Watson. *Madroño* 4: 171-176.
- SHELLY, J. S., P. LESICA, P. G. WOLF, P. S. SOLTIS and D. E. SOLTIS. 1998. Systematic studies and conservation status of *Claytonia lanceolata* var. *flava* (Portulacaceae). *Madroño* 45:64-74.
- SOLTIS, D. E., P. S. SOLTIS, D. L. NICKRENT, L. A. JOHNSON, W. J. HAHN, S. B. HOOT, J. A. SWEERE, R. K. KUZOFF, K. A. KRON, M.W. CHASE, S. M. SWENSEN, E. A. ZIMMER, S. M. CHAW, L. J. GILLESPIE, W. J. KRESS and K. J. SYTSMA. 1997. Angiosperm phylogeny

- inferred from 18S ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* 84: 1-49.
- STEWART, D. and D. WIENS. 1971. Chromosome races in *Claytonia lanceolata* (Portulacaceae). *American Journal of Botany* 58: 41-47.
- SWANSON, J. R. 1966. A synopsis of relationships in Montioideae (Portulacaceae). *Brittonia* 18: 229-240.
- SWOFFORD, D. L. 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods). Sunderland: Sinauer Associates.
- THORNE, R. F. 1972. Major disjunctions in the geographic ranges of seed plants. *The Quarterly Review of Biology* 47: 365-411.
- TOLMACHEV, A. L. and B. A. YURTSEV. 1996. Portulacaceae. Pp. 195-205 in *Flora of the Russian Arctic. Vol. III: Salicaceae - Ranunculaceae*, ed. J. G. Packer, translated by G. C. D. Griffiths. Edmonton: University of Alberta Press.
- VON POELLNITZ, K. 1932. *Claytonia* Gronov. and *Montia* Mich. *Feddes Repertorium Specierum Novarum Regni Vegetabilis*. 30: 279-325.
- WIENS, J. J. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution* 58: 193-197.
- YURTSEV, B. A. and N. N. TSVELYOV 1972. New taxa from north-east Asia. *Botanicheski Zhurnal*. 57: 644-647.
- . 1981. Seven new taxa of flowering plants from the northeastern Asia and adjacent territories. *Botanicheski Zhurnal*. 66: 1041-1043.

TABLE 1. Taxonomic treatments of Montieae.

Swanson (1966)	Davis (1951, 1966); treated perennials only, except for <i>M. fontana</i>	Nilsson (1966a, b, c; 1967; 1970; 1971a, b) did not treat <i>Claytonia</i> .	McNeill (1975)	Miller (2003); treated only North American taxa.	O'Quinn and Hufford (present paper)
<i>Claytonia</i>	<i>Claytonia</i>	<i>Claytonia</i>	<i>Claytonia</i>	<i>Claytonia</i>	<i>Claytonia</i>
Sect. <i>Claytonia</i>	<i>caroliniana</i>		Sect. <i>Claytonia</i>	<i>caroliniana</i>	Sect. <i>Claytonia</i>
<i>caroliniana</i>	<i>virginica</i>	<i>Montiastrum</i>	<i>caroliniana</i>	<i>virginica</i>	<i>caroliniana</i>
<i>virginica</i>	var. <i>virginica</i>	<i>vassilievii</i>	var. <i>caroliniana</i>	<i>lanceolata</i>	<i>virginica</i>
<i>lanceolata</i>	var. <i>simsii</i>	<i>bostockii</i>	var. <i>lewisii</i>	<i>multiscapa</i>	<i>lanceolata</i>
<i>tuberosa</i>	var. <i>lutea</i>	<i>linearis</i>	<i>czukczorum</i> (= <i>C. multiscapa</i>)	<i>rosea</i>	var. <i>peirsonii</i>
<i>umbellata</i>	var. <i>robusta</i>	<i>dichotoma</i>	<i>virginica</i>	<i>tuberosa</i>	<i>multiscapa</i>
Sect. <i>Caudicosae</i>	<i>lanceolata</i>		var. <i>virginica</i>	<i>ogilviensis</i>	<i>rosea</i>
<i>megarhiza</i>	var. <i>lanceolata</i>	<i>Maxia</i>	var. <i>acutiflora</i>	<i>umbellata</i>	<i>tuberosa</i>
<i>acutifolia</i>	var. <i>flava</i>	<i>howellii</i>	(= var. <i>simsii</i>)	<i>megarhiza</i>	<i>ogilviensis</i>
<i>arctica</i>	(= <i>C. multiscapa</i>)	<i>Neopaxia</i>	<i>lanceolata</i>	<i>acutifolia</i>	<i>umbellata</i>
<i>eschscholtzii</i> (= <i>C. acutifolia</i> subsp. <i>graminifolia</i>)	var. <i>sessilifolia</i>	<i>australasica</i>	var. <i>lanceolata</i>	<i>arctica</i>	<i>megarhiza</i>
<i>sibirica</i>	var. <i>peirsonii</i>		var. <i>chrysantha</i>	<i>scammaniana</i>	var. <i>megarhiza</i>
Sect. <i>Rhizomatosae</i>	var. <i>rosea</i> (= <i>C. rosea</i>)	<i>Limnalsine</i>	(= <i>C. multiscapa</i>)	<i>sarmentosa</i>	var. <i>bellidifolia</i>
<i>nevadensis</i>	var. <i>idahoensis</i>	<i>diffusa</i>	var. <i>flava</i> (= <i>C. multiscapa</i>)	<i>cordifolia</i>	var. <i>nivalis</i>
<i>scammaniana</i>	<i>tuberosa</i>		var. <i>idahoensis</i>	<i>nevadensis</i>	<i>acutifolia</i>
<i>sarmentosa</i>	<i>megarhiza</i>	<i>Naiocrene</i>	var. <i>multiscapa</i>	<i>arenicola</i>	Sect. <i>Rhizomatosae</i>
<i>cordifolia</i>	var. <i>megarhiza</i>	<i>parvifolia</i>	(= <i>C. multiscapa</i>)	<i>palustris</i>	<i>arctica</i>
<i>heterophylla</i>	var. <i>bellidifolia</i>		var. <i>pacifica</i>	<i>sibirica</i>	<i>joanneana</i>
(= <i>C. sibirica</i>)	var. <i>nivalis</i>	<i>Crunocallis</i>	var. <i>virginica</i>	<i>exigua</i>	<i>scammaniana</i>
Sect. <i>Limnia</i>	<i>eschscholtzii</i> (= <i>C. acutifolia</i> subsp. <i>graminifolia</i>)	<i>chamissoi</i>	var. <i>peirsonii</i>	subsp. <i>exigua</i>	<i>porcildii</i>
<i>arenicola</i>		<i>calcicola</i>	var. <i>sessilifolia</i>	subsp. <i>glauca</i>	<i>sarmentosa</i>
<i>spathulata</i> (= <i>C. parviflora</i> subsp. <i>viridis</i>)	<i>umbellata</i>		var. <i>rosea</i>	<i>gypsophiloides</i>	<i>cordifolia</i>
<i>saxosa</i>	<i>arctica</i>	<i>Mona</i>	<i>tuberosa</i>	<i>saxosa</i>	<i>nevadensis</i>
<i>perfoliata</i> (includes <i>C. parviflora</i> and all subspecies)	<i>scammaniana</i>	<i>meridensis</i>	<i>ogilviensis</i>	<i>perfoliata</i>	<i>arenicola</i>
<i>depressa</i> (= <i>C. rubra</i> subsp. <i>depressa</i>)	<i>bostockii</i>		<i>umbellata</i>	subsp. <i>perfoliata</i>	Sect. <i>Limnia</i>
	<i>sarmentosa</i>	<i>Montia</i>	Sect. <i>Caudicosae</i>	subsp.	<i>palustris</i>
<i>Montia</i>	<i>parvifolia</i>	<i>fontana</i>	<i>megarhiza</i>	<i>intermontana</i>	<i>sibirica</i>
Sect. <i>Naiocrene</i>	var. <i>parvifolia</i>		var. <i>megarhiza</i>	subsp. <i>mexicana</i>	var. <i>sibirica</i>
<i>parvifolia</i>	var. <i>flagellaris</i>		var. <i>bellidifolia</i>	<i>parviflora</i>	var. <i>bulbillifera</i>
Sect. <i>Montiastrum</i>	<i>sibirica</i>		var. <i>nivalis</i>	subsp. <i>viridis</i>	<i>exigua</i>
<i>linearis</i>	var. <i>cordifolia</i> (= <i>C. cordifolia</i>)		<i>acutifolia</i>	subsp. <i>utahensis</i>	subsp. <i>exigua</i>
<i>dichotoma</i>	<i>nevadensis</i>		<i>eschscholtzii</i> (= <i>C. acutifolia</i> subsp. <i>graminifolia</i>)	subsp. <i>parviflora</i>	subsp. <i>glauca</i>
Sect. <i>Limnalsine</i>	<i>fontana</i>		<i>arctica</i>	subsp. <i>grandiflora</i>	<i>gypsophiloides</i>
<i>diffusa</i>			<i>joanneana</i>	<i>washingtoniana</i>	<i>saxosa</i>
Sect. <i>Montia</i>			Sect. <i>Rhizomatosae</i>	<i>rubra</i>	<i>perfoliata</i>
<i>australasica</i>			<i>scammaniana</i>	subsp. <i>rubra</i>	subsp. <i>perfoliata</i>
<i>chamissoi</i>			<i>sarmentosa</i>	subsp. <i>depressa</i>	subsp.
<i>fontana</i>			<i>cordifolia</i> (= <i>C. asarifolia</i>)	<i>Montia</i>	<i>intermontana</i>
<i>howellii</i>			<i>nevadensis</i> (incl. <i>C. chenopodina</i>)	<i>bostockii</i>	subsp. <i>mexicana</i>
			Sect. <i>Limnia</i>	<i>linearis</i>	<i>parviflora</i>
			<i>arenicola</i>	<i>dichotoma</i>	subsp. <i>viridis</i>
			<i>spathulata</i> (= <i>C. parviflora</i>)	<i>howellii</i>	subsp. <i>utahensis</i>
			var. <i>spathulata</i>	<i>diffusa</i>	subsp. <i>parviflora</i>
			var. <i>exigua</i>	<i>parvifolia</i>	subsp. <i>grandiflora</i>
			var. <i>rosulata</i>	<i>chamissoi</i>	<i>washingtoniana</i>
			var. <i>tenuifolia</i>	<i>fontana</i>	<i>rubra</i>
			var. <i>viridis</i> (= <i>C. parviflora</i> subsp. <i>viridis</i>)		subsp. <i>rubra</i>
			<i>gypsophiloides</i>		subsp. <i>depressa</i>
			<i>saxosa</i>		
			<i>perfoliata</i>		<i>Montia</i>
			var. <i>perfoliata</i>		Sect. <i>Montiastrum</i>
			var. <i>angustifolia</i>		<i>vassilievii</i>
					<i>bostockii</i>
					<i>linearis</i>
					<i>dichotoma</i>
					Sect. <i>Australiensis</i>
					<i>howellii</i>
					<i>australasica</i>
					<i>erythrophylla</i>
					<i>racemosa</i>
					<i>sessilifolia</i>

	<i>drucei</i>
var. <i>depressa</i> (= <i>C.</i> <i>rubra</i> subsp. <i>depressa</i>)	<i>campylostigma</i> <i>calycina</i>
var. <i>nubigena</i> (= <i>C.</i> <i>gypsophiloides</i>)	<i>linearifolia</i>
var. <i>parviflora</i> (= <i>C.</i> <i>parviflora</i>)	Sect. <i>Montia</i> <i>diffusa</i>
<i>heterophylla</i> (= <i>C.</i> <i>sibirica</i>)	<i>parvifolia</i> (incl. <i>flagellaris</i>)
<i>sibirica</i>	<i>chamissoi</i>
var. <i>sibirica</i>	<i>calcicola</i>
var. <i>bulbillifera</i>	<i>fontana</i> <i>meridensis</i>
<i>Montia</i>	<i>incertae sidis</i>
Sect. <i>Claytoniella</i>	<i>biapiculata</i>
<i>vassilievii</i>	
<i>bostockii</i>	
Sect. <i>Montiastrum</i>	
<i>linearis</i>	
<i>dichotoma</i>	
Sect. <i>Maxia</i>	
<i>howellii</i>	
Sect. <i>Australiensis</i>	
<i>australiensis</i> (incl. <i>calycina</i>)	
Sect. <i>Limnalsine</i>	
<i>diffusa</i>	
Sect. <i>Naiocrene</i>	
<i>parvifolia</i>	
<i>flagellaris</i>	
Sect. <i>Alsinastrum</i>	
<i>chamissoi</i>	
<i>calcicola</i>	
Sect. <i>Mona</i>	
<i>meridensis</i>	
Sect. <i>Montia</i>	
<i>fontana</i>	

TABLE 2. Habitat character state assignments for perennial *Claytonia*. SD = seasonally dry, PW = persistently wet; H = high elevation/latitude, L = low elevation/latitude.

Taxon	Moisture	Elevation/Latitude
Section		
<i>Rhizomatosae</i>		
<i>C. arenicola</i>	SD	L
<i>C. nevadensis</i>	PW	H
<i>C. cordifolia</i>	PW	L
<i>C. arctica</i>	PW	H
<i>C. scammaniana</i>	PW	H
<i>C. sarmentosa</i>	PW	H
<i>C. joanneana</i>	PW	H
Section <i>Claytonia</i>		
<i>C. acutifolia</i>	PW	H
<i>C. umbellata</i> OR	SD	L
<i>C. umbellata</i> CA	SD	H
<i>C. megarhiza</i>	SD	H
<i>C. ogilviensis</i>	SD	H
<i>C. lanceolata</i>	SD	L
<i>C. virginica</i>	SD	L
<i>C. tuberosa</i>	PW	H
<i>C. caroliniana</i>	SD	L

TABLE 3. Habitat state reconstructions for perennial *Claytonia* from MacClade and DIVA for maximum parsimony and Bayesian inference trees. Node numbers refer to figure 3 A-E. SD = seasonally dry, PW = persistently wet; H = high elevation/latitude, L = low elevation/latitude (; = “or” and / = “both”).

Maximum Parsimony Tree (Figs. 3A, C)					
Node #	Moisture		Elevation/Latitude		
	MacClade Del./Acc.	DIVA	MacClade Del.	Acc.	DIVA
1	SD	SD; PW	L; H	L; H	L; L/H
2	SD	SD; PW	L; H	H	L; H; L/H
3	SD	SD/PW	L; H	H	L; L/H
4	PW	PW	L; H	H	H; L/H
5	PW	PW	L; H	H	L/H
6	PW	PW	H	H	H
7	PW	PW	H	H	H
8	PW	PW	H	H	H
9	SD	SD/PW	L; H	H	H;L/H
10	SD	SD	L; H	H	L; H
11	SD	SD	L; H	H	L/H
12	SD	SD	L	L	L
13	SD	SD	L	L	L
14	SD	SD/PW	L	L	L/H
15	SD	SD	H	H	H
16	SD	SD	L; H	H	L/H

Bayesian Inference Tree (Figs. 3B, D, E)					
Node #	Moisture		Elevation/Latitude		
	MacClade Del./Acc.	DIVA	MacClade Del.	Acc.	DIVA
1	SD	SD	L	L	L
2	SD	SD	L	L	L
3	SD	SD/PW	L	L	L
4	PW	PW	L	H	L;H/L
5	PW	PW	L	H	H;H/ L
6	PW	PW	H	H	H
7	PW	PW	H	H	H
8	PW	PW	H	H	H
9	SD	SD	L	L	H/L
10	SD	SD	L	L	L
11	SD	SD	L	L	L
12	SD	SD	L	L	H/L

13	SD	SD	L	L	L
14	SD	SD/PW	L	L	H/L
15	SD	SD	H	H	H
16	SD	SD/PW	H	H	H

FIGURE LEGENDS

FIGURE 1. Cladograms from maximum parsimony analyses of Montieae. Bootstrap proportions $\geq 50\%$ are above clades. Decay values are in parentheses below clades. Geographic locations for multiple samples of the same taxon appear after the name. A-B. ITS topologies. A. Strict consensus of 420 most parsimonious trees from analysis of full ITS data set (C. I. = 0.5767, R. I. = 0.8364, R. C. = 0.5298). B. Strict consensus of 28 most parsimonious trees from analysis of the reduced ITS data set (C. I. = 0.5879, R. I. = 0.7951, R. C. = 0.5110). C. Strict consensus of 12 most parsimonious trees from analysis of the *trnK/matK* data set (C. I. = 0.7200, R. I. = 0.8694, R. C. = 0.7023).

FIGURE 2. Maximum parsimony, maximum likelihood and Bayesian inference cladograms from analyses of the combined ITS and *trnK/matK* data sets. Geographic locations for multiple samples of the same taxon appear after the name. A. Strict consensus of 56 most parsimonious trees (C. I. = 0.6229, R. I. = 0.8131, R. C. = 0.5693). Bootstrap proportions $\geq 50\%$ are above clades. Decay values are in parentheses below clades. B. Maximum likelihood phylogram (-ln likelihood = 9109.71). Bootstrap proportions $\geq 50\%$ are above clades. C. Bayesian inference 50% majority rule consensus tree. Averaged posterior probabilities from five independent analyses are above clades.

FIGURE 3. Biogeographic reconstructions on two randomly selected, trees from maximum parsimony (MP) and Bayesian inference (BI) analyses of the combined ITS and *trnK/matK* data. A-B. DIVA reconstructions. Perennial clades are highlighted in gray. Letters represent geographic character state assignments (see key). Numbers at nodes refer to ecological

reconstructions (Tables 2, 3). A. MP tree. B. BI tree. C-E. MacClade reconstructions for perennial *Claytonia*. Branch shading corresponds to the geographic states described in the key. D. MP tree showing the identical results of ACCTRAN and DELTRAN optimizations. E. BI tree with ACCTRAN optimization. C. BI tree with DELTRAN optimization.

FIGURE 4. Geographic distributions for perennial *Claytonia*. A. *Claytonia* sect. *Claytonia*. B. *Claytonia* sect. *Rhizomatosae*.

APPENDIX 1. Montieae sampled for phylogenetic study, including collection and voucher location and Genbank accession numbers for ITS (AY764037-AY764087) and *trnK/matK* (AY764088-AY764127).

INGROUP:

Claytonia

- C. acutifolia* Pallas ex Willd. (C. L. Parker, A. R. Batten, M. Duffy & J. Cole 7322, Norton Bay Quad, AK) [ALA]; ITS AY764047; *trnK/matK* AY764097
- C. arctica* Adams (B. F. Freidman 82/67, Atka Quad, AK) [ALA]; ITS AY764046; *trnK/matK* AY764096
- C. arenicola* Henderson (R. O'Quinn 480, Whitman Co., WA) [WS]; ITS AY764037; *trnK/matK* AY764088
- C. caroliniana* Michaux. (C. E. Hellquist 1086, Berkshire Co., MA) [WS]; ITS AY764049; *trnK/matK* AY764099
- C. caroliniana* Michaux. (S. Bartos s.n., Grafton Co., NH) [WS]; ITS AY764048; *trnK/matK* AY764098
- C. cordifolia* S. Watson (L. Kinter 3252, Latah Co., ID) [WS]; ITS AY764050; *trnK/matK* AY764100
- C. exigua* subsp. *exigua* (L.) Chambers (R. O'Quinn 306, Humboldt Co., CA) [WS]; ITS AY764038; *trnK/matK* AY764089
- C. gypsophiloides* Fischer & C. A. Meyer (R. O'Quinn 348, grown from seed K. L. Chambers 6182) [WS]; ITS AY764039; *trnK/matK* AY764090
- C. joanneana* Roem. & Schult. (T. Elias, S. Shetler and D. Murray 8026, West Sayan Mtns., southern Siberia, Russia) [ALA]; ITS AY764051; *trnK/matK* AY764101
- C. lanceolata* Pursh. (R. O'Quinn & C. R. Björk 227, Latah Co., ID) [WS]; ITS AY764052; *trnK/matK* AY764102
- C. megarhiza* (A.Gray) Parry ex S. Watson var. *megarhiza* (C. R. Björk 4765, Union Co., OR) [WS]; ITS AY764053; *trnK/matK* AY764103
- C. megarhiza* var. *ivalis* (English) C. L. Cronquist (R. O'Quinn 517, Chelan Co., WA) [WS]; ITS AY764054
- C. megarhiza* (A.Gray) ITS- L78027
- C. nevadensis* Watson (R. O'Quinn 479, Harney Co., OR) [WS]; ITS AY764055; *trnK/matK* AY764104
- C. ogilviensis* McNeill (W. J. Cody & J. H. Ginns 34165, Ogilvie & Werneke Mtns., YT, CAN) [DAO]; ITS AY764056; *trnK/matK* AY764105
- C. palustris* Kelley & Swanson (R. O'Quinn 330, Butte Co., CA) [WS]; ITS AY764057; *trnK/matK* AY764106
- C. parviflora* Hook. subsp. *grandiflora* J. M. Miller & Chambers (R. O'Quinn s.n., grown from seed K. L. Chambers 5398) [WS]; ITS AY764041; *trnK/matK* AY764092
- C. parviflora* Hook. subsp. *parviflora* (ROQ 311, Lake Co., CA) [WS]; ITS AY764042
- C. perfoliata* Donn ex Willd. (ROQ 237, Multnomah Co., OR) [WS]; ITS AY764040; *trnK/matK* AY764091
- C. porsildii* Jurtz. (A. P. Khokhryakov, B.A. Yurtsev & D. F. Murray 6557, Philip Mtns. Quad, AK) [ALA]; ITS AY764058
- C. rubra* (Howell) Tidestrom (R. O'Quinn 267, Siskyou Co., CA) [WS]; ITS AY764043; *trnK/matK* AY764093

C. saxosa Brandegee (R. O'Quinn 324, Colusa Co., CA) [WS]; ITS AY764044; *trnK/matK* AY764094

C. sarmentosa C. A. Meyer (R. O'Quinn 401, Eagle Summit, AK) [WS]; ITS AY764059; *trnK/matK* AY764107

C. scammaniana Hultén (C. L. Parker & C. R. Meyers 10592, Noatak National Preserve, Howard Pass Quad, AK) [WS]; ITS AY764060; *trnK/matK* AY764108

C. sibirica L. var. *sibirica* (R. O'Quinn 235, Multnomah Co., OR) [WS]; ITS AY764061; *trnK/matK* AY764109

C. sibirica L. var. *sibirica* (R. O'Quinn 477, Shasta Co., CA) [WS]; ITS AY764062

C. tuberosa Pallas ex Willd. (R. O'Quinn 462, Mt. Fairplay, AK) [WS]; ITS AY764063; *trnK/matK* AY764110

C. tuberosa Pallas ex Willd. (R. O'Quinn 434, Dempster Hwy., YT, CAN) [WS]; ITS AY764064

C. umbellata S. Watson (R. O'Quinn 527, Wasco Co., OR) [WS]; ITS AY764065; *trnK/matK* AY764112

C. umbellata S. Watson (Bell and Johnson 711, Mono Co., CA) [WS]; ITS AY764066; *trnK/matK* AY764111

C. virginica L. (L. Kinter 3251, Berkeley Co., SC) [WS]; ITS AY764067; *trnK/matK* AY764113

C. washingtoniana (Suksd.) Suksd. (R. O'Quinn 291, Del Norte Co., CA) [WS]; ITS AY764045; *trnK/matK* AY76495

Neopaxia

N. australasica (Hook. f.) Ö.Nilss. (A. Strid 22081, Victoria, AUS) [CHR]; ITS AY764079

N. calycina (Colenso) Heenan (M. Rixon, P. Thomas, V. Tregidda, M. F. Watson, ENZAT # 161, Tongariro Evol. Region, N. Z.) [CHR]; ITS AY764080

N. campylostigma Heenan (P. B. Heenan and M. Leiffering 54/95, Canterbury Land District, Cameron River, N. Z.) [CHR]; ITS AY764081

N. erythrophylla Heenan (P. B. Heenan, Canterbury Land District Torlesse Range, N. Z.) [CHR]; ITS AY764082; *trnK/matK* AY764123

N. linearifolia Heenan (E. Edgar, Canterbury Land District, Red Hills, N. Z.) [CHR]; ITS AY764083

N. racemosa (Buchanan) Heenan (P. B. Heenan, Marlborough Land District, N. Z.) [CHR]; ITS AY764084; *trnK/matK* AY764124

Montia

M. bostockii A. E. Porsild (R. O'Quinn 419, Kluane National Park, YT, CAN) [WS]; ITS AY764068; *trnK/matK* AY764114

M. chamissoi Ledebour ex Sprengel (R. O'Quinn 478, Harney Co., OR) [WS]; ITS AY764069; *trnK/matK* AY764120

M. dichotoma (Nuttall.) Howell (R. O'Quinn s.n., grown from seed M. Fishbein 4294) [WS]; ITS AY764070; *trnK/matK* AY764115

M. diffusa (Nuttall.) Greene (F. Bowcutt 1987, Mendocino Co., CA) [DAV]; ITS AY764071; *trnK/matK* AY764121

M. fontana L. (S. Marcus s.n., Umatilla Co., OR) [WS]; ITS AY764073; *trnK/matK* AY764119

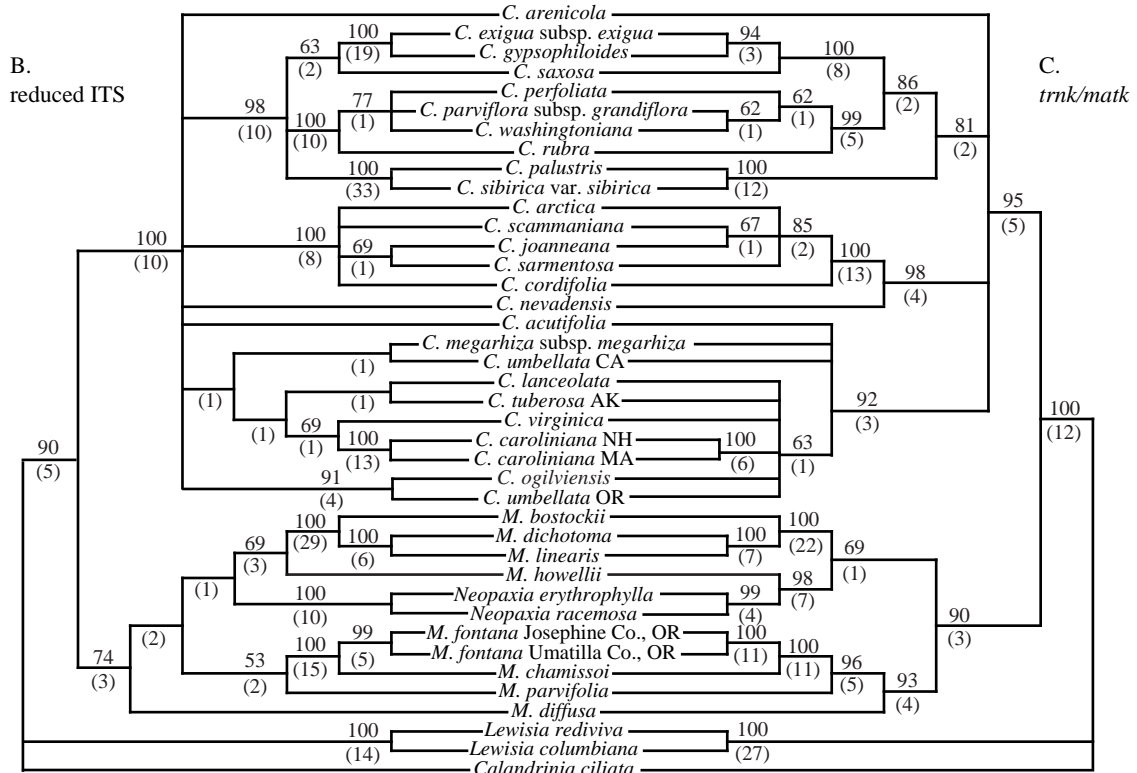
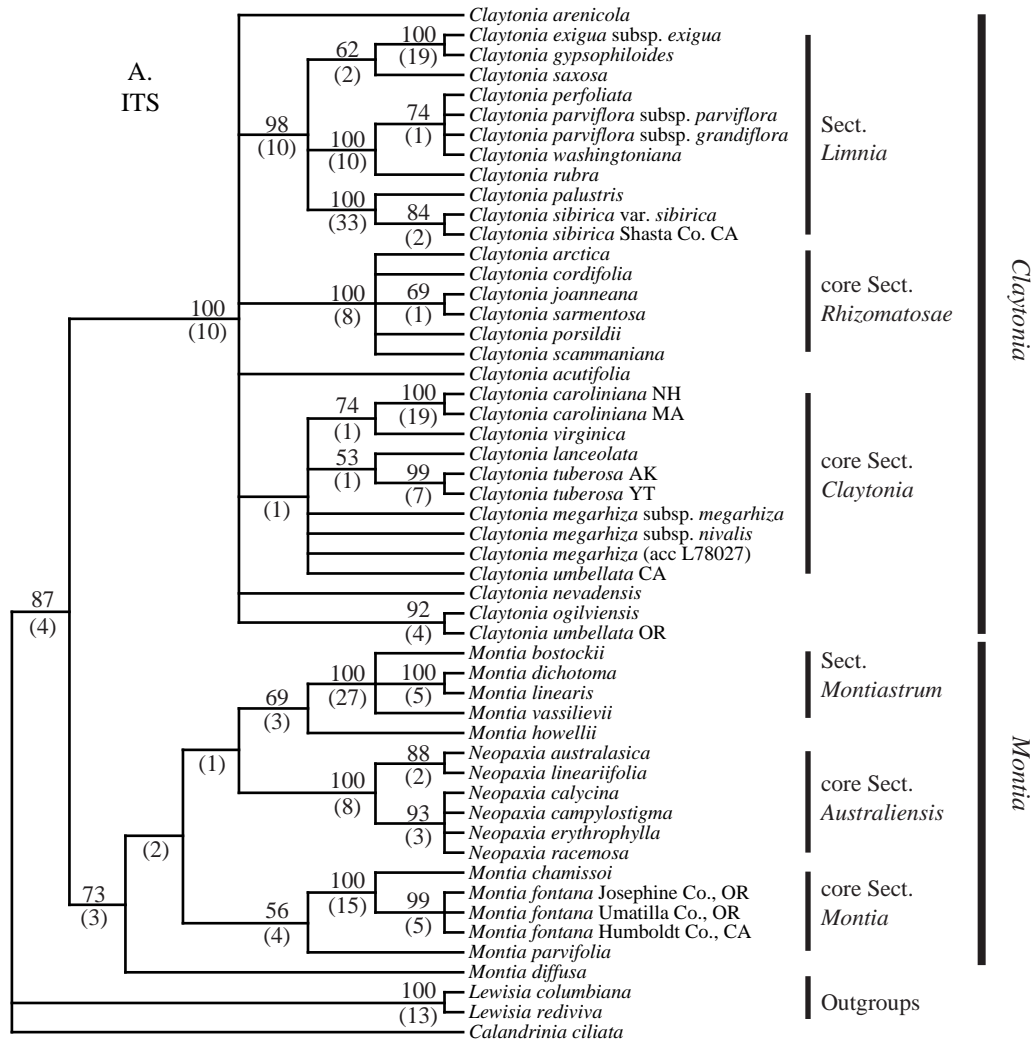
M. fontana L. (R. O'Quinn 542, Josephine Co., OR) [WS]; ITS AY764072; *trnK/matK* AY764118

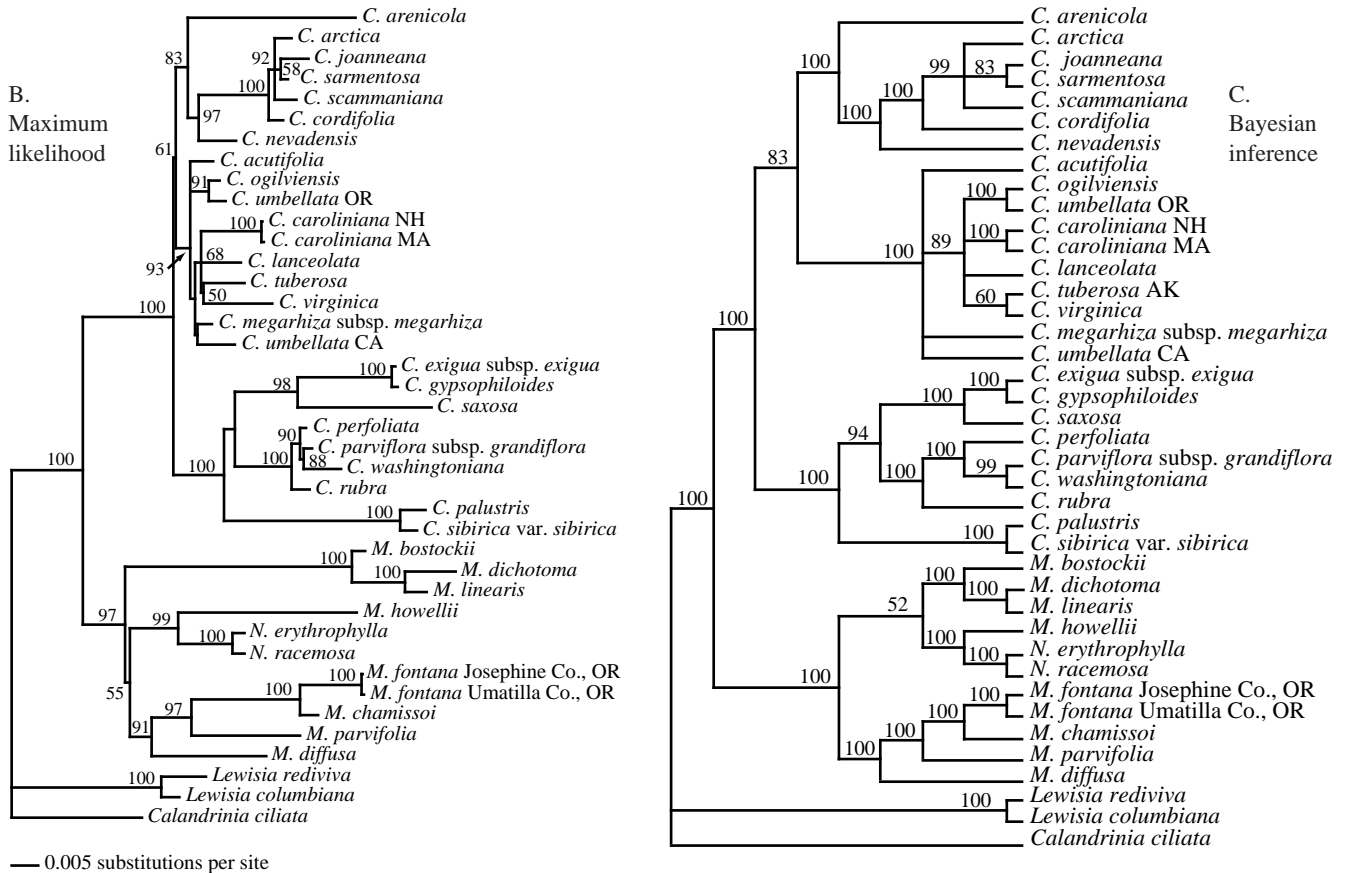
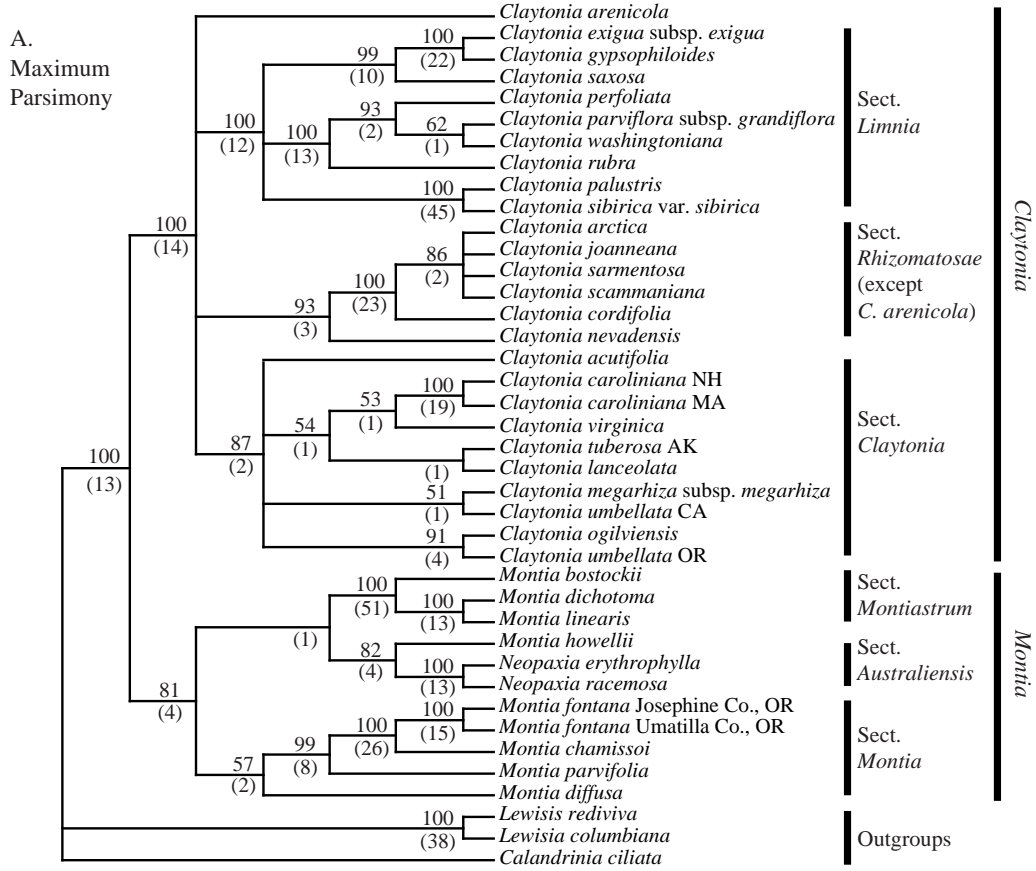
M. fontana L. (J. Pykälä 1623, Humboldt Co., CA) [DAO]; ITS AY764074

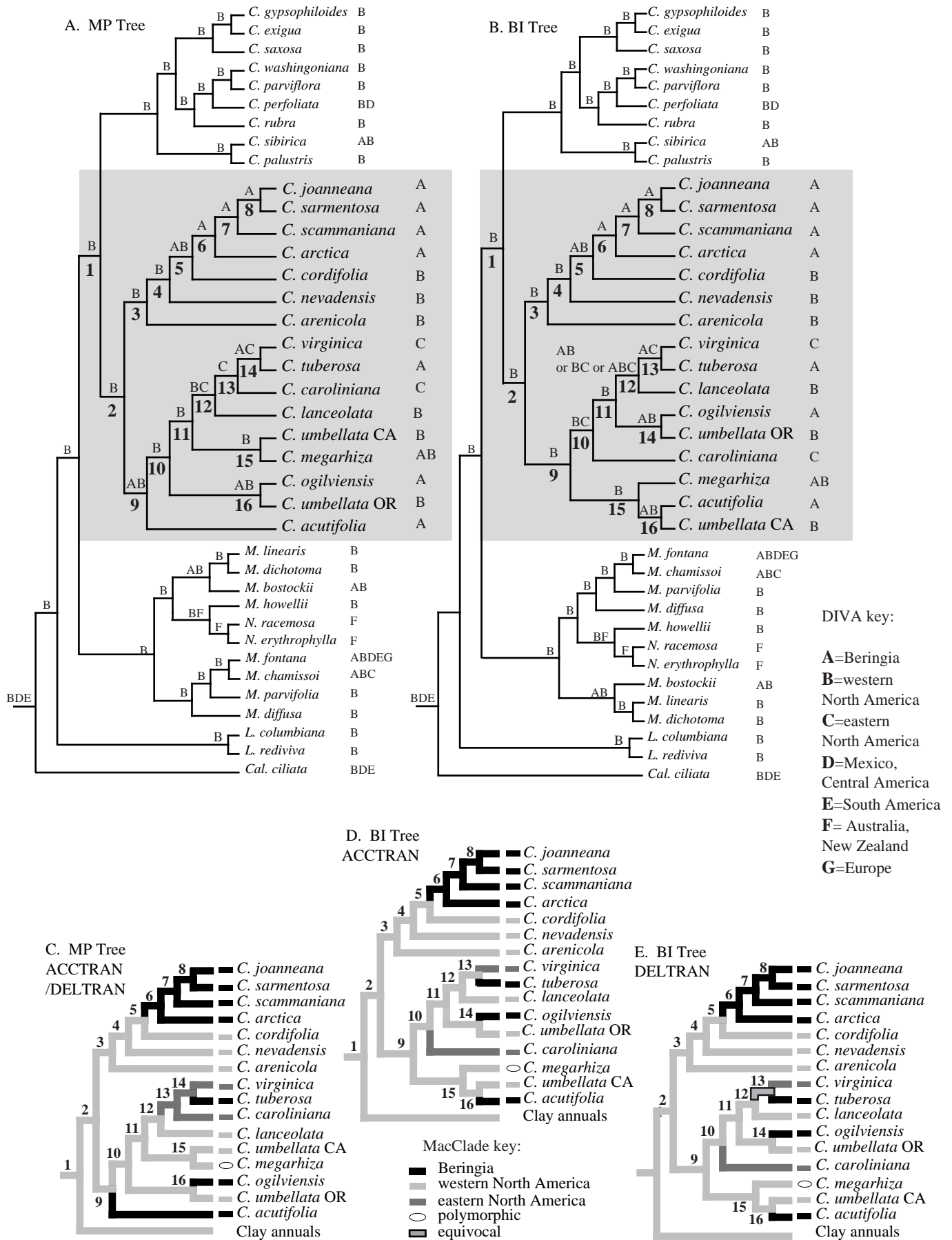
M. howellii S. Watson (R. O'Quinn & K. L. Chambers 531, Benton Co. OR) [WS]; ITS AY764075; *trnK/matK* AY764117
M. linearis (Doug. ex Hook.) Greene (R. O'Quinn & C. R. Björk 226, Latah Co., ID) [WS]; ITS AY764076; *trnK/matK* AY764116
M. parvifolia (de Candolle) Greene (R. O'Quinn 241, Multnomah Co., OR) [WS]; ITS AY764077; *trnK/matK* AY764122
M. vassilievii (Kuzeneva) McNeill (B. Yurtsev, P. G. Zukova, V. Y. Raszivin, N. A. Sekretareva, Koryzak Mtns.) [ALA]; ITS AY764078

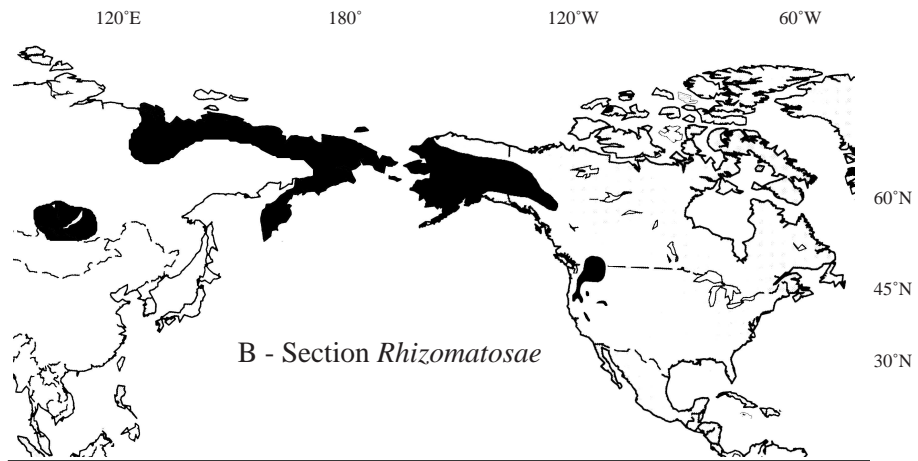
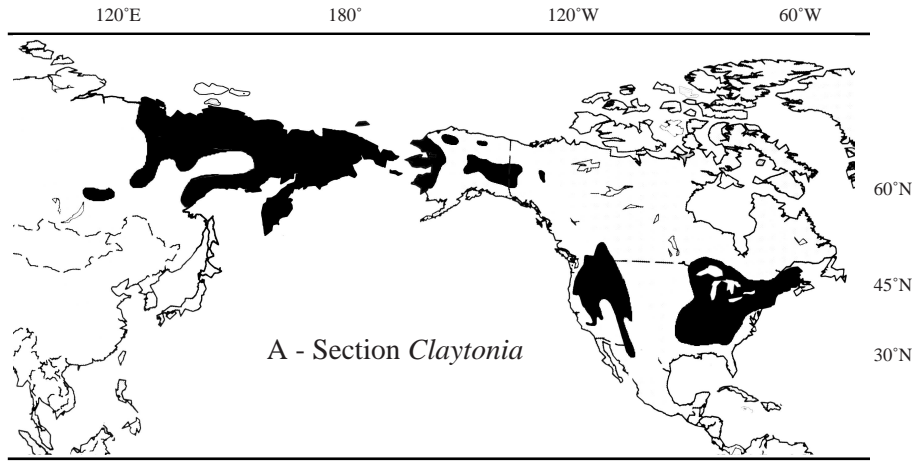
OUTGROUPS:

Lewisia rediviva Pursh var. *rediviva* (R. O'Quinn 272, Siskiyou Co. CA) [WS]; ITS AY764086; *trnK/matK* AY764125
Lewisia columbiana (Howell ex A. Gray) B. L. Robinson var. *columbiana* (R. O'Quinn s.n. Kittitas Co., WA) [WS]; ITS AY764085; *trnK/matK* AY764126
Calandrinia ciliata (Ruiz & Pavón) de Candolle (M. Fishbein 4429, Municipio de Cucurpe, Sonora, MEX) [WS]; ITS AY764087; *trnK/matK* AY764127









CHAPTER TWO

HOMOLOGY OF SUBTERRANEAN PERENNATION STRUCTURES IN *CLAYTONIA* SECT. *CLAYTONIA*

(PORTULACACEAE)

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Abstract

Perennation structures in *Claytonia* exhibit considerable morphological diversity, which has figured prominently in traditional taxonomic classifications. Recent molecular analyses indicate that these classifications do not reflect monophyletic groups and raise questions about inferences of structural homology. We use anatomical characters of roots and shoots to assess the structural homologies of perennation structures in *Claytonia* section *Claytonia* to better understand the basis of morphological diversity. Fully subterranean, globose to ovoid perennation structures of *C. lanceolata*, *C. tuberosa*, *C. umbellata*, and *C. virginica* are predominantly shoot, whereas the elongate perennation structures of *C. megarhiza* consist of both root and shoot. A critical difference among taxa centers on persistence of the primary root beyond the first season. We hypothesize that the loss of the primary root is associated with ecological shifts in substrate and from alpine to lower elevation habitats.

Shoot architectural variations, expressed as specializations for perennation, in *Claytonia* (Portulacaceae) represent novel solutions for persistence in diverse environments. Since Gray's (1887) revision of *Claytonia*, architectural variations have been especially important for delimiting subgeneric groups (Gray 1887, Swanson 1966, McNeill 1975). Gray (1887) distinguished taxa that had underground perennation structures (i.e. corms, thickened caudices with taproots, or "rootstocks") from those lacking them, and he further divided the former group by the morphology of their perennation structures. The resulting classification, which recognized three sections of perennial *Claytonia* has been largely followed by subsequent workers (Howell 1889; Greene 1891; Swanson 1966; McNeill 1975). Recent molecular phylogenetic analyses (O'Quinn and Hufford in press), however, found Gray's (1887) species groups were not monophyletic. Notably, O'Quinn and Hufford (in press) found Gray's (1887) sect. *Caudicosae*, which he distinguished by elongate perennation structures, to be polyphyletic; members of Gray's *Caudicosae* are phylogenetically part of the two perennial clades designated as sections *Claytonia* and *Rhizomatosae*. The phylogenetic results call into question the homology assessments that earlier workers have relied on as a basis for classifications and imply that the similar forms once grouped in sect. *Caudicosae* may have evolved independently. Such errors of homology assessment can lead to underestimates of morphological diversification in clades.

Chambers (1963) recognized the taxonomic value of morphological diversity in the underground structures of the *Claytonia* and *Montia* and called attention to the need for thorough morphological investigations for rigorous homology assessment. Morphological interpretations of underground structures in *Claytonia* sect. *Claytonia* alone have varied widely among previous workers. For example, the globose perennation structures of *C. caroliniana*, *C. lanceolata*, and

C. virginica were identified as roots by Holm (1905, 1913), one of the few workers to investigate the anatomy of *Claytonia*; Swanson (1966) referred to them as both corms (modified shoots) and roots; and Grandtner and Gervais (1990) determined they were stems. Resolving basic questions about the construction of perennation structures and homologies among taxa are essential for understanding the diversity of *Claytonia*. The phylogenetic hypotheses of O'Quinn and Hufford (in press) provide a framework for focusing comparative morphological studies on monophyletic groups, namely here on *Claytonia* sect. *Claytonia*. Our objectives are to characterize the anatomy and morphology of perennation structures in sect. *Claytonia* to better distinguish whether they consist of root and/or shoot systems and to hypothesize structural homologies as a basis for inferences of diversification.

Materials and Methods

Morphological analyses of perennation structures were conducted using scanning electron (SEM) and light (LM) microscopy. Specimens from natural populations (Table 1) were fixed in formalin-acetic acid. Specimens for SEM were dehydrated in a graded ethanol series, critical-point dried, mounted on aluminum stubs and gold coated for examination at an accelerating voltage of 15 kV. Images were captured digitally using the program Quartz PCI (Quartz Imaging Corp. 1993-1998). Specimens for LM were dehydrated in a graded tertiary-butyl alcohol series (Johansen 1940), infiltrated and embedded in Paraplast™, sectioned at 10-20 μm , mounted on glass slides, and stained with safranin-O and fast green.

Transverse and longitudinal sections were made for 3-5 reproductively mature individuals of *C. virginica*, *C. tuberosa*, *C. umbellata*, *C. lanceolata* and *C. megarhiza*. Additional younger (i.e. non-reproductive) material was sampled for *C. lanceolata* and *C. megarhiza*, whose growth forms represent the extremes of form found in perennation structures

in this clade. This taxon sampling represents five of the ten species of sect. *Claytonia* (*sensu* O'Quinn and Hufford in press), encompassing the morphological disparity of the section. Additional data on morphology are provided for a broader range of species from an examination of herbarium specimens.

Results

Organography

Shoot systems of sect. *Claytonia* are orthotropic and monopodial. They are characterized by the annual production of aerial foliage leaves and inflorescences (in plants of reproductive age) and the formation of long persistent subterranean storage (= perennation) structures (figs. 1; 2). Perennation structures are fully subterranean in *C. caroliniana*, *C. lanceolata*, *C. multiscapa*, *C. ogilviensis*, *C. rosea*, *C. tuberosa*, *C. umbellata* and *C. virginica* (fig. 1). In these taxa with fully subterranean perennation structures, aerial shoot systems are ephemeral and produced at the apex of the perennation structures. In contrast, aerial shoot systems in *C. megarhiza*, and presumably *C. acutifolia* (fig. 2) are perennial, and their perennation structures extend slightly above the soil surface. Persistent taproots extend from the proximal portions of perennation structures of *C. acutifolia*, *C. megarhiza*, *C. ogilviensis*, and *C. umbellata* (figs. 1D, 1H-J; 2A, 2B), whereas the roots of other members of the section are more transient.

Perennation structures have diverse forms (figs. 1; 2). They are globose in *C. lanceolata*, *C. caroliniana*, *C. virginica* and *C. rosea* (figs. 1F, 1G, 1K-M), ovoid (vertically elongated) in *C. ogilviensis* (fig. 1D) and *C. umbellata* (fig. 1H-J). *Claytonia multiscapa* and *C. tuberosa* are more complex in having an ovoid (radially elongated) basal portion and a distal neck (figs. 1A-C, 1E). Histological preparations also revealed a slight distal neck in *C. lanceolata* and *C. umbellata* (figs. 4; 5A). Perennation structures of *C. tuberosa* have warty projections on the

ovoid basal portion (figs. 1A, 1B). Perennation structures of *C. acutifolia* and *C. megarhiza* have elongate, often branched, axis-like forms, which are thickened nearly uniformly from the base of the basal leaf rosette to the base of the primary taproot (fig. 2).

Perennation structures in *Claytonia* sect. *Claytonia* enlarge and persist over multiple seasons. They are not replaced annually. Asexual reproduction, involving perennation structures, has not been observed, although ramet formation through fragmentation of caudex branches in *C. megarhiza* and *C. acutifolia* may occur. Perennation structures vary in size from 10–15 mm in *C. rosea* to ≥ 3.0 cm in *C. tuberosa* (figs. 1A, 1B). In *C. acutifolia* and *C. megarhiza* the girth and length of mature perennations structures vary in width to ≥ 3.0 cm and in length to ≥ 15 cm (fig. 2). *Claytonia umbellata* and *C. ogilviensis* (figs. 1D, 1 H-J) have sizes ranging from 1-5 cm in diameter to ≥ 10 cm in length.

Shoot system growth is rhythmic. All examined taxa preform foliage leaves and inflorescences at the end of each growing season. In taxa that have fully underground perennation structures, preformed appendages are surrounded by a series of tightly overlapping scale leaves (fig. 3A). The emergence of foliage leaves and inflorescences in the spring displaces the overlapping scales, which persist as tattered remnants on top of the perennation structure (fig. 3B). Shoot apices in *C. lanceolata*, *C. rosea*, *C. multiscapa*, *C. umbellata* and *C. tuberosa* produce relatively few foliage and scale leaves per season, and foliage leaf expansion and senescence frequently precedes inflorescence expansion, resulting in the absence of evident foliage leaves at the time when flowers are open. In *C. virginica*, however, a greater number of lateral appendages develop and foliage leaves and inflorescence branches expand simultaneously. Senescence of aerial biomass follows seed dispersal.

Claytonia lanceolata, *C. rosea*, *C. tuberosa*, *C. multiscapa*, *C. caroliniana* and *C. virginica* have clusters of thin, ephemeral, shoot-borne roots that arise along the sides of perennation structures (figs. 1B, 1C, 1E-G, 1K-M). In mature *C. tuberosa*, root clusters arise only in the warty globose region and do not arise on the flanks of the neck. In addition to ephemeral clusters of shoot-borne root along their sides, we found that some specimens of *C. virginica* also have a cluster of roots positioned at the base of the perennation structure. *Claytonia umbellata* (and presumably *C. ogilviensis*) have either a single dense cluster of ephemeral roots in a basal position or a persistent primary root and infrequently have clusters of ephemeral shoot borne roots along the sides of perennation structures. *Claytonia megarhiza* has a well-developed, highly branched primary taproot that is continuous with the perennation structure, and lateral roots emerge along the branches of the primary root.

Apical zone

Shoot apical meristems (SAM) in *Claytonia* sect. *Claytonia* are located at the apex of short necks (figs. 4; 5A) or on the flattened to slightly sunken apices of perennation structures (figs. 5B; 6). The apical zone is characterized by a SAM surrounded by leaf primordia/foilage leaves with axillary inflorescences axes and scale leaves or their persistent remnants (figs. 3; 4; 5; 6; 7A-C). In late season (November) collections of *C. lanceolata*, scale leaves envelop developing lateral appendages (fig. 3A). Scale leaves are white and semi-fleshy (tissue-like) with numerous folds on their adaxial surfaces. The scale leaves seen in the aerial shoots of taxa with fully subterranean perennation structures are not produced in *C. megarhiza*. Internode elongation is minimal for all taxa, which contributes to the shallow breadth and depth of the apical zone; this is especially pronounced in the shoot apical zone of *C. megarhiza*, which is broad and never associated with a neck region (fig. 6). Subjacent to the short apical zone, the

perennation structure is much broader. This is a consequence of cell expansion in the pith and cortex and the initiation of secondary growth close to the SAM. Pith and cortex expansion is associated with starch storage in parenchyma cells (fig. 8C).

Arrangement of primary tissues

Vascular tissue arrangement at the distal end of all perennation structures is shoot-like and consists of a cylinder of procambium subjacent to the SAM that differentiates to form a ring of typical collateral vascular bundles. The shoot-like ring of vascular tissues, dissected by leaf gaps and surrounding pith, is found throughout the length of the perennation structures of *C. lanceolata* and *C. tuberosa* (figs. 7A-C; 8B). No transition to root-like vasculature occurs at lower levels of the perennation structures for *C. lanceolata* (fig. 7B) and *C. tuberosa*. Vascular tissue arrangement in *C. virginica* and *C. umbellata* is like that of *C. lanceolata* and *C. tuberosa* throughout the perennation structure, except that some specimens of *C. virginica* have vascular bundles in the lower quarter of the perennation structure that merge to form a single bundle (fig. 7C, 7D), and some individuals of *C. umbellata* have a persistent taproot (figs. 1H, 1J). In *C. megarhiza*, only the distal one third of the perennation structure has shoot-like arrangement of vasculature, the proximal two thirds have a transitional and root-like vascular arrangement.

Seedlings of *C. megarhiza* have hypocotyls that are slightly larger in diameter than the primary root, providing an abrupt morphological transition. This transition zone from shoot to root remains distinguishable as a slightly swollen portion of the perennation structure in non-reproductive specimens, however, in fully mature specimens this distinction is not visible and very little change in the diameter of the perennation structure occurs over the transition zone. In the hypocotyls of seedlings, a root-like arrangement of vascular tissue (solid central core of xylem surrounded by pockets of phloem) extends from the root apex to the cotyledons. A shoot-

like ring of vascular bundles is characteristic only of epicotyl. In young, non-reproductive specimens of *C. megarhiza*, the procambium subjacent to the SAM differentiates collateral vascular bundles. The vascular bundles are separate for only a short distance before they anastomose to form composite bundles that have an amphicribal vascular tissue arrangement. In the lower part of the perennation structure, these composite vascular bundles converge (fig. 9B) to form the single central cylinder of the root (fig. 9C).

Secondary growth

Modifications that occur during secondary growth differ among species, although all form both secondary dermal and vascular tissues. *Claytonia lanceolata*, *C. virginica*, *C. umbellata* and *C. tuberosa* have a discontinuous vascular cambium, that forms between some vascular bundles, but not others (fig. 10B-E). In interfascicular regions where cambium forms, xylem is produced centripetally. Xylem consists of vessel elements and tracheids that have annular or helical secondary wall thickenings and xylem parenchyma. The interfascicular cambium produces centrifugally only thin walled, starch-filled parenchyma (fig. 10D, 10E). Secondary phloem, consisting of conducting cells and parenchyma, is confined to the fascicular regions (fig. 10D-F). No fibers are produced in either xylem or phloem. The interfascicular cambium is short lived, relative to that of the fascicular cambium, which results in spoke-like bundles of secondary vascular tissue between broad bands of primary cortex (fig. 10F). As perennation structures age, the fascicular regions expand to become fan-shaped in transverse sections; this results in the crushing of primary cortical parenchyma in the interfascicular regions. Radial expansion of secondary phloem also crushes the cortex.

In *C. megarhiza*, secondary vascular growth is centered in the composite vascular bundles subjacent to the anastomoses of the short, distal primary vascular bundles. Secondary

growth in these amphicribal bundles produces secondary xylem centrifugally and secondary phloem centripetally. There is no formation of interfascicular secondary vascular tissue.

Periderm is initiated in subepidermal cells close to the apical zone, extending proximally from the scale leaves in fully subterranean taxa and from the base of the foliage leaves of *C. megarhiza*. The periderm consists of several layers of phellum and phelloderm that are produced in radial rows from a bifacial phellogen (e.g. fig. 8A). Periderm thickness differs among taxa, but maintains a uniform thickness over the life of the organ.

Discussion

Structural homologies of perennation structures

Assessments of structural homology require inferences of correspondence or “equivalence” in comparative data. Workers have differed in the criteria considered acceptable as the basis for hypotheses of structural homology (Wagner 1989; Donoghue 1992). Some workers, such as Patterson (1982) and Brower (2000), accept only positional similarity as consistent with a hypothesis of structural homology; whereas most workers have applied the three criteria of Remane (1952): (1) similarity in position, (2) transitional forms (evident either in ontogeny or in morphoclines), and (3) similarity in special attributes, such as anatomical attributes. The relatively simple forms of perennation structures in sect. *Claytonia* offer few landmarks that can be used to characterize positional similarity. For example, all have shoot apical meristems and a cluster of leaves at one pole of the perennation structure, but this offers little insight into the homologies of perennation structures aside from the presence of shoot tissue at the distal end. We apply here Remane’s (1952) third criterion of similarity in special attributes, namely anatomical characters, to hypothesize structural homologies for perennation structures.

Earlier hypotheses identified perennation structures in sect. *Claytonia* as either shoot or root (Holm 1905, 1913; Grandtner and Gervais 1990; Miller 2003), or both in the case of Swanson (1966). Anatomical data can be applied to distinguish between these two regions of plant bodies. For example, vascular tissue arrangement and differentiation differ between shoots and roots for most eudicots. During primary growth most eudicots have stem vascular tissue in a single ring (eustele) of discrete bundles with a collateral arrangement of phloem and xylem (Cutter 1971; Esau 1977; Mauseth 1988). In contrast, vascular tissue in the root forms a single central cylinder in which xylem tissue is located at the center of the region and is generally transectionally lobed with phloem tissue positioned between the xylem lobes. The differentiation of phloem is centripetal in both shoot and root, however, xylem maturation is endarch (centrifugal) in shoots but exarch (centripetal) in roots (Cutter 1971; Esau 1977; Mauseth 1988). In the hypocotyl, there is a transition between shoot and root arrangements of vascular tissue.

Our results suggest that at least the distal portions of all examined perennation structures in sect. *Claytonia* are shoots and some are entirely shoot-like. A shoot-like arrangement of vascular tissue characterized the full length of the perennation structures of *C. lanceolata* and *C. tuberosa* and most of the length of the perennation structures for *C. umbellata* and *C. virginica*. This inference is consistent with the suggestion of Grandtner and Gervais (1990) that perennation structures of *C. caroliniana* were shoots, but it differs from the conclusions of Holm (1905, 1913) for *C. virginica*. Holm (1905) inferred that the perennation structures of *C. virginica* were roots, stating that they consisted of the enlarged basal portion of the primary root whose apex was ephemeral. The specimens of *C. virginica* we examined were clearly shoot-like through most of the perennation structure but showed a transition to root-like characteristics in

the lower quarter in some specimens (fig. 6C, 6D). In contrast to the fully subterranean forms discussed above, the perennation structures of *C. megarhiza* include both shoot and root axes. A persistent taproot forms a major part of the perennation structure, however, the hypocotyl and epicotyl are incorporated as well. This type of storage organ, derived from the combination of both swollen shoot and root, has been well characterized for *Daucus carota* (Havis 1939; Esau 1940). Although Holm (1905) recognized that much of the swollen portion of *C. megarhiza* perennation structures were root, he did not comment on the role of the shoot axis in the formation of these organs.

Origins of diversity

A key difference that distinguishes among perennation structures in *Claytonia* sect. *Claytonia* is primary root persistence (fig. 12A-D). We hypothesize that loss of the primary root was associated with ecological shifts in substrate and from alpine to lower elevation habitats. *Claytonia megarhiza*, for example, is restricted to alpine rock crevices and talus slopes and has an elongate perennation structure with long and often highly ramified primary roots. Root architecture in *C. megarhiza* has an asymmetrical (bilaterally fan-shaped) form that is associated with slope-grown species (Chiatante et al. 2003). This growth form may be specialized for long term, secure positioning in unstable substrates. *Claytonia acutifolia* also has elongate perennation structures but has a wider range of habitat tolerance than *C. megarhiza*. A greater percentage of the perennation structure in *C. acutifolia* appears to be shoot rather than root, although this needs further investigation, and the primary root is less highly ramified. Perennation structures of *C. umbellata* are intermediate in form between the elongate structures of *C. megarhiza* and *C. acutifolia*, and the globose/pyriform structures of *C. lanceolata*, *C. tuberosa* and *C. virginica*. *Claytonia umbellata*, like *C. acutifolia*, is adapted to a wider range of

habitats and substrates. The globose/ovoid structures of *C. tuberosa*, *C. lanceolata* and *C. virginica*, in contrast, are consistently found on stable substrates and often at lower elevations.

A second difference that distinguishes among perennation structures in *Claytonia* sect. *Claytonia* is the presence of a neck region at the distal end of the perennation structure. We distinguish between the short neck seen in younger specimens of *C. lanceolata* (figs. 7A, 7B), which consist of unexpanded cortical cells and phellogen derivatives that will ultimately form part of the spherical body of the perennation structure, and the prominent necks of *C. multiscapa* and *C. tuberosa*. The necks of the latter two species do not appear to be incorporated in the larger ovoid basal part of the perennation structure as it expands, and they have extensive development of secondary tissues. We hypothesize that a fully geophytic condition has resulted in selection for mechanisms to adjust position in the soil. Geophytes are known to adjust their depth in the soil in response to environmental parameters (Rimbach 1895, Galil 1958, 1980; Jacoby and Halvey 1970) and development of a neck region may provide a means of adjusting the depth of the apical meristem without the expansion of the entire, nutrient rich basal portion of the perennation structure.

Secondary growth

All examined members of *Claytonia* sect. *Claytonia* are relatively long-lived and form both secondary dermal and vascular tissues. Most of the secondary vascular growth in the perennation structures of *C. lanceolata*, *C. umbellata*, *C. virginica*, and *C. tuberosa* is centered in individual vascular bundles (fig. 10). Mauseth (1988) has noted that this type of secondary growth is common in succulents and most herbaceous eudicots that undergo secondary growth; wood resulting from this type of secondary growth forms vertical cylinders or woody networks (Mauseth 1988) embedded in a matrix of thin-walled parenchyma cells. This type of secondary

growth reduces mechanical support (Carlquist 1975), which may be a reasonable compromise in these relatively short perennation structures of *Claytonia* sect. *Claytonia* that are embedded in soil.

In the perennation structures of *C. megarhiza* secondary growth is also confined to vascular bundles, however, in this taxon primary vascular bundles coalesce to form composite amphicribal bundles (figs. 9A, 9B). Secondary growth occurs within each composite bundle and multiple bundles anastomose in the transition region to form a central cylinder of vascular tissue that is continuous with the root. These amphicribal bundles have been interpreted as lateral roots that remain in the primary plant body by Holm (1905). However, Holm's (1905) interpretation of these bundles as lateral roots is inconsistent with our observations regarding the position of multiple bundles in the primary plant body. We find these bundles above the single central core of vascular tissue, which we interpret as the primary root, and continuous with the collateral vascular bundles of the shoot. Thus, we interpret these composite bundles as a modified mode of secondary growth in the primary vascular bundles of the shoot that accomplish the transition of vascular tissues between the shoot and root.

The secondary xylem characteristics in *Claytonia* match closely those of other herbaceous members of the portulacaceous alliance (Gibson 1973; Carlquist 1962; Mauseth 1988, 1993; Mauseth and Plemmons-Rodriguez 1998; Landrum 2000, 2001) in having narrow vascular tracheids and parenchyma that are generally more plentiful than vessels and in being fiberless and rayless. In particular, fiberless woods with vascular tracheids and vessel elements that have annular or helical secondary wall thickenings are characteristic of cereoid cacti (Gibson 1973, Carlquist 1975, Mauseth 1988, 1993). Carlquist (1975) interprets the syndrome of fiberlessness in conjunction with vessel elements and vascular tracheids that have annular or

helical secondary wall thickenings to indicate vascular flexibility during drought conditions. Annular or helical secondary wall thickenings, in contrast to pitted secondary walls, allow movement of the cell walls, which prevents breakage of the conducting cells. Succulents that express this syndrome are hypothesized to avoid the need for the strongly reinforced secondary cell walls required to withstand highly negative water potentials by storing water. Helical secondary cell walls are characteristic of desert and high latitude herbaceous plants as well (Carlquist 1975). Mauseth (1993) has suggested this syndrome is derived in Cactaceae as an adaptation for extreme arid environments. However, the widespread presence of this syndrome in members of the core Caryophyllales, especially the portulacaceous alliance, indicates that a reappraisal of the origin of these characters in a broader phylogenetic context may be warranted.

Conclusions

The morphologically disparate perennation structures of *Claytonia* sect. *Claytonia* differ in form and especially in the degree to which root system contributes to these bodies. The spherical to ovoid perennation structures that are fully subterranean are predominantly shoot and generally have an ephemeral primary root. The more elongate, axis-like perennation structure of *C. megarhiza* that extends slightly above the soil surface consists of both shoot and root, and the root forms a substantial portion of the structure. The evolutionary shift to an ephemeral primary root appears to be associated with ecological changes. Despite the morphological disparity in the group, they are anatomically similar. Secondary vascular tissues tend to be limited only to fascicular regions, markedly so in *C. megarhiza* and in the more basal portions of taxa with fully subterranean, spherical/ovoid perennation structures, such as *C. tuberosa*.

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Literature Cited

- Brower AVZ 2000. Homology and the inference of systematic relationships: some historical and philosophical perspectives. Pages 10-21 in R Scotland, RT Pennington eds. Homology and systematics: coding characters for phylogentic analysis. Taylor and Francis, London.
- Carlquist S 1975 Ecological strategies of xylem evolution. University of California Press, Berkeley.
- Chambers KL 1963 *Claytonia nevadensis* in Oregon. Leaflet W Bot 10:1-8.
- Chiatante D, SG Scippa, A Di Iorio, and M Sarnataro 2003 The influence of steep slopes on root system development. J Plant Growth Regul 21:247-260.
- Cutter EG 1971 Plant anatomy: experiment and interpretation. Part 2 Organs. Edward Arnold Ltd., London.
- Donoghue MJ 1992 Homology. Pages 170-179 in EF Keller, EA Lloyd (eds.), Keywords in evolutionary biology. Harvard University Press. Boston.
- Esau K 1977 Anatomy of seed plants, 2nd ed. John Wiley & Sons, Inc., New York.
- 1940 Developmental anatomy of the fleshy storage organ of *Daucus carota*. Hilgardia 13:175-

226.

Gibson AC 1973 Comparative anatomy of secondary xylem in Cactoideae (Cactaceae).

Biotropica 5:29-65.

Grandtner MM, C Gervais 1990 Étapes initiales du développement *in situ* de *Claytonia caroliniana* var. *caroliniana*. Can J Bot 68:726-730.

Gray A 1887 Contributions to American Botany. XV. 1. Revision of some polypetalous genera and orders precursory to the Flora of North America. Proc Am Acad 22:270-314.

Greene EL 1891 Flora Franciscana. Cubery & Co., San Francisco.

Havis L 1939 Anatomy of the hypocotyl and roots of *Daucus carrota*. J Agric Res 58: 557-564.

Holm T 1905 *Claytonia* Gronov. A morphological and anatomical study. Mem Nat Acad 10: 27
37.

— 1913 Types of *Claytonia* Gronov. Minde J Steenstrup 21: 1-11.

Johansen DA 1940 Plant microtechnique. McGraw-Hill Book Co.Inc., New York.

Landrum JV 2002 Four succulent families and 40 million years of evolution and adaptation to xeric environments: What can stem and leaf anatomical characters tell us about their phylogeny? Taxon 51:463-47.

—2001 Wide-band tracheids in leaves of genera in Aizoaceae: the systematic occurrence of a novel cell type and its implications for the monophyly of the subfamily Ruschioideae. Plant Syst Evol 227:49-61.

Mauseth JD 1993 Water storing and cavitation-preventing adaptations in wood of cacti. Ann Bot 72:81-89.

—1988 Plant Anatomy. Benjamin/Cummings Publishing Co., Inc., Menlo Park.

—BJ Plemons-Rodriguez 1998 Evolution of extreme xeromorphic characters in wood: a study of

- nine evolutionary lines in Cactaceae. *Am J Bot* 85:209-218.
- Miller JM 2003 *Claytonia* L. and *Montia* L. (Portulacaceae). Pages 465-475; 485-488 in *Flora of North America* Committee eds. *Flora of North America north of Mexico*, Volume 4 Magnoliophyta: Caryophyllidae, part 1. Oxford University Press, New York.
- O'Quinn RL, L Hufford In press Molecular systematics of Montieae (Portulacaceae): implications for taxonomy, biogeography and ecology. *Sys Bot*
- Quartz PCI 1993-1998 Scientific Image Management Systems. Quartz Imaging Corp. Version 4.20.
- Remane A 1952 *Die Grundlagen des natürlichen Systems der vergleichenden Anatomie und der Phylogenetik*. Geest and Portig, Leipzig.
- Rimbach A 1895 Zur Biologie der Pflanzen mit unterirdischem Sprosse. *Ber Dtsch Bot Ges.* 13:141-155.
- Swanson JR 1966 A synopsis of relationships in Montioideae (Portulacaceae). *Brittonia* 18:229-240.
- Wagner GP 1989 The origin of morphological characters and the biological basis. *Evolution* 43:1157-1171.

Table 1

Claytonia collected for morphological study, including collection and voucher locations

Taxon name	Population location	Collection voucher and date
<i>Claytonia lanceolata</i> Pursh	Texas Ridge Road, Latah Co. ID ¹	R. O'Quinn 353; 4/18/01
	Forks Road, Latah Co., ID ¹	R. O'Quinn 545; 4/20/03
	Kamiak Butte, Whitman Co., WA ¹	R. O'Quinn 352; 4/18/01
		L. Hufford 858; 4/20-21/01
<i>Claytonia megarhiza</i> var. <i>nivalis</i> (English) C. L. Cronquist	Paddy-Go-Easy Trail, Chelan Co. WA ¹	R. O'Quinn 517; 9/02/02
	Wallowa Co. OR	C. Björk 4765; 8/29/99
<i>Claytonia megarhiza</i> var. <i>megarhiza</i> (A. Gray) Parry ex S. Watson	La Sal Mtns. San Juan Co. UT	L. Hufford 4249; 8/5/04
<i>Claytonia umbellata</i> S. Watson	Wasco Co. OR	R. O'Quinn 527; 3/18/03
<i>Claytonia virginica</i> L.	Farmington, Van Buren Co., IA	L. Hufford 263; 3/29/89 and 3/90
	Oktribbeha Co., MS	M. Fishbein s.n. 3/17/04
	Berkeley Co., SC	L. Kinter 3251; 3/19/00
<i>Claytonia tuberosa</i> Pallas ex Willd.	Steese Hwy., AK	R. O'Quinn 411; 7/5/01
	Dempster Hwy. YT CAN	R. O'Quinn 434; 7/12/01
	Dempster Hwy. YT CAN	R. O'Quinn 444; 7/15/01
	Mt. Fairplay, AK	R. O'Quinn 462; 7/17/01

¹ Collection data for voucher represents one of multiple collections made between March 2000 and November 2004.

Figure legends

Fig. 1 Growth habit and perennation structures for taxa in *Claytonia* section *Claytonia*. *A-B*, *C. tuberosa*. *A*, Mature ramet. *B*, Mature perennation structure, showing elongate neck and warty, globose/ovoid basal region. *C*, Mature ramet of *C. multiscapa*. *D*, Mature perennation structure of *C. ogilviensis*. *E*, Mature perennation structure of *C. multiscapa*, showing elongate neck region above a basal globose/ovoid region. *F*, Mature perennation structure of *C. caroliniana*. *G*, Mature perennation structure of *C. virginica*. *H - J*, *C. umbellata*. *H*, Mature ramet. *I*, Growth habit. *J*, Mature perennation structure. *K - L*, *C. lanceolata*. *K*, Mature ramet. *L*, Mature perennation structure. *M*, Mature ramet of *C. virginica*. Scale bars for *A-G* = 1.0 cm; *H* and *K* = 1.5 cm; *I* and *J* = 3.0 cm; *L* = 0.5 cm; *M* = 2.0 cm.

Fig. 2 Growth habit and perennation structures for taxa with perennial aerial shoots in *Claytonia* section *Claytonia*. *A*, *C. megarhiza*. *B*, *C. acutifolia*. Scale bars for *A* = 2.0 cm; *B* = 1.0 cm.

Fig. 3 Apex of perennation structure of *C. lanceolata*. *A*, Terminal bud, showing scale leaves (November collection). *B*, Apex after bud expansion, showing remnants of scale leaves around dissected petioles of expanded foliage leaves (April collection). sc = scales, p = petiole. Scale bar for *A* = 0.86 mm; *B* = 1.50 mm.

Fig. 4 Apical zones of perennation structures in *Claytonia* sc *Claytonia*. *A*, *Claytonia lanceolata*. *B*, *Claytonia tuberosa*. SAM = shoot apical meristem, pi = pith, cx = cortex, p = periderm. Arrows indicate vascular strands. Scale bars = 1.0 mm.

Fig. 5 Apical zones of perennation structures in *Claytonia* section *Claytonia*. *A*, *Claytonia umbellata*. *B*, *Claytonia virginica*. See legend of figure 4 for abbreviation definitions. Arrows indicate vascular strands. Scale bars = 1.0 mm.

Fig. 6 Apical zone of perennation structures of *Claytonia megarhiza*. See legend of figure 4 for abbreviation definitions. Arrows indicate vascular strands. Scale bar = 1.0 mm.

Fig. 7 Longitudinal sections of perennation structures of *C. lanceolata* and *C. virginica*. *A-B*, *C. lanceolata*. *A*, Upper half of perennation structure, showing neck (n) with SAM and extending vascular bundles (arrows) from apical zone into globose lower portion. *B*, Whole perennation structure. *C-D*, *C. virginica*. *C*, Vasculature converges to root near base of medium sized perennation structure. *D*, Base of perennation structure, showing convergence of shoot vascular strands into root trace. pi = pith, cx = cortex, p = periderm, n = neck, sc = scale leaf, rt = root trace. Arrows indicate vascular strands. Scale bars for *A - C* = 1.36 mm; *D* = 0.25 mm.

Fig. 8 Sections of perennation structures of *Claytonia* section *Claytonia*. *A*, Periderm of *C. megarhiza*, showing bifacial phellogen producing phellum to the outside and phelloderm to the inside. Crushed cortical cells abut the phellogen. *B*, Transection through middle of *C. lanceolata* perennation structure showing distribution of collateral bundles (already in secondary

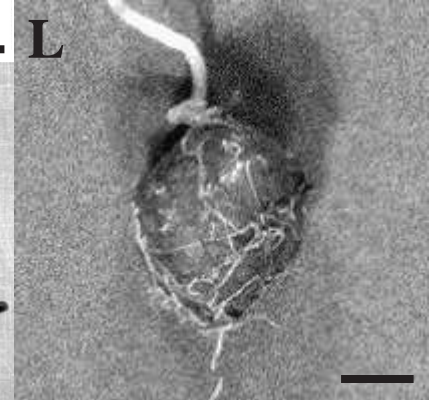
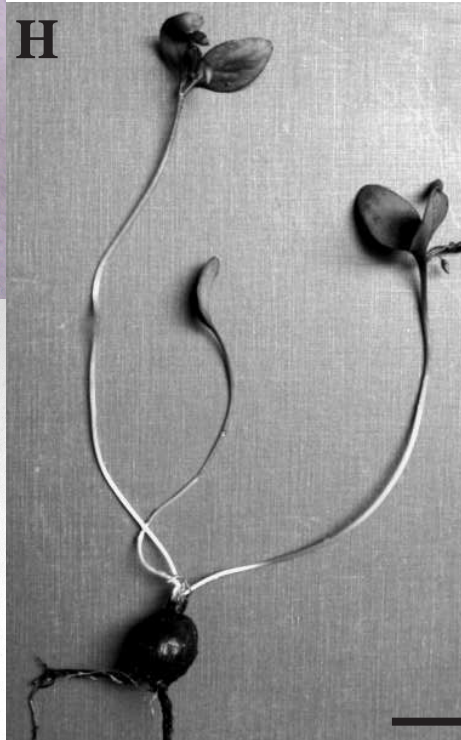
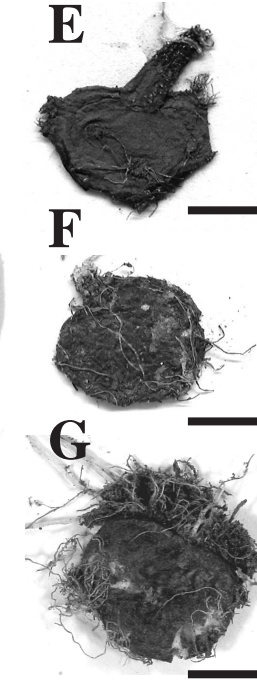
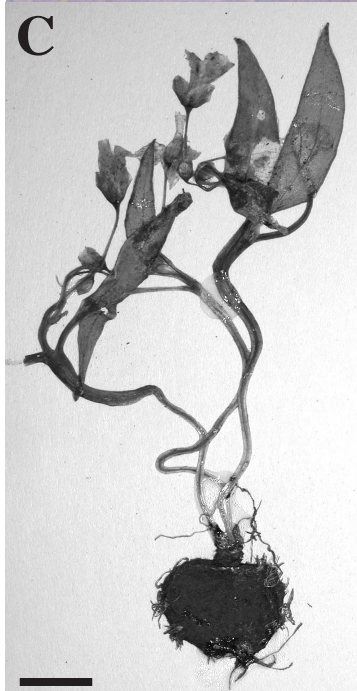
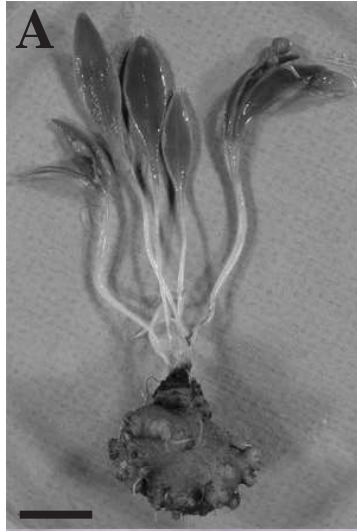
growth). *C*, Thin walled, starch-filled cortical cells in *C. virginica*. ph = phellum, pg = phellogen, pd = phelloderm, cx = cortex, p = periderm, vb = vascular bundles, st gr = starch grains. Scale bar for *A* = 0.1 mm; *B* = 1 mm; *C* = 60 μ m.

Fig. 9 Vasculature of perennation structure of *C. megarhiza*, showing transition from shoot to root. *A*, Two vascular bundles in upper hypocotyl. *B*, Merging of vascular bundles in lower hypocotyl. *C*, Magnified view of root vascular cylinder, showing vascular cambium, secondary xylem and phloem. ph = phloem, xy = xylem, vc = vascular cambium, ve = vessel element, vt = vascular tracheid. Scale bars for *A* and *B* = 0.2 mm; *C* = 0.05 mm.

Fig. 10 Secondary growth in perennation structures of *C. umbellata* and *C. tuberosa*. *A - C*, Transverse sections through distal half of a mature *C. umbellata* perennation structure. Secondary xylem shown as solid black areas, dotted line is the transition between the periderm and the ground tissue, empty spaces are shaded gray and the cortex and periderm are shown in white. *A*, Section at proximal end of neck region, showing discrete vascular bundles. *B*, Section mid-way between *A* and *C*, showing an early phase of fascicular cambial growth only. *C*, Section through middle of perennation structure. Cambial activity localized to the fascicular cambia has produced a characteristic spoke-like growth pattern. *D - E*, Details of secondary vasculature from transverse sections of mature *C. tuberosa* perennation structures. *D*, Early stage in transition from continuous cambial growth to fascicular only cambial growth. *E*, Older stage in secondary growth, showing radiating bundles. *F*, Transverse section through middle of mature perennation structure of *C. tuberosa*, showing late stage secondary growth in which spoke-like regions of vascular tissue are separated by torn regions of primary cortex that create

channels of empty space. fc = fascicular cambium, ir = interfascicular cambium cx = cortex, vb = vascular bundle, pi = pith, p = periderm. Scale bar for *A – C* = 0.8 mm; *D* = 0.2mm; *E* = 0.05 mm; *F* = 1.00 mm.

Fig. 11 Regional correspondences among perennation structures in *Claytonia* sect. *Claytonia*. *A*, *C. tuberosa* and *C. lanceolata*. *B*, *C. virginica*. *C*, *C. umbellata*. *D*, *C. megarhiza*.

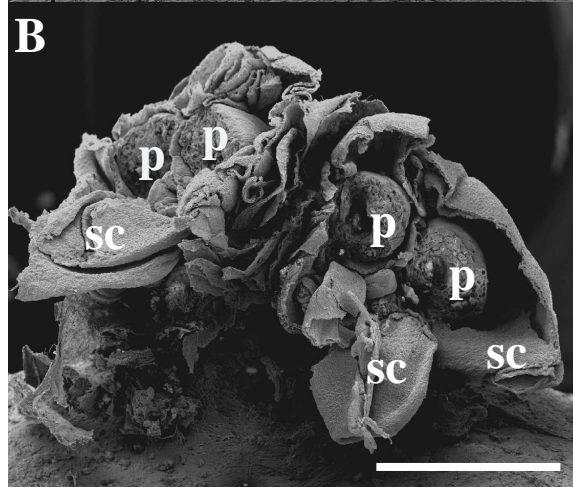
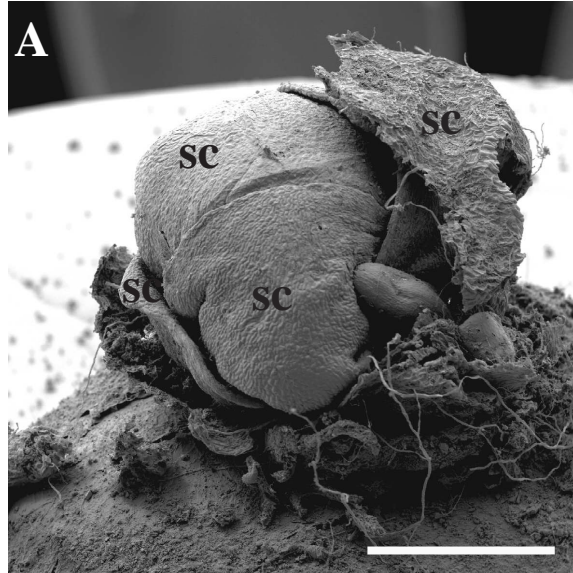


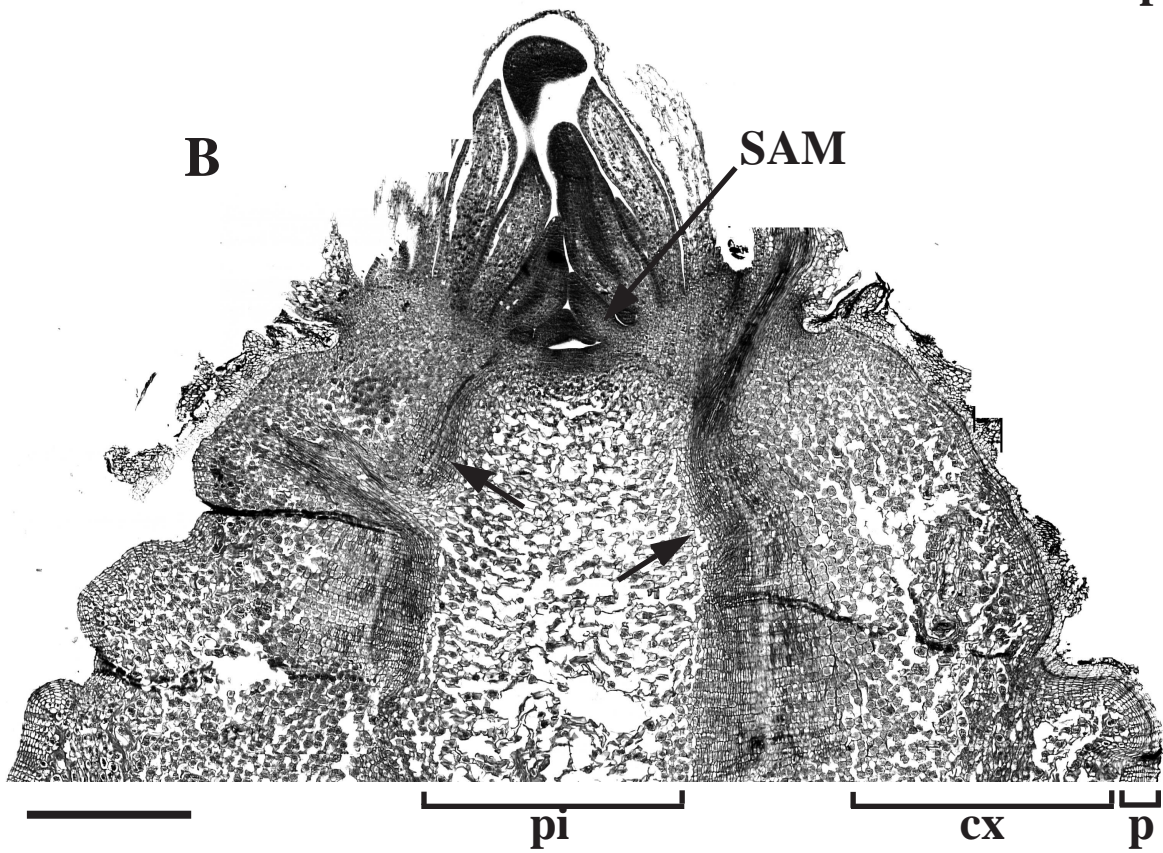
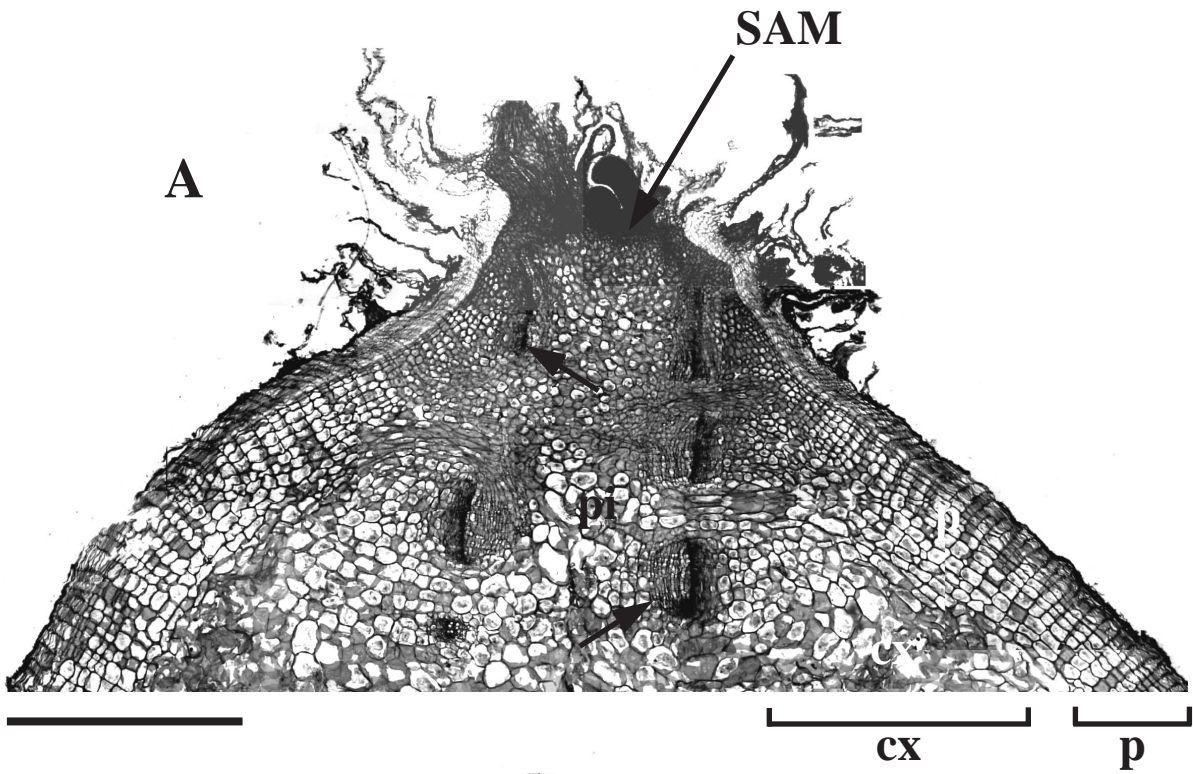
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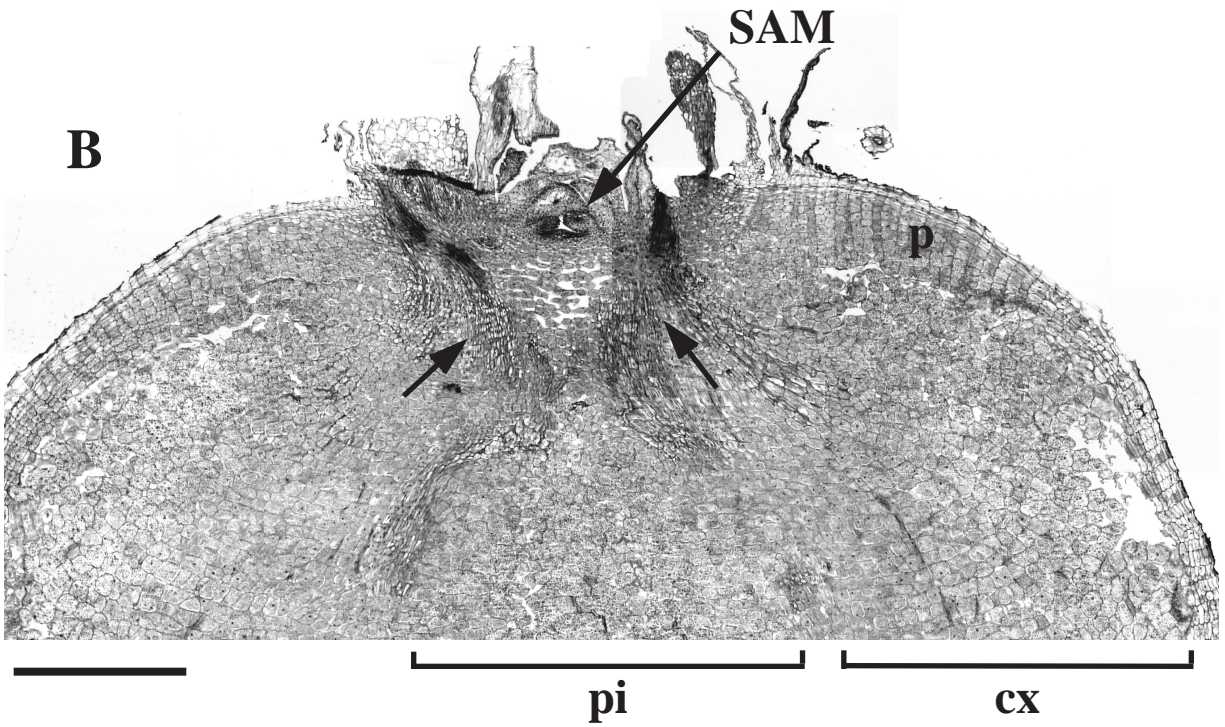
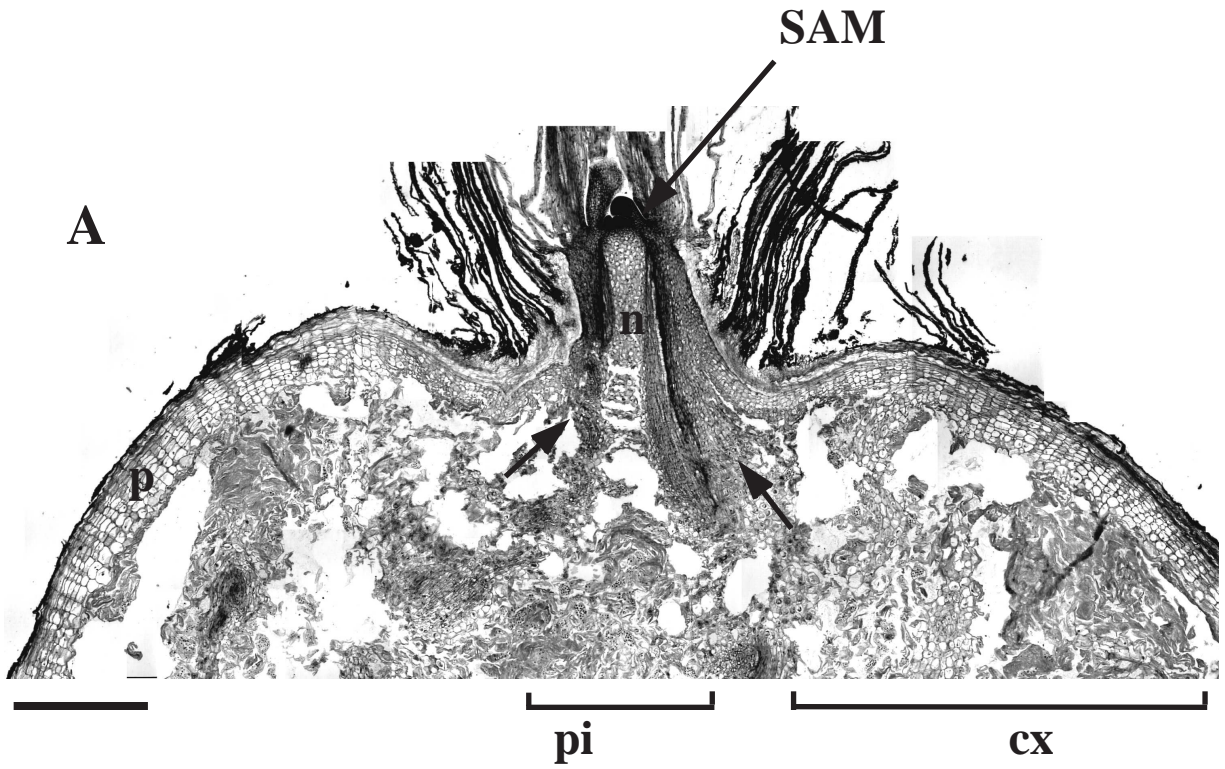


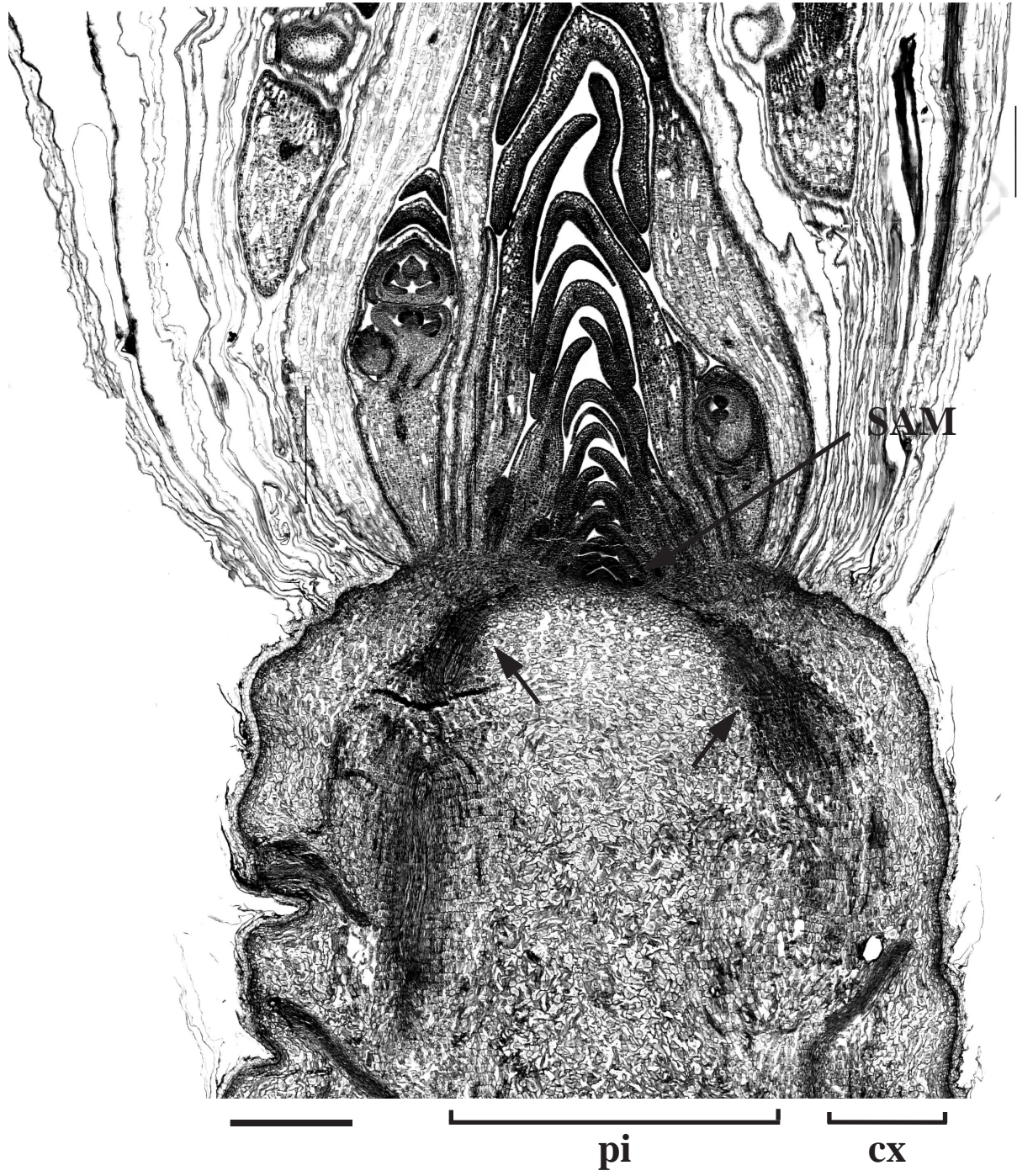
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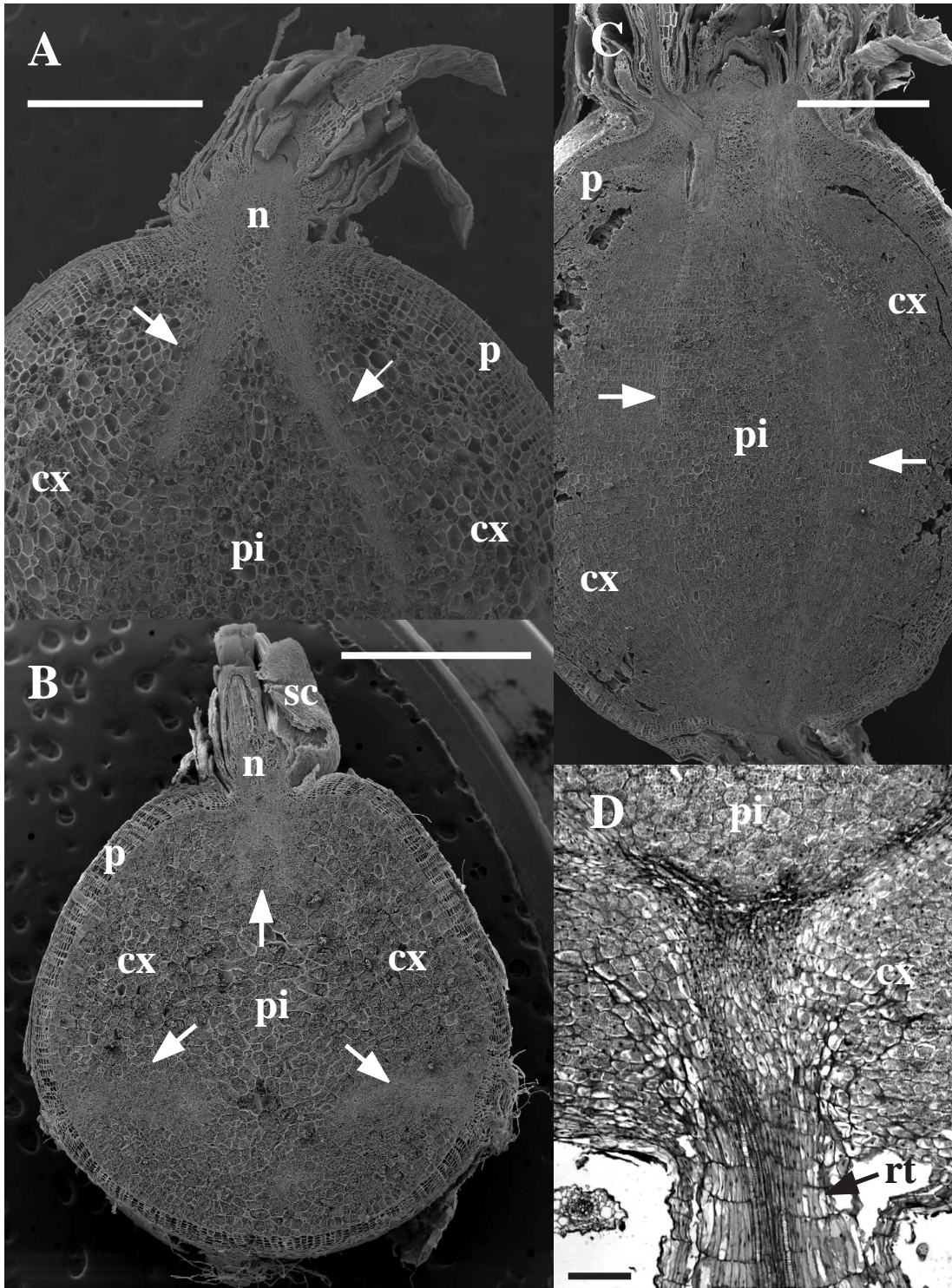


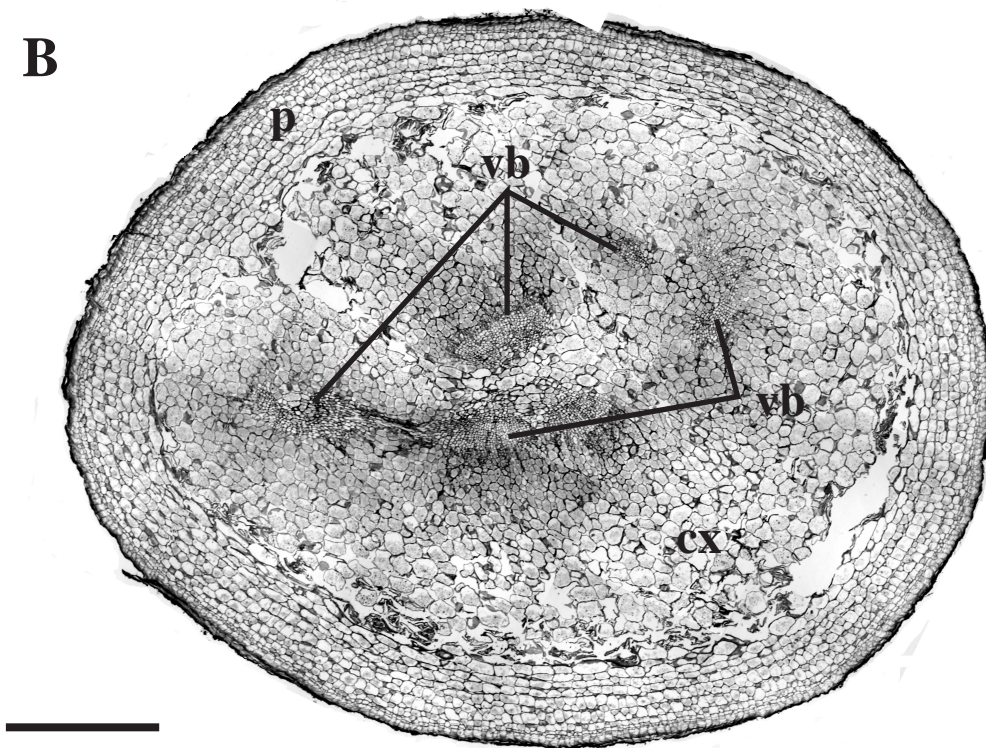
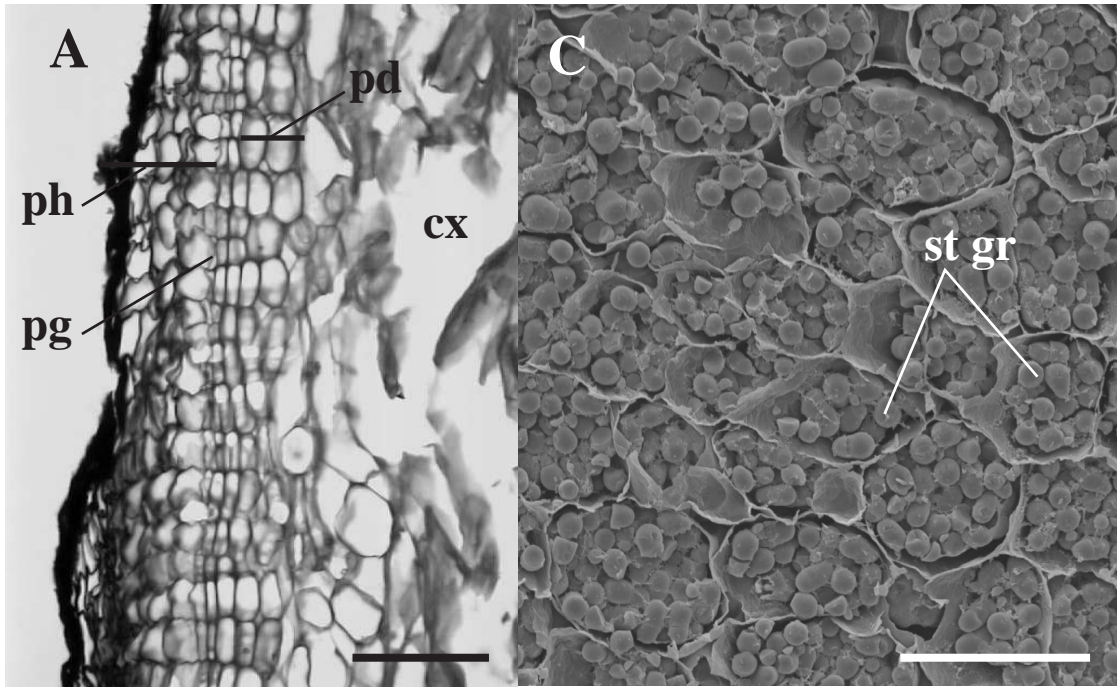


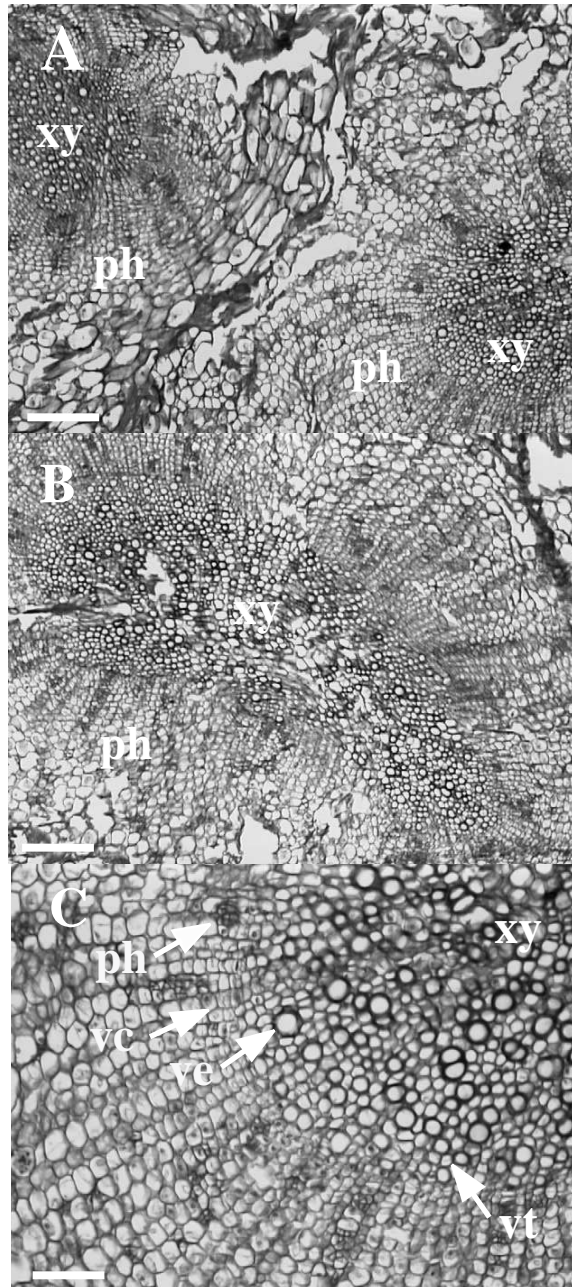


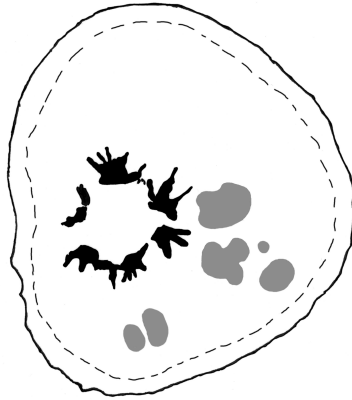
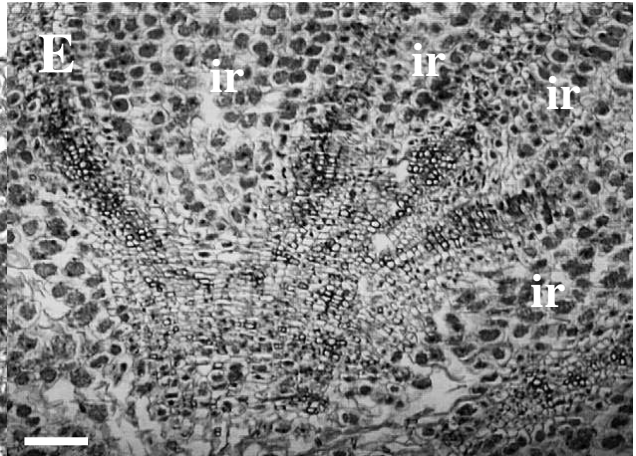
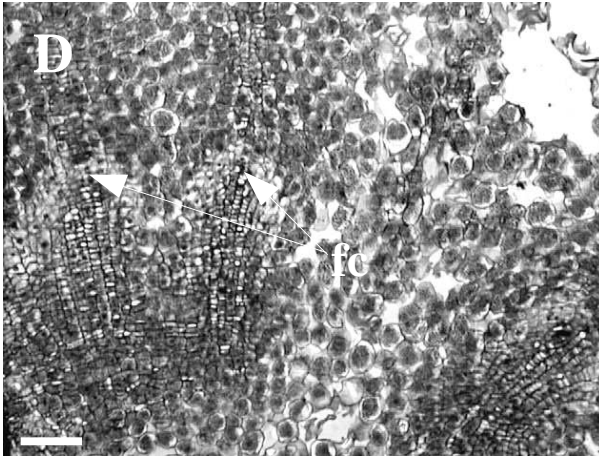
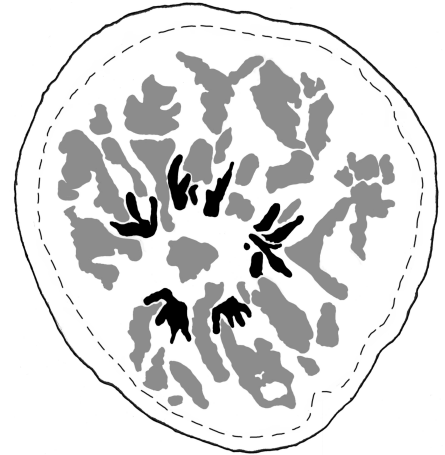
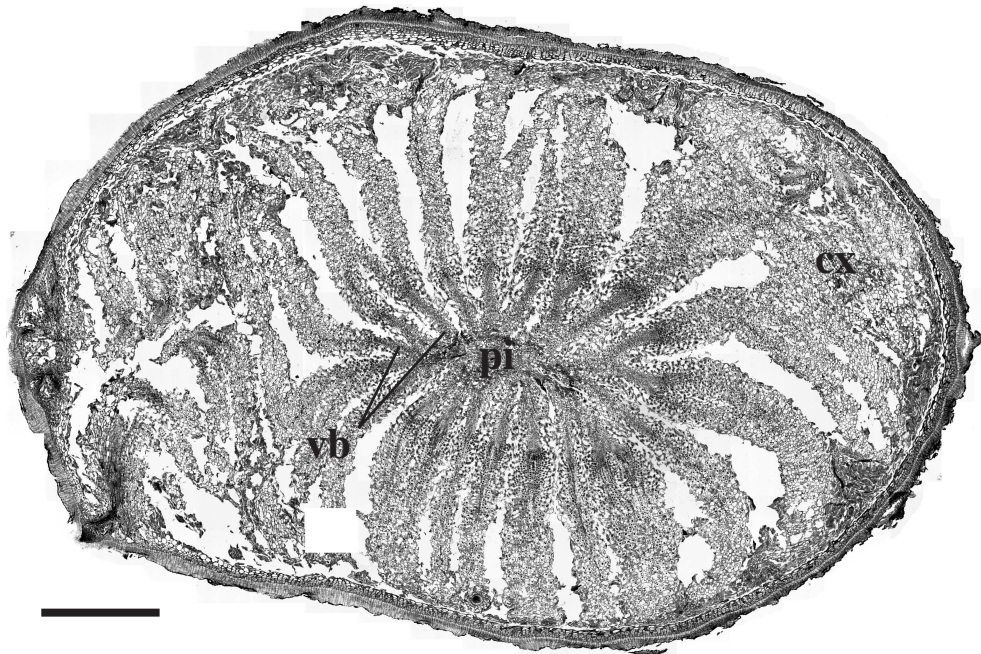


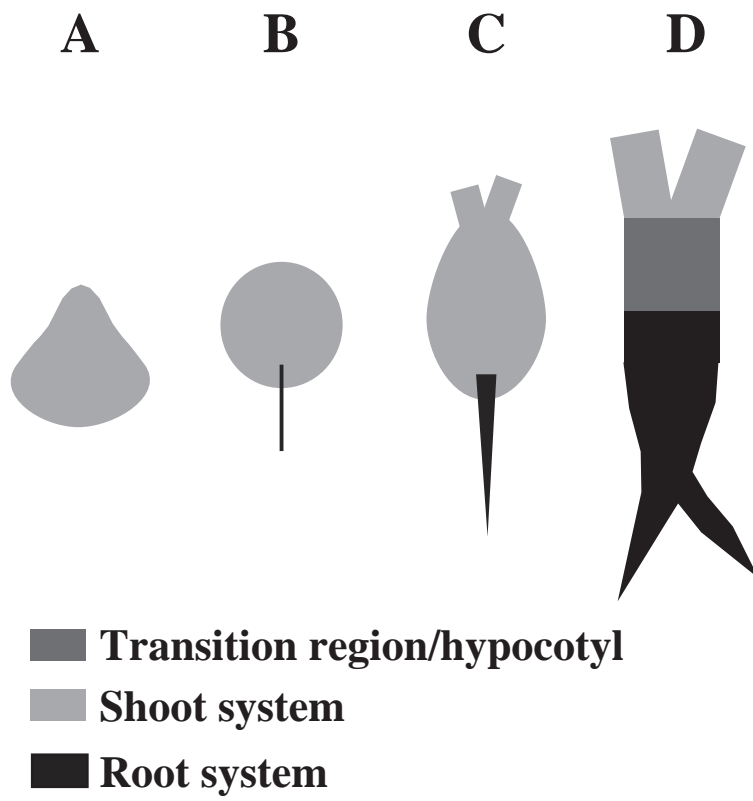








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CHAPTER THREE

SHOOT MORPHOLOGY IN THE *CLAYTONIA SIBIRICA* COMPLEX (PORTULACACEAE)

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ABSTRACT

The *Claytonia sibirica* complex, including *C. sibirica* and *C. palustris*, exhibits considerable morphological variation that encompasses ecological diversity over a wide geographic range. Shoots are basically rhizomatous in the complex and least specialized in *C. sibirica* var. *sibirica*. *Claytonia sibirica* var. *bulbillifera*, a serpentine endemic of southern Oregon and northern California, forms succulent, storage scale leaves distal to its foliage leaves each growing season. These scale leaves, which consist primarily of leaf base, are generally lacking in other members of the *sibirica* complex and give the shoot systems of *C. sibirica* var. *bulbillifera* a bulb morphology. *Claytonia palustris*, like *C. sibirica* var. *sibirica*, forms an apically swollen rhizome, but differs in its habit by forming renewal shoots, born in the axils of the basal leaves, at the ends of plagiotropic, single long internodes.

Key Words: Bulb, homology, leaf specializations, perennation, shoot architecture

Claytonia sibirica L. is a common understory herb of coastal and mesic inland forests extending from northern Santa Cruz County, California, to coastal northeastern Siberia (Miller et al. 1984; Chambers 1993a; Miller 2003). *Claytonia sibirica* can be annual or perennial and exhibits considerable morphological, ecological and cytological variation over its range. Its shoot systems have been most often described as rhizomatous. In the Klamath region (KR) of northwestern California and southwestern Oregon, however, *C. sibirica* have specialized underground structures involved in perennation (Gray 1877, 1887; Miller et al. 1884). Gray (1877) first described this KR form as *C. bulbifera* Gray and suggested it resembled *C. sibirica* but produced densely crowded perennating bulbs in a basal rosette. Gray's (1887) revision of the North American Portulacaceae treated *C. bulbifera* as *C. sibirica* L. var. *bulbillifera* Gray and described it as "...only a form of *C. sibirica* with thickened bases of the radical leaves, which persist on the crown as bulblet-scales."

Miller et al. (1984) identified a polyploid assemblage as the *C. sibirica* complex having as its core three diploid morphotypes. These morphotypes are largely allopatric, except for a region of overlap in southern Oregon and northern California. Variability among these morphotypes is expressed "in climatic adaptation, habitat, shape of basal leaves and the presence or absence of basal bulblets and rhizomes" (Miller et al. 1984, p. 266). One morphotype recognized by Miller et al. (1984) fits Gray's (1877, 1887) description of *C. sibirica* var. *bulbillifera* in being bulbiferous and geographically localized to southern Oregon and northern California (Fig. 1), and we will use this name to refer to the specialized KR morphotype. Miller et al. (1984) further distinguished the KR morphotype by its elliptical basal leaves and frequent occurrence on serpentine substrates. The second morphotype is found in shaded mesic habitats and has the deltoid basal leaf shape of the type specimen for *C. sibirica* var. *sibirica*. Variety

sibirica is the name we apply to populations distributed in the Pacific Northwest along the Cascadian cordillera, the Columbia River Gorge, coast ranges northward from Santa Cruz county, California, to Alaska, and the Aleutian and Commander Islands (Fig. 1). It overlaps with *C. sibirica* var. *bulbillifera* in the KR (Fig. 1). *Claytonia sibirica* var. *sibirica* also has disjunct populations in the inland Northwest (northern Idaho, western Montana and surrounding portions of Oregon and British Columbia; Fig. 1). Miller et al. (1984) characterized *C. sibirica* var. *sibirica* as bulbiferous as well, but less so than the endemic KR morphotype (= *C. sibirica* var. *bulbillifera sensu* Gray 1887; O'Quinn and Hufford this paper); however, disjunct eastern populations of *C. sibirica* var. *sibirica* are reported to lack swollen leaves. We distinguish the morphological variation in *C. sibirica* var. *sibirica* as western and eastern morphotypes. The third morphotype discussed by Miller et al. (1984) was later described as *C. palustris* Swanson and Kelley by Swanson and Kelley (1987). *Claytonia palustris* is narrowly endemic to two small, mid-elevation regions at the northern and southern ends of the Sierra Nevada and in Siskiyou County, California, where it overlaps with *C. sibirica* var. *bulbillifera* at its eastern edge (Fig. 1). This taxon is unique in the complex in preferring perennially wet, sunny habitats and in being strongly stoloniferous (Swanson and Kelley 1987).

O'Quinn and Hufford (in press) found robust support for the monophyly of the Miller et al. (1984) *C. sibirica* complex based on plastid and nuclear ribosomal DNA sequence data (Fig. 2). Notably, all members of the complex share a unique 10 base pair motif that includes a 3 base pair insertion in the internal transcribed spacer region of the nuclear ribosomal DNA.

Phylogenetic results recovered a sister taxon relationship between *C. palustris* and *C. sibirica*, but lineages within *C. sibirica* were not resolved.

We characterize shoot morphology of the *C. sibirica* complex, with a particular emphasis on specializations for nutrient storage and perennation. Beyond Gray's initial description of *C. sibirica* var. *bulbillifera*, the morphology of the so-called bulbiferous morphotype of the KR populations has not been studied. This comparative study of the shoot systems in the *C. sibirica* complex addresses especially the morphological identity of structures described as bulbs and bulbiferous and presents hypotheses the origins of morphological specializations.

MATERIALS AND METHODS

We sampled specimens of the western morphotype of *C. sibirica* var. *sibirica* from the Willamette Valley, Columbia River Gorge and foothills of the Hood River valley, and of the eastern morphotype from the Lochsa and Clearwater River valleys. *Claytonia sibirica* var. *bulbillifera* was collected in the Illinois and Rogue River valleys of southern Oregon where this variety is the most common morphotype. Samples of *C. palustris* were collected at the type locality at Jones Creek in Butte County, California, and seeds for greenhouse grown material were collected from a population at Stubbs Lake, Butte County, California (Table 1). Based on the cytogeographic results of Miller et al. (1984), we assume that our collections of the eastern morphotype of *C. sibirica* var. *sibirica*, *C. sibirica* var. *bulbillifera*, and *C. palustris* are diploid. Collections of the western morphotype of *C. sibirica* var. *sibirica* are potentially either diploid or tetraploid. Miller et al. (1984) suggested that diploids and polyploids have the same shoot morphologies.

Comparative morphology of shoot systems for the four forms of perennial sibiricas used scanning electron (SEM), and light (LM) microscopy. Specimens from natural, greenhouse and common garden populations were sampled in May or June and August (Table 1) for fixation in formalin-acetic acid (FAA). Specimens for SEM were dehydrated in a graded ethanol series,

critical-point dried, and mounted on aluminium stubs prior to gold coating. We examined 5-8 individuals per morphotype for SEM. Specimens were examined at an accelerating voltage of 15-20 kV. Images were captured digitally using the program Quartz PCI (Quartz Imaging Corp. 1993-1998). Specimens for LM were dehydrated in a graded tertiary-butyl alcohol series (Johansen 1940), infiltrated and embedded in Paraplast™, sectioned at 16μm, mounted on glass slides, stained with safranin-O and fast green, and examined with a Leitz light microscope. Microtomed sections were photographed or drawn using a drawing tube. To characterize shoot architecture and leaf base shape over ontogeny, we made cross and longitudinal sections through the basal rosettes of 3-5 individuals per examined population (Table 1) of western and eastern *C. sibirica* var. *sibirica*, *C. sibirica* var. *bulbillifera*, and greenhouse grown specimens of *C. palustris*.

RESULTS

Shoot Architecture

Claytonia sibirica var. *sibirica*. Perennials form an orthotropic to plagiotropic shoot with short internodes that bear helically arranged leaves, forming a rosette of photosynthetic leaves at the base of the newly elongating axis early in the growth season. Inflorescence branches and renewal shoots form in the axils of the basal leaves (Fig. 3A). The main axis of the shoot enlarges in length to approximately 1-2 cm over the growth season and becomes globose/ovoid (0.5-1.0 cm in diameter) at its distal end (Fig. 3A; 4A); however, shoot size is variable and appears to depend on the age and growth conditions of the individual. Shoots older than one season have a distal globose/ovoid region and a proximal cylindrical region that consists of stem produced in the preceding one or two growth seasons (Figs. 3A; 4B). Shoots consist rarely of more than three seasons of the main axis. Some shoots were observed to retain their taproot up

to their third growth season (4B); however, more commonly the younger shoot axes will disarticulate from older portions of rhizomes with taproots. The younger shoot axes will form shoot-borne roots associated with nodes of the basal leaves.

When a new growth cycle commences, several whorls of foliage leaves expand before the first inflorescences emerge. Each inflorescence has a pair of opposite, sessile leaves (Fig. 4C) and each flower is subtended by a small, oblanceolate bract. Inflorescences initially develop from the axils of distal leaves in the basal rosette, although late season lateral branches that expand from axillary buds at proximal nodes of the basal rosette can form inflorescences or renewal shoots (Fig. 3A). Renewal shoots have a basal rosette of helically arranged leaves. Elongation in the lower internodes of axillary branches (below their rosette of foliage leaves) can create aerial rhizomes that extend renewal shoots 1-5 cm away from the main axis (Fig. 3A). Axillary, aerial rhizomes have shoot-borne roots associated with the nodes of the basal rosette leaves.

In most shoots examined, all leaves were foliage leaves and had a leaf base, petiole and lamina. Foliage leaves have a range of forms, varying in size depending on growing conditions and probably ploidy level, but range from 3-30 cm in overall length and 5-8 cm in blade width (Fig. 4C). Leaf bases are crescentic in cross-section and the width to thickness ratio increases as they age (Fig. 4D-F). Petioles are terete in cross-section and roughly twice the length of the lamina. The laminas of basal leaves in *C. sibirica* var. *sibirica* are generally deltoid (Fig. 4C); however, Miller et al. (1984) illustrate a wide range of variation in lamina shape in tetraploid and hexaploid populations (see Miller et. al 1984, figs. 5-20, pp. 270). Foliage leaf color is consistently bright green for both morphotypes.

Some examined ramets produced late season scale leaves in addition to foliage leaves. These scale leaves consisted largely of leaf base with a rudimentary petiole and lamina (Fig. 4G). Although this heteroblastic shift was found uncommonly in populations of the western morphotype of *C. sibirica* var. *sibirica*, it was not observed among any individuals from populations of the eastern morphotype.

Claytonia sibirica var. *bulbillifera*. This variety has shoot morphology distinct from that of *C. sibirica* var. *sibirica* in stature, habit, perennation strategy and leaf specialization. Its shallow, subterranean shoot system is consistently smaller than that of *C. sibirica* var. *sibirica*, and its growth habit more lax (Fig. 5A). *Claytonia* var. *bulbillifera* shoot systems are generally similar to those of var. *sibirica* in producing annually a globose/ovoid, orthotropic axis (Fig. 3B; 5B) that has a basal rosette of helically arranged leaves, renewal shoots formed in the axils of the earliest basal leaves that can elongate as rhizomes and axillary inflorescences (Fig. 3B).

Claytonia sibirica var. *bulbillifera* produces specialized storage leaves that have a swollen, succulent leaf base and an unexpanded petiole and lamina (Figs. 3B; 5C-G) at nodes distal to the foliage leaves in the latter part of the growing season (Figs. 3B). At the beginning of the next growing season, these storage leaves can be either decaying or still turgid (Fig. 5D). With the resumption of shoot growth, the axis thickens and elongates distal to the storage leaf zone, new foliage leaves expand as a basal rosette, and inflorescences elongate from those rosette leaf axils. Foliage leaves have a distinct leaf base, petiole and narrowly to broadly elliptic lamina (Fig. 5A), and are often gray green with a reddish hue, especially when associated with sunny, serpentine sites. Shoot-borne roots emerge in the region between the storage leaves and the newly expanding foliage leaves. By late spring, storage leaves are produced distal to the foliage leaf zone (Fig. 3B). During the summer, shoot systems of *C. sibirica* var. *bulbillifera*

produce a range of leaf types from the typical foliage leaf described above to a modified form of foliage leaf, which has a succulent leaf base and expanded petiole (Fig. 5F) and lamina, as well as storage leaves (Fig. 5C-G). Inflorescences continue to expand from axillary buds of all leaf types throughout the growth season, which is extended for plants growing in more mesic sites. On drier sites, however, the above ground biomass withers and dies by late summer, leaving a shallowly subterranean shoot system that has prominent storage leaves (Fig. 5E). At the end of the growing season, *C. sibirica* var. *bulbillifera* preforms the foliage leaves and inflorescence buds that will expand during the next growing season.

Claytonia palustris. *Claytonia palustris* is shallowly subterranean to often submerged and differs from the rest of the *sibirica* complex in habit, degree of internode elongation, vegetative reproduction, production of modified leaves and size. Shoot systems are weakly orthotropic to plagiotropic, consisting of a swollen ovoid stem with alternately arranged, sheathing leaves in an open basal rosette (i.e. with longer internodes than those of the *sibirica* varieties; Fig. 3C; 6A-E). *Claytonia palustris* has an alternate rather than helical leaf arrangement (Fig. 6A, B) and produces fewer foliage leaves along longer internodes than other members of the complex. Under natural and greenhouse growth conditions, *C. palustris* has a size comparable to *C. sibirica* var. *bulbillifera*. In *C. palustris*, leaf bases are dorsiventrally flattened and sheathing around a swollen stem (Fig. 6B). No leaf specializations were observed in greenhouse grown or field-collected material. Greenhouse grown material grew only vegetatively. Renewal branches are formed in the axils of the lowermost leaves of the basal rosette and inflorescences in the axils of the uppermost (Fig. 3C). Inflorescence axes have a subequal pair of oblanceolate to broadly elliptic leaves and flowers that are subtended by small oblanceolate bracts.

The axillary buds that form renewal shoots extend plagiotropically from the axils of rosette leaves and become highly elongated (5-15 cm) (Fig. 6D, E). Most of this elongation is in a single, basal internode that initially has a slightly swollen apical zone with unexpanded leaf primordia (Fig. 6E). The apical zone, (Fig. 6A) which consists of few nodes, becomes orthotropic, undergoes radial thickening in the axis, and foliage leaves expand. Shoot-borne roots are formed at nodes of these swollen, orthotropic renewal shoots, which then replicate the architecture of primary shoots over the course of the growing season.

Modified Leaves

A heteroblastic shift from foliage leaves to scale leaves was observed in all examined ramets of *C. sibirica* var. *bulbillifera* (Fig. 3B; 5D; 7) but was uncommon among ramets of *C. sibirica* var. *sibirica*. The scale leaves of both varieties have rudimentary laminas that have a primordial shape and size and are frequently dislodged from the leaf base at maturity (Figs. 4D, G; 5C-F; 7A). All scale leaves are supplied by a single vascular strand, which broadens to form one medial and two lateral bundles that are embedded in a ground tissue of large, starch-filled, isodiametric cells. The epidermis is a single cell layer thick.

Scale leaf form, however, differs between the two varieties. Scale leaves of *C. sibirica* var. *sibirica* are similar in size and shape to the leaf bases of foliage leaves (Fig 4D, F, G). In contrast, the scale leaves of *C. sibirica* var. *bulbillifera* are radially thicker than the bases of most foliage leaves, although transitional leaf forms that had a thickened base, short petiole, and small lamina were found among early season foliage leaves directly preceding the formation of foliage leaves (Fig. 5F; 7D). The thickening of scale leaves of *C. sibirica* var. *bulbillifera* is centered primarily in cells adaxial to the primary vascular strand, producing a flattened adaxial surface (Fig. 5C, G). In contrast, scale leaves of *C. sibirica* var. *sibirica* had limited adaxial thickening

and retained the adaxial concavity of foliage leaf bases (Fig. 4D-F). Modified leaves in the western morph of *C. sibirica* var. *sibirica* were found only in late season collections and always in the distal portion of the shoot. This contrasts with our observations of *C. sibirica* var. *bulbillifera*, in which the late-forming scale leaves persisted through the winter attached to the stem axis and were subjacent to the expanding foliage leaves and inflorescences of the next growing season (Fig 5D).

DISCUSSION

Being a Bulb

Perennial sibiricas have similar globose to ovoid primary shoot axes that bear annually a basal rosette of leaves, from which axillary inflorescences and renewal shoots are formed (Fig. 3A-C). Although these shoot systems are fundamentally rhizomatous (*sensu* Bell 1991), some variants in the *C. sibirica* complex have been described as having bulbs, bulblets, or bulbils, and being bulbiferous (Gray 1877, 1887; Miller et al. 1984). Thus, it is important to clarify the bulb aspects of shoot systems in the *C. sibirica* complex to understand how they represent modifications of the basic rhizomatous shoot system. Bulbs and bulblets are usually described as orthotropic shoot systems that bear fleshy (especially enlarged) scale leaves along very short internodes (Arber 1925; Rees 1972; Dahlgren and Clifford 1982; Bell 1991). Shoot systems of *C. sibirica* var. *bulbillifera* meet the criteria for bulb morphology. The production of relatively large, fleshy scale leaves during late season growth of *C. sibirica* variety *bulbifera* results in a bulb morphology that presumably serves as an overwintering specialization of the basic rhizomatous form shared with other members of the complex. Gray's characterization of the KR form as having bulbs in a basal rosette (Gray 1877) and a crown of bulblet-scales (Gray 1887) calls attention to architectural variation: renewal shoots that formed in the axils of foliage leaves

can have the form of bulbs when distal scale leaves swell late in the growing season and on the primary axis new succulent scale leaves of the current growing season would be formed as a crown distal to the foliage leaves. Bulbs of *C. sibirica* var. *bulbillifera* differ from those found commonly among various geophytic monocots. For example, geophytic monocots often have a thin, dry scale leaf or leaves (the tunic) that surrounds the entire bulb (Mann 1952; Rees 1972; McNeal and Ownbey 1973). Because they have very short internodes and leaves that lack petioles, it can appear that foliage leaves of geophytic monocots emerge from the rosette of fleshy scale leaves (Arber 1925; Dahlgren and Clifford 1982). Both of these distinctive aspects of monocotyledonous bulbs are lacking in *C. sibirica* var. *bulbillifera*. Despite Dahlgren and Clifford's (1982) assertion that bulbs are a specialization found only in monocotyledons, we and others (Rees 1972; Cronquist 1981; Bell 1991) have recognized that a few clades of dicotyledons have also converged on bulb morphology.

Kruckeberg (1984) discussed the general infertility of serpentine soils and the low turnover of nitrogen and phosphorus in communities associated with these soils. He emphasized that these unique nutritional and chemical characteristics have not only ecological but also evolutionary consequences, namely the origin of endemic species and subspecific ecotypes of plants adapted strictly to the serpentine environment. *Claytonia sibirica* var. *bulbillifera* appears to be a serpentine endemic, and we hypothesize that the serpentine environment provided the selection for its bulb morphology. In the KR region, the growing season is limited largely to the late winter and spring and the above-ground foliage of herbaceous perennials has generally senesced by later summer. This relatively short growing season for herbaceous perennials in the KR is reminiscent of that faced by spring ephemerals of eastern deciduous forests. Lapointe (2001) emphasized that subterranean perennating structures, including bulbs, corms, thick

rhizomes, and tubers, were evolutionary responses to the strong selection that spring ephemerals face for the rapid allocation of high levels of nutrients for shoot growth during the early spring when cool temperatures may limit enzymatic activity for photosynthesis. Herbaceous perennials of the KR region would face similar selection; moreover, this selection would be enhanced by the nutrient limitation of the serpentine environment. Thus, selection for a bulb morphology in this complex, in which ancestral heteroblastic variation would have included the formation of thick scale leaves as exemplified by *C. sibirica* var. *bulbillifera*, would help to circumvent the early season need for the rapid uptake of nutrients in a nutrient-limited environment by making them available largely from scale leaves that are specialized for nutrient storage (and were provisioned over the course of the preceding growing season).

Morphological Transitions and Homology

Claytonia sibirica. Arber (1925) emphasized the morphological continuity between bulbs and rhizomes, and we observe this transition in *C. sibirica*. The shoot architecture of both varieties of *C. sibirica* is largely the same, but in var. *bulbillifera* we find specialization in the consistent formation of swollen scale leaves distal to the foliage leaves. At the end of the growing season, the bulb of var. *bulbillifera* consists of a tight aggregation of swollen leaves clustered around the preformed, but unexpanded, leaves and inflorescences of the next growing season. Not all ramets of *C. sibirica* var. *sibirica* form scale leaves at the end of the growing season, but when scale leaves develop they have largely the size and shape of foliage leaf bases and are arranged in a relatively loose rosette at the tip of the shoot and are fewer in number than the swollen scale leaves of var. *bulbillifera* (cf. Fellows 1971). Given the positional and morphological similarity of scale leaves in both varieties, we hypothesize that they are homologous.

Miller et al. (1984) suggested that attributes of diploids, such the morphotypes described here for varieties *sibirica* and *bulbillifera*, could have been combined in hybrid populations and this could account for the presence of scale leaves in some ramets of var. *sibirica*. Alternatively, the formation of scale leaves by some perennial ramets of var. *sibirica* may simply represent variation in populations irrespective of hybridization or polyploidy. Instead these bulb-like modifications may be similar to the precursors of the distinctly bulbous var. *bulbillifera*. Additional populations of var. *sibirica* over its geographic range and habitat conditions need to be sampled for morphological variation, ploidy level, and ancestry to ascertain the phylogenetic homology of shoot system variants.

Claytonia palustris. In contrast to Miller's (1984) description of *C. palustris* as having "...branched rhizomes that are bulbiferous," we did not observe shoot systems in our sampling of this species that had the morphology of bulbs (cf. also Swanson and Kelley 1987). Primary and renewal axes of *C. palustris* become swollen and have short internodes that bear scale leaves at the end of the growth season, but these scale leaves do not enlarge as storage structures, a critical feature of bulbs. The initial elongation of axillary renewal shoots is centered in a single internode, a hypopodium (sensu Bell 1991), that is functioning in a manner similar to the droppers of various monocotyledonous geophytes, (e.g. *Erythronium*) in positioning the orthotropic portion of the renewal axis at a distance from the parent shoot (Arber 1925, McLean and Ivimey-Cook 1951). Aside from the formation of hypopodia during the initial elongation of renewal shoots, shoot architecture is very similar in *C. palustris* and *C. sibirica*. However, *C. palustris* is further distinguished from *C. sibirica* by the formation of leaf bases that completely ensheath the shoot axis, and these leaf bases lack the radial thickening that is common in *C. sibirica*.

Taxonomic Implications

Miller et al. (1984) did not recognize the morphotypes in the *C. sibirica* complex as different taxonomic entities, and Chambers (personal communication) has suggested that *C. sibirica* may simply exhibit high phenotypic plasticity over its wide latitudinal range. However, plants cultivated from seed and grown over successive years under uniform greenhouse conditions show that plants from the KR maintain a strongly bulbiferous phenotype (O'Quinn unpublished data), from which we infer that shoot system plasticity in the formation of enlarged, fleshy scale leaves is limited. Because of their distinctive bulb morphology, discrete geographic distribution and preference for serpentine soils, we have followed Gray's (1887) treatment in recognizing KR populations as *C. sibirica* var. *bulbillifera*.

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LITERATURE CITED

- ARBER, A. 1925. *Monocotyledons: a morphological study*. Cambridge University Press, Cambridge.
- BELL, A. D. 1991. *Plant form: an illustrated guide to flowering plant morphology*. Oxford University Press, New York, NY.
- CHAMBERS, K. L. 1993. *Claytonia*. Pp. 898-900. in J. C. Hickman (ed.), *The Jepson manual: higher plants of California*. University of California Press, Berkeley, CA.

- CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, NY.
- DAHLGREN, R. M. T. AND H. T. CLIFFORD. 1982. The monocotyledons: a comparative study. Academic Press, London.
- FELLOWS, C. E. 1971. A cytotaxonomic study of the origin of *Claytonia washingtoniana*. M.S. thesis. Humboldt State University, Arcata, CA.
- GRAY, A. 1877. Characters of new species etc. Proceedings of the American Academy of Arts and Sciences 12:54.
- 1887. Contributions to American Botany. XV. 1. Revision of some polypetalous genera and orders precursory to the Flora of North America. Proceedings of the American Academy of Arts and Sciences 22: 270-314.
- JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Company, Inc., New York, NY.
- MANN, L. K. 1952. Anatomy of the garlic bulb and factors affecting bulb development. Hilgardia 21: 195-251.
- MCLEAN, R. C. AND W. R. IVIMEY-COOK. 1951. Textbook of theoretical botany, Volume 1. Longmans, Green and Company, London.
- MCNEAL, D. W. AND M. OWNBEY. 1973. Bulb morphology in some western North American species of *Allium*. Madroño 22: 10-24.
- MILLER, J. M. 2003. *Claytonia* L. Pp. 485-488. in Flora of North America Committee (eds.), Flora of North America north of Mexico, Volume 4 Magnoliophyta: Caryophyllidae, part 1. Oxford University Press, New York, NY.

- K. L. CHAMBERS AND C. E. FELLOWS. 1984. Cytogeographic patterns and relationships in the *Claytonia sibirica* complex (Portulacaceae). *Systematic Botany* 9:266-271.
- O'QUINN, R. L. AND L. HUFFORD. In press. Molecular systematics of Montieae (Portulacaceae): implications for taxonomy, biogeography and ecology. *Systematic Botany*.
- QUARTZ PCI. 1993-1998. Scientific Image Management Systems. Quartz Imaging Corporation. Version 4.20.
- REES, A. R. 1972. The growth of bulbs. Academic Press, London.
- SWANSON, J. R AND W. A. KELLEY. 1987. *Claytonia palustris* (Portulacaceae), a new species from California. *Madroño* 34: 155-161.

TABLE 1. COLLECTION DATA FOR SAMPLED POPULATIONS OF THE *C. SIBIRICA* COMPLEX. All vouchers are at WS.

Taxon name	Population location	Collection voucher	Collection date	
<i>Claytonia sibirica</i> var. <i>sibirica</i> Eastern morphotype	Lochsa River sites: (pooled)	R. O'Quinn 483	17 May 2002	
	Glade Creek	R. O'Quinn 488	17 May 2002	
	Eagle Summit	R. O'Quinn s.n	25 Aug 2002	
	Ashpile Creek			
<i>Claytonia sibirica</i> var. <i>sibirica</i> Western morphotype	Columbia River sites: Herman Creek (low)	R. O'Quinn 492 R. O'Quinn 528	20 May 2002 19 Mar 2003	
	Pine Mountain Road (high)	R. O'Quinn 490 R. O'Quinn s.n.	20 May 2002 31 Aug 2002	
	Bridal Veil Falls (low)	R. O'Quinn 529	19 Mar 2003	
	Willamette River site: Corvallis, OR	J. Schenk 774 J. Schenk 773	28 June 2004 27 June 2004	
	<i>Claytonia sibirica</i> var. <i>bulbillifera</i>	Southern Oregon sites: Davis Creek	R. O'Quinn 494	22 May 2002
		Brigg's Valley Road	R. O'Quinn 504	23 May 2002
		Cave's Camp Road	R. O'Quinn 365 R. O'Quinn 474	29 May 2001 05 Aug 2001
Eight Dollar Mtn. Road		R. O'Quinn 290 R. O'Quinn 508	24 May 2000 24 May 2002	
<i>Claytonia palustris</i>		Northern California sites: Jonesville (type location)	R. O'Quinn 330	4 June 2000
	Stubb's Lake (seed source for greenhouse grown collections)	C. Björk 5704b	Multiple collections between 2001 and 2004	

FIGURE LEGENDS

FIGURE 1. Geographic distribution of the *Claytonia sibirica* complex.

FIGURE 2. Maximum likelihood cladogram from combined ITS and *trnK/matK* data showing phylogenetic relationships of the *Claytonia sibirica* complex (O'Quinn and Hufford in press)

FIGURE 3. Diagram of shoot system architecture in the *Claytonia sibirica* complex. A. *C. sibirica* var. *sibirica* B. *C. sibirica* var. *bulbillifera* C. *C. palustris*. Arrows labeled 'a' show aerial rhizomes, arrows labeled 'b' show hypopodia.

FIGURE 4. Shoot system of *Claytonia sibirica* var. *sibirica*. A. Globose/ovoid shoot from active growth phase with foliage leaves and inflorescences removed (western morphotype). B. Shoot system of the eastern morphotype that has three seasons of growth and retains a taproot. C. Habit. D. Leaf base of foliage leaf (left) and scale leaf (right). E. Apex of a shoot system, showing the broad leaf base of a foliage leaf and two developing leaves. F. Cross section through the distal portion of a shoot showing the transectional shapes of leaf bases. G. Scale leaves that formed distally to the foliage leaves. lp = leaf primordium, agr = active growth rhizome, psr = preceding season's rhizome, ar = aerial rhizome, tr = taproot, ol = opposite leaves on inflorescence axis, dl = deltoid lamina, infl = inflorescence axis, sl = scale leaf, slb = scale leaf base, flb = foliage leaf base, fl = foliage leaf. Scale bar = 3.0 mm in A; 2.0 mm in D, G; 1.86 mm in E; 1.0 mm in F; and 1 cm in B, C.

FIGURE 5. Shoot system of *C. sibirica* var. *bulbillifera*. A. Habit. Arrow shows elliptical lamina of the foliage leaf. B. Dissected shoot system showing two seasons of growth. The stem is thicker in the region of active growth than for the preceding season. Foliage leaves, inflorescences and shoot-borne roots have been removed. C. Swollen scale leaves attached to distal portion of a rhizome. D. Shoot system showing overwintered scale leaves proximal to newly expanding foliage leaves and inflorescences. E. Subterranean bulb. F. Shoot system showing characteristics of transition from foliage to scale leaf zones. G. Cross section through distal portion of shoot showing transectional shape of scale leaves. lp = leaf primordium, agr = active growth rhizome, psr = preceding season's rhizome, tr = taproot, infl = inflorescence axis, sl = storage leaf, rl = rudimentary lamina, p = petiole, slb = storage leaf base. Scale bar = 1.0 cm in A; 2.0 mm in B, C, E; 5.0 mm in D; 1.0 mm in F, G.

FIGURE 6. Shoot system of *Claytonia palustris*. A. Renewal shoot apex showing two leaf primordia at apical meristem. B. Renewal shoot showing prominently swollen axis apex. C. Renewal shoots. D. Stoloniferous habit. E. Renewal shoot with hypopodia. h = hypopodium, rs = renewal shoot, lp = leaf primordium, am = apical meristem, sl = scale leaf, fl = foliage leaf. Scale bars = 100 μ m in A; 1.2 mm in B; 1.0 cm in D; 5 mm in C, E.

FIGURE 7. Heteroblastic leaf series from one individual ramet of *Claytonia sibirica* var. *bulbillifera* (collected July 2004). A = Swollen scale leaves proximal to foliage leaves of the active growing season. B = Transition leaves with swollen bases, short petioles and small laminas. C = Foliage leaves. D = Swollen scale leaves at distal end of shoot.

